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Bone Abstracts

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# ECTS 2014

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# CONTENTS

**POSTER PRESENTATIONS**

# Clinical Update

## Postmenopausal osteoporosis, vitamins and minerals

### CU1.1

**Calcium and vitamin D: an update on benefits and risks**

Paul Lips
Amsterdam, The Netherlands.

Abstract unavailable.

DOI: 10.1530/boneabs.3.CU1.1

### CU1.2

**Selenium**

Graham Williams
London, UK.

Selenium (Se) is an essential trace element active in the catalytic sites of many redox enzymes, including glutathione peroxidase and the iodothyronine deiodinases that metabolize thyroid hormones. In humans 25 genes encode selenoproteins, in which a specific tRNA incorporates the amino acid selenocysteine during protein translation. Although specific roles for many selenoproteins are unclear, studies suggest an important role for Se in skeletal development and bone maintenance. In animal studies deletion of the Se transport protein selenoprotein P, or key components of the selenocysteine incorporation machinery during protein translation, results in growth retardation and defects of endochondral ossification. Kashin–Beck disease is an osteoarthropathy that is endemic in Se- and iodine-deficient areas of Asia and which may result from oxidative damage to skeletal tissues due to Se deficiency or in response to combined deficiencies of both elements. Dietary supplementation studies in rodents indicate an important role for Se in bone, but they also identify interactions between Se and iodine that could be confounded by the critical role of both Se and iodine in thyroid hormone metabolism.

We investigated Se status in a well-defined cohort of 1144 healthy euthyroid postmenopausal women from the Osteoporosis and Ultrasound Study (OPUS) and determined its relationship to thyroid status, bone turnover markers, bone mineral density (BMD) and vertebral, hip and non-vertebral fractures. Higher Se and selenoprotein P levels were positively associated with higher BMD and negatively associated with lower levels of bone formation and resorption markers. These associations persisted after adjustment for thyroid status, vitamin D levels and PTH. Effects of changes in thyroid status on BMD and fracture risk were similarly independent of Se status.

Overall, these studies demonstrate that variation in Se status is related to BMD and bone turnover in healthy postmenopausal women.

DOI: 10.1530/boneabs.3.CU1.2

### CU1.3

**Vitamin K and bone health**

Cees Vermeer
Maastrict, The Netherlands.

Vitamin K is a group name for a number of closely related compounds. Relevant structures for this presentation are phylloquinone (vitamin K1), menaquinone-4 (MK-4) and MK-7. The latter two are members of the vitamin K2 family. The function of all forms of vitamin K is to catalyze the formation of Gla-residues, which are strong calcium-binding groups and essential for the activity of the proteins in which they occur. For calcium metabolism three Gla-proteins are of particular interest: osteocalcin, matrix Gla-protein (MGP) and Gla-rich protein (GRP). Population-based studies have demonstrated that poor vitamin K status is associated with accelerated bone loss, low BMD and osteoporosis, but human vitamin K intervention studies have shown controversial outcomes. We have performed three independent vitamin K RCTs in postmenopausal women using vitamin K1, MK-4, or MK-7, and using DEXA BMD and BMC as a clinical endpoint. All three studies showed a positive effect of vitamin K and the effect of MK-7 was observed at a relatively low dose (180 μg/day). Besides its effect on bone, vitamin K has also a positive effect on vascular characteristics (elasticity and pulse-wave velocity). MGP, the main calcification inhibitor in the vasculature, is undercarboxylated in the healthy population suggesting subclinical vitamin K insufficiency. Calcium supplements may increase the vascular calcium load, which is consistent with reports in the literature claiming increased arterial calcification as a result of calcium supplements. Whereas osteocalcin is a direct marker for bone metabolism, uncarboxylated MGP is a marker for vascular calcification risk and mortality. We have designed an assay for circulating uncarboxylated MGP with which we have demonstrated a strong association between vascular vitamin K status and cardiovascular disease. The assay is now available in an automated form (IDS-iSYS inaKtiv MGP assay). The assay was found to be highly predictive for calcifications in chronic kidney disease and peripheral artery disease. We conclude that increased vitamin K intake helps protect against both postmenopausal bone loss and arterial calcification. In case calcium supplements (with or without vitamin D) are prescribed for the prevention of bone loss, it is recommended to combine this with vitamin K.

DOI: 10.1530/boneabs.3.CU1.3

### CU1.4

**Postmenopausal osteoporosis: when to treat and what with?**

Stuart Ralston
Edinburgh, UK.

Abstract unavailable.

DOI: 10.1530/boneabs.3.CU1.4

### CU1.5

**Treatment of postmenopausal osteoporosis: long-term benefit and risk**

Bo Abrahamsen
Copenhagen, Denmark.

Effective treatments are available to reduce fracture risk in patients with postmenopausal osteoporosis. Though factors have been identified that modify the effect of treatment with one or more the currently available drugs, as a rule patients will benefit more from treatment the higher their base risk of fracture. From a societal and clinical point of view it is surprising that many guidelines for postmenopausal osteoporosis do not target treatment to patients at the highest absolute risk, favouring treatment in younger over older postmenopausal women. Further, there is a real need for long-term data on efficacy of osteoporosis drugs. Their mechanism of action may well in many cases permit fairly short durations of treatment – perhaps one or two infusions in the case of zoledronic acid – followed by long periods off treatment.

As for long-term safety it is important to be aware that the harm:benefit ratio may not be constant over time. Indeed, some side effects – such as DVT or MI (raloxifene or strontium ranelate) or oesophagitis (oral bisphosphonates) - may precede any perceivable benefits whereas others may develop only with a long durations of treatment. In recent years, extension studies and real-world pharmacoepidemiology data have provided some data to fill in the gaps but uncertainty remains; perhaps especially about atypical femur fractures. Extrapolating from short term Swedish data on atypical femur fractures, excess atypical femur fractures could outweigh saved hip fractures after as little as 7 years of treatment. Danish data suggest no increase in the total number of subtrochanteric and shaft fractures (atypical and typical combined) after up to 10 years of alendronate treatment. Race and proximal femur geometry are important risk factors for atypical femur fractures so the risk will be much lower in some patients than in others. New data on bone quality using microindentation analysis find equally impaired biomechanical competence in atypical femur fractures and typical osteoporotic fractures but no impairment in long-term bisphosphonate users.

In conclusion, given the relative paucity of long-term efficacy and safety data, physicians should regularly re-assess the indication for treatment. Treatment with antiresorptive drugs for more than five years should be reserved for high risk patients only.

DOI: 10.1530/boneabs.3.CU1.5

## Management of parathyroid diseases

### CU2.1

**Hypoparathyroidism: diagnosis and management**

Natalie Cusano
New York, New York, USA.

Hypoparathyroidism is a disorder characterized by hypocalcemia, low or absent parathyroid hormone (PTH), and abnormal bone remodeling. The diagnosis is made in the setting of hypocalcemia with markedly reduced or absent PTH levels. Postoperative hypoparathyroidism is the most common etiology. Other causes of hypoparathyroidism include autoimmune disease and rarely, congenital syndromes of parathyroid dysgenesis such as DiGeorge syndrome. Standard treatment of hypoparathyroidism consists of oral calcium and vitamin D supplementation at various doses with the goal of ameliorating symptoms of hypocalcemia, maintaining normal serum calcium, and reducing urinary calcium excretion. Patients are typically not easily controlled with standard therapy. Hypoparathyroidism is the only classic endocrine deficiency disease for which the missing hormone is not yet an approved treatment. Subcutaneous recombinant human (rh) PTH(1–34) and rhPTH(1–84) have been studied. In hypoparathyroidism, both rhPTH(1–34) and rhPTH(1–84) lower calcium and vitamin D requirements while maintaining serum calcium levels. Other potential benefits of PTH therapy include an improvement in skeletal dynamics toward more normal levels and improvement in quality of life.

DOI: 10.1530/boneabs.3.CU2.1

### CU2.2

**Primary hyperparathyroidism: epidemiology and diagnosis**

Richard Eastell
Sheffield, UK.

The purpose of this presentation is to present the guidelines for recommending surgery for asymptomatic primary hyperparathyroidism, based on a workshop held in 2013. The indications for surgery were: i) a serum calcium more than 1 mg/dl (or 0.25 mmol/l) above the reference interval; ii) a low bone density (*T*-score $-2.5$ or less at the lumbar spine, total hip, femoral neck, or distal radius 1/3) or presence of vertebral fracture; iii) chronic kidney disease stage 3 (eGFR $< 60$ cc/min) or presence of kidney stones (X-ray, ultrasound or CT) or risk of kidney stones (high urinary calcium of 400 mg/day or 10 mmol/day, and positive kidney stone work-up); iv) age $< 50$. Both second and third generation parathyroid hormone assays may be used but it is important to establish reference intervals based on vitamin D-replete population. It was recognized that a proportion of patients with this disorder will have normal PTH but high serum calcium, and that a proportion will have normal serum calcium and a raised PTH (in the absence of obvious causes such as vitamin D deficiency and CKD), the so-called normocalcaemic hyperparathyroidism. Vitamin D deficiency is commonly associated with this disorder and should be treated as it may be linked to the severity of the disease. More than 10% or more of patients may have a genetic basis, especially individuals who are young, or have multiple glands affected, or have other endocrine disorders already. They also recognized the importance of searching carefully for occult kidney stones as there is some evidence that the risk of kidney stones is reduced by surgery, and for osteoporosis, as there is some evidence that fracture risk may be reduced by surgery.

DOI: 10.1530/boneabs.3.CU2.2

### CU2.3

**Treatment of primary hyperparathyroidism**

Claudio Marcocci
Pisa, Italy.

The aim of management is to normalize serum calcium and reduce PTH levels, leading to improvement in any associated symptoms. Parathyroidectomy (PTx) is the only curative treatment and should be recommended for PHPT patients with symptomatic disease, but also considered in asymptomatic patients who meet surgical criteria defined by international guidelines. Studies of the natural history of asymptomatic PHPT indicate that in the absence of PTx some patients show stability in serum calcium and PTH levels and bone mineral density (BMD) for several years. The guidelines recommend annual monitoring of serum calcium and PTH, and bi-annual measurement of three-site BMD.

Alternative treatment options in patients ineligible for, or unwilling to undergo PTx or failed PTx include the off-label use of bisphosphonates, and the recently approved calcimimetics cinacalcet. Bisphosphonates increase BMD and decrease bone turnover, but have no impact on serum calcium or PTH levels. The calcimimetic cinacalcet reduces serum calcium and PTH and raises serum, but has no effect on BMD. Medical management should be offered to patients with contraindication to surgery or unwilling to have PTx. It could also be considered in selected asymptomatic PHPT patients who meet the surgical criteria for PTx.

DOI: 10.1530/boneabs.3.CU2.3

### CU2.4

**Parathyroid diseases and multiple endocrine neoplasia**

Raj Thakker
Oxford, UK.

Primary hyperparathyroidism (PHPT) may occur as part of a complex syndrome or as an isolated (non-syndromic) disorder, and both of these forms can occur as hereditary (i.e. familial) or non-familial (i.e. sporadic) diseases. Syndromic forms of PHPT include multiple endocrine neoplasia (MEN) types 1–4 (MEN1–MEN4), and the hyperparathyroidism–jaw tumour (HPT–JT) syndrome. MEN1 is characterised by the combined occurrence of parathyroid tumours, pancreatic neuroendocrine tumours (NETS), anterior pituitary tumours, adrenal tumours and gastro-intestinal NETS; MEN2a is characterised by the occurrence of medullary thyroid carcinoma (MTC), phaeochromocytomas, and parathyroid tumours; MEN2b, also called MEN3, is characterised by occurrence of MTC and phaeochromotyomas in association with a marfanoid habitus, mucosal neuromas, medullated corneal fibers and intestinal autonomic ganglion dysfunction; MEN4 may be associated with tumours of the parathyroids, anterior pituitary, adrenals and gonads; and the HPT–JT syndrome is associated with parathyroid tumours, which in 15% of patients are carcinomas, fibro-osseous jaw tumours, renal tumours and uterine tumours. MEN1 is caused by abnormalities of the *MEN1* gene which encodes a tumour suppressor; MEN2 and MEN3 are due to mutations of the rearranged during transfection (*RET*) proto-oncogene, which encodes a tyrosine kinase receptor; MEN4 is due to mutations of a cyclin-dependent-kinase inhibitor (*CDNK1B*); and HPT–JT is due to mutations of cell division cycle 73 (*CDC73*) which encodes parafibromin. Non-syndromic PHPT, which may be familial and referred to as familial isolated hyperparathyroidism (FIHP), may also be due to mutations of the *MEN1*, *CDC73*, or calcium-sensing receptor (*CASR*) genes. In addition, $\sim$10% of patients presenting below the age of 45 years with non-syndromic (sporadic) PHPT may have *MEN1*, *CDC73* or *CASR* mutations, and overall more than 10% of patients with PHPT will have a mutation in one of 11 genes.

DOI: 10.1530/boneabs.3.CU2.4

### CU2.5

**Secondary and tertiary hyperparathyroidism**

Marie-Helene Lafage-Proust
Saint-Etienne, France.

The pathophysiology of secondary hyperparathyroidism (II HPT) in the absence of renal disease is mainly due to lack of vitamin D, calcium deficiency being extremely rare. In case of chronic kidney disease (CKD), phosphate retention and increase in secretion of FGF23 by bone are among the earliest factors associated with II HPT occurrence. Then, the deficit in calcitriol synthesis induces lack of calcium intestinal absorption which worsens II HPT. As renal function declines, the loss of receptors for FGF (FGFR), vitamin D (VDR) and calcium (CaSR) in parathyroid glands is responsible for the progressive autonomisation of these glands leading, together with increase in parathyroid cell proliferation, to tertiary HPT. Tertiary HPT correspond to two histology types: hyperplasia or adenoma, both lesion being potentially associated. The primary management of II HPT is based upon natural vitamin D supplementation (ego/colecalciferol) in association with calcium. In non uremic patients, the use of other forms of vitamin D is not recommended, except when hypocalcemia is present, then treatment with 25OH vitamin D may be used. In CKD, both natural and 1 $\alpha$ vitamin D derivatives may be used in order to down regulate PTH secretion. Cinacalcet, a calcium sensing receptor agonist has, in few years, substantially changed the management of II HPT in CKD and considerably reduced the indication for parathyroid surgery.

DOI: 10.1530/boneabs.3.CU2.5

# Allied Health Professionals Session

## AHP1

**Epidemiology of ageing, fracture and falls. Geographic and ethnic disparities in osteoporotic fractures**

Jane Cauley
Pittsburgh, Pennsylvania, USA.

Osteoporotic fractures are a major worldwide epidemic. Worldwide, age-standardized rates in hip fracture vary >200-fold in women and >140-fold in men when comparing incidence rates in the highest vs lowest country. Median age standardized rates are highest in North America and Europe followed by Asia, Middle East, Oceania, Latin America, and Africa. Globally rates of hip fracture are greater in women than men with an average ratio of ~2:1. In all populations studied to date, hip fracture rates increase with age in both men and women. However, the correlation in age-specific rates was greatest in older individuals (> age 75) with weak correlations between older (> age 75) and younger (< age 60) individuals. The incidence of radiographic vertebral fractures is much higher than hip fractures while incidence rates of clinical vertebral fractures mirror hip fracture rates in most countries. Methodological challenges of defining and ascertaining vertebral fractures limits the interpretation of these data. Secular declines in hip fracture have been reported in populations from North America, Europe and Oceania. These declines are especially notable in women, suggesting that reproductive factors might contribute to this reduction. By contrast, hip fracture rates are increasing in parts of Asia and Latin America. Global indicators of health, education and socioeconomic status are positively correlated with fracture rates suggesting that lifestyles in developed countries might contribute to hip fractures. Most fractures occur because of a fall and the differences in the incidence of falls and risk factors for falls may also contribute to the geographic variability in hip fractures. Improvements in fracture assessment, in particular non-hip fractures and identification of factors that contribute to this variability, might substantially impact our understanding of osteoporotic fracture aetiology and provide new avenues for prevention.

DOI: 10.1530/boneabs.3.AHP1

## AHP2

**Imaging the older patient**

Claus Glüer
Kiel, Germany.

Abstract unavailable.

DOI: 10.1530/boneabs.3.AHP2

## AHP3

**Self-perceived fracture risk among post-menopausal women: how to communicate fracture risk (IS3)**

Mette Juel Rothmann
Odense, Denmark.

Abstract unavailable.

DOI: 10.1530/boneabs.3.AHP3

## AHP4

**Vitamin D and bone health in the older patient**

Paul Lips
Amsterdam, The Netherlands.

Vitamin D deficiency is common in aging people and is associated with secondary hyperparathyroidism, bone loss, osteoporosis and fractures in epidemiological studies. Vitamin $D_3$ is produced in the skin through u.v. irradiation of the sun in spring and summer, and it can also be obtained from food, especially fatty fish, such as herring or mackerel. Vitamin D is activated by liver and kidney. Its main action is stimulation of calcium absorption from the gut. Vitamin D status is positively related to bone mineral density. Many randomized clinical trials studying the effect of vitamin D with or without calcium vs placebo have been done with fracture incidence as main outcome. About one-third of these showed a decrease of hip fracture and/or non-vertebral fracture incidence in the order of 10–20%. The vitamin D dose in these trials was 400–1000 IU/day. Many meta-analyses have been performed generally concluding to a modest preventative effect. The effect was stronger in older persons, in the institutionalized, and when baseline calcium intake and serum 25-hydroxyvitamin D were low. On the other side, an increase in fracture incidence was seen in two trials where vitamin D was given in a very high dose once per year. In some clinical trials, vitamin D with or without calcium also led to a decrease of the incidence of falling. Severe longstanding vitamin D deficiency may result in mineralisation defects and osteomalacia (English disease) but this is relatively rare. The optimal blood level of 25-hydroxyvitamin D is above 50 nmol/l. To obtain this level without sunshine exposure the vitamin D intake should be 800 IU/day.

**Learning objectives**

Vitamin D is produced in the skin under the influence of sunlight and can be ingested with some foods, e.g. fatty fish.

Vitamin D stimulates the absorption of calcium from the gut.

Vitamin D deficiency is related to osteoporosis and fractures.

Vitamin D and calcium supplements can decrease fracture incidence.

DOI: 10.1530/boneabs.3.AHP4

# Main Symposium

## Osteocytes and calcium homeostasis

### S1.1

**Periosteocytic osteolysis in preclinical models**
Alberta Zallone
Bari, Italy.

Abstract unavailable.

DOI: 10.1530/boneabs.3.S1.1

### S1.2

**Osteocytes and cortical bone quality in human bone diseases**
Björn Busse
Hamburg, Germany.

While a strong emphasis has been put on the characteristics of trabecular bone due to its' high metabolic rate, the human skeleton actually consists of ~80% cortical bone. Furthermore, the cortical bone supports a major proportion of the mechanical load (i.e. upto 96%) at common fracture sites, such as the base of the femoral neck and the intertrochanteric region. In these regions of the hip, large compressive stresses concentrate during walking as well as during falls and may result in bone fracture. Bone's ability to resist fracture originates from the quality of the trabecular and cortical compartments. Bone quality encompasses many aspects of the bone's state including the multi-length-scale composition, mass, architecture, microdamage, bone turnover and osteocytic mechanosensitivity. When aspects of the bone quality are altered due to disease-specific disorders, the risk of fracture may be attributed to changes in bone quality endangering the bone's normal organization and is particularly relevant in cases with diseased bone and its subsequent treatment. Here, our data shows how osseous and cellular characteristics of the cortical compartment vary with age, disease and treatment strategies (e.g. osteoporosis, vitamin D-deficiency, Paget's disease of bone, bisphosphonate treatment, total hip replacement, etc.) and may influence the risk of bone fracture. To assess changes in bone quality across bone's hierarchical structure, a combination of techniques was carried out at the nano- to micron-level including 2D histomorphometry, 3D-microcomputed tomography, scanning electron microscopy/backscattered electron microscopy, Raman spectroscopy, Fourier transform infrared microscopy, micro-mechanical and materials testing. In this presentation, summarized findings emphasize how the structure of mineralized bone including the underlying osteocytic network, bone's composition, as well as the mineral and collagen distribution may influence changes in the bone quality framework and the risk of fracture in a cohort of subjects suffering from skeletal aging, major bone diseases and/or pharmacologic treatment.

DOI: 10.1530/boneabs.3.S1.2

## Imaging – from cell to structure

### S2.1

**Imaging and fracture risk prediction**
Claus Glüer
Kiel, Germany.

Abstract unavailable.

DOI: 10.1530/boneabs.3.S2.1

### S2.2

**Multiphoton microscopy of cell migration**
Peter Friedl
Nijmegen, The Netherlands.

Abstract unavailable.

DOI: 10.1530/boneabs.3.S2.2

## Vascular calcification

### S3.1

**Mechanisms of vascular calcification**
Catherine Shanahan
London, UK.

Vascular calcification is a ubiquitous pathology in the aged and diseased vasculature occurring at two sites in the vessel wall; in the intima in association with atherosclerosis and in the media in association with ageing, diabetes, and end-stage renal disease. Calcification at either site is associated with adverse cardiovascular outcomes including increased risk of myocardial infarction and arterial stiffness. Numerous studies have observed that vascular calcification and cardiovascular mortality are increased in association with osteoporosis suggesting a possible link between these two processes. Indeed, overwhelming evidence has now established that vascular calcification is an actively regulated, cell-mediated process, similar to bone formation, that is orchestrated by vascular smooth muscle cells (VSMCs). VSMCs modulate to an osteo/chondrocytic phenotype in response to injury, and this is associated with upregulation of the obligate bone/cartilage transcription factors Runx2 and Sox9. VSMCs also release matrix vesicles and these small membrane-bound bodies, derived from both apoptotic and stressed VSMCs are capable of nucleating hydroxyapatite. Under normal conditions, these vesicles are loaded with potent inhibitors of mineralization, including matrix Gla protein (MGP) and fetuin-A, which act to block or control crystal nucleation and growth. However, if these inhibitory proteins are lacking or dysfunctional, or vesicle release is overwhelming calcification ensues. Importantly, a number of key proteins and pathways have been shown to regulate both vascular calcification and bone mineralization. These include osteoprotegerin, bone morphogenetic proteins (BMPs), and inflammatory pathways. Recent data suggests that the DNA damage response may activate a number of these pathways to drive vascular calcification locally and may potentially also induce systemic changes that concomitantly impact on bone homeostasis.
Learning objectives
Vascular calcification is a cell-mediated process with similarities to bone formation.
VSMCs orchestrate vascular calcification.
Calcification involves cell death, loss of inhibitors and osteo/chondrogenic differentiation of VSMCs.
Ageing is a key driver of vascular calcification.
A number of key signaling pathways drive VSMC osteo/chondrogenic differentiation.
Evidence that vascular calcification and bone mineralization are co-regulated.

DOI: 10.1530/boneabs.3.S3.1

### S3.2

**Clinical aspects**
Keith Hruska
St Louis, Missouri, USA.

Abstract unavailable.

DOI: 10.1530/boneabs.3.S3.2

## Maintaining muscle during life

### S4.1

**Making muscle in the embryo and the adult, role of muscle stem cells**
Didier Montarras
Paris, France.

The formation of skeletal muscle depends on myogenic regulatory factors of the MyoD family that are essential for entry into the myogenic programme and subsequent differentiation of muscle fibres. More recently attention has focussed on upstream regulators of muscle progenitor cells, prior to activation of the myogenic determination genes Myf5 and MyoD. In this context, Pax3 and Pax7 play an important role. In the embryo, Pax3 marks muscle stem cells that give rise to trunk and limb muscles. Pax7 is subsequently co-expressed with Pax3 in this cell population that fuels muscle growth during development. In adult muscle, Pax7/3 positive cells, now present as quiescent satellite cells on muscle fibres, are

activated on injury and ensure regeneration of the tissue. The behaviour of skeletal muscle stem cells will be discussed in the context of developmental and regenerative myogenesis.

DOI: 10.1530/boneabs.3.S4.1

### S4.2

**New targets for pharmacological treatment of sarcopenia in old age**

David Glass
Boston, Massachusetts, USA.

Abstract unavailable.

DOI: 10.1530/boneabs.3.S4.2

## Anabolic treatment of osteoporosis

### S5.1

**Limitations to anabolic stimulation of bone formation**

Roland Baron
Boston, Massachusetts, USA.

Abstract unavailable.

DOI: 10.1530/boneabs.3.S5.1

### S5.2

**Clinical use of anabolic drugs**

Adolfo Diez-Perez
Barcelona, Spain.

Anabolic drug (AD) options are currently limited to PTH analogs. With potent bone-forming effects, AD offer marked increases in BMD. However, the demonstration of their efficacy on fracture risk reduction was possibly limited by the early discontinuation of the pivotal trial for safety concerns. Therefore, their use must rely both in the available evidence as well as in the clinical judgment for the individual patient. Indeed, potential contraindications are the first step in deciding if we put our patient on AD. Not least, cost of treatment should be also weighted when indicating this treatment.

As a first-line option can be considered in cases of very advanced disease, measured in terms of BMD and existing fractures, with the objective of inducing a 'remission' of the disease to, later on, continue with a maintenance therapy for consolidating the achieved gains in bone density and strength. Two options are possible, used alone or in combination with a potent antiresorptive even though the latter option remains in an investigational phase. More evidence on antifracture efficacy is needed for the combined use although offers a promising efficacy profile in cases at substantial risk of hip fracture.

Another scenario when AD can be considered as a first alternative might be in cases where risk factors for treatment failure to oral antiresorptives accumulate in a single patient. In this case the clinician could choose between an AD or a potent injected antiresorptive. Very similar decision is faced by the clinician when a patient has failed to oral antiresorptives, again with similar alternatives for switching therapy to an AD or a potent injected AR.

Finally, a third possibility is in cases of intolerance or, more importantly, side effects associated with the use of antiresorptives. Two main situations are under active research, cases of osteonecrosis of the jaw and atypical femoral fractures, when the patient still is at high risk of common fragility fractures.

New, more potent anabolics, better combined treatments or regimens, including non pharmacological interventions are the future for achieving the cure of osteoporosis, not just a decrease in risk, the best we can currently achieve.

DOI: 10.1530/boneabs.3.S5.2

## New approaches to fracture prediction

### S6.1

**Virtual physiological human**

Marco Viceconti
Firenze, Italy.

Abstract unavailable.

DOI: 10.1530/boneabs.3.S6.1

### S6.2

**Goals of treatment for osteoporosis**

Steven Cummings
San Francisco, California, USA.

Drug treatment for osteoporosis typically begins with an oral bisphosphonate, regardless of initial BMD or fracture risk and decisions to continue or change treatment are often based on evidence of 'response' to treatment based on changes in BMD, bone turnover markers, and occurrence of fractures. Treatment is often continued for fixed periods of time, perhaps stopped for a 'drug holiday'.

This approach differs from preventive therapy for other conditions, such as hypertension, where treatment is based on achieving a goal. The goal could be a certain risk of fracture or level of BMD. Goal-directed treatment would individualize the initial choice of treatment based on the probability that alternatives would achieve the patient's goal. In contrast to changing treatments based on years of use or failure to respond, the patient's BMD and risk would be reassessed periodically and decisions to stop or change treatment would be based on achieving or maximizing the chance of reaching an acceptable level of fracture risk or BMD.

A task force of the ASBMR and US National Osteoporosis Foundation, including clinicians and scientists from many specialties and countries, has been developing recommendations about goal-directed treatment for osteoporosis. I will provide an update on the issues and recommendations being considered by the task force.

DOI: 10.1530/boneabs.3.S6.2

# Clinical Debate

## D1.1

**Debate: there are more benefits than risks associated with HRT in postmenopausal women: for the motion**

John Stevenson
London, UK.

Hormone replacement therapy (HRT) comprises estrogen, with or without progestogen addition, given to postmenopausal women. HRT is by far the most effective treatment for the relief of menopausal symptoms such as vasomotor and genito-urinary symptoms. It is as effective as alternative treatments for the prevention of postmenopausal osteoporosis. It increases bone mass, reduces bone turnover, and reduces fracture risk at all the important skeletal sites. It has a beneficial effect on collagen in bone and skin, and helps preserve inter-vertebral discs. It is now increasingly realised that low doses of oestrogen may be effective for bone conservation in most women. There is no increased incidence of osteonecrosis of the jaw or femoral fragility fractures as are seen with some alternative treatments. HRT should remain the first-line therapy for primary prevention of osteoporosis. Concerns about increased cardiovascular risks were inappropriately raised by the Women's Health Initiative, but the totality of current data shows coronary benefits and no increased stroke risks when HRT is initiated early in the menopause, and there is a reduction in mortality. Other benefits associated with HRT use include prevention of type 2 diabetes, improvements in cognitive function and a possible reduction in colo-rectal cancer. Increased venous thrombo-embolic events can be avoided with non-oral HRT which may also reduce stroke risk, Any possible increased risk of breast cancer remains controversial and unproven with estrogen-progestogen HRT, and the magnitude of any increased risk is extremely small, being less than many lifestyle factors. With estrogen-alone HRT there is a significant reduction in breast cancer. Finally, the benefits of HRT on quality of life are profound, and this should leave no doubt that there are far more benefits than risks.

HRT learning objectives

Understand the benefits such as relief of menopausal symptoms, prevention of osteoporosis, prevention of coronary heart disease, reduced incidence of diabetes, reduced mortality and improvement in quality of life.

Understand the magnitude of any risks such as stroke, venous thrombo-embolism, and possibly breast cancer, and learn how to avoid or minimise these risks.

DOI: 10.1530/boneabs.3.D1.1

## D1.2

**Debate: there are more benefits than risks associated with HRT in postmenopausal women: against the motion**

Jane Cauley
Pittsburgh, USA.

Currently menopausal hormone treatment (HT) is not recommended for the prevention of chronic disease. This recommendation is based on reputable science relying largely on the Women's Health Initiative (WHI), a large methodologically sound, long-term, randomized clinical trial (RCT) of 27 347 women age 50–79 years. A RCT is considered level two evidence in the hierarchy of evidence only below a systematic review of several RCTs. The WHI HT trials were designed to determine the risks and benefits of HT when taken for chronic disease prevention by predominantly healthy postmenopausal women. Specific targets for age were instituted to insure a wide representative sample of postmenopausal women. At the time the WHI was designed (1990–1992), menopausal HT had been increasingly viewed as a way to prevent chronic diseases of aging, including coronary heart disease (CHD) and cognitive impairment. Women with an intact uterus received conjugated equine estrogen (CEE; 0.625 mg/day) plus medroxyprogesterone acetate (MPA; 2.5 mg/day) or placebo. Women with prior hysterectomy received CEE or placebo. The intervention lasted a median of 5.6 years in the CEE + MPA trial and 7.2 years in the CEE-alone trial with 13 years of cumulative follow-up. During the intervention phase of CEE + MPA trial, the hazard ratio (HR) for the primary endpoints were CHD, HR = 1.18; 95% CI, 0.95–1.45 and invasive breast cancer, HR = 1.24; 95% CI, 1.10–1.53. Additional risks included an increased stroke, pulmonary embolism, deep vein thrombosis (DVT), dementia, gall bladder disease and urinary incontinence. For the CEE trial, the HR for the primary endpoints were CHD, HR = 0.94; 95% CI, 0.78–1.14 and breast cancer, HR = 0.79; 95% CI, 0.6–1.02. In the post-intervention period the increased risk of breast cancer in the CEE + MPA trial persisted and in CEE-alone, the decreased risk was statistically significant. Of importance, however, the risk of stroke, DVT, gall bladder and urinary incontinence remained elevated among women randomized to CEE-alone. These risks outweighed observed benefits for hip fracture, diabetes and vasomotor symptoms. In summary, in both HT trials, the observed increased risk of stroke and DVT as well as gall bladder disease and urinary incontinence remain a concern in both younger and older women on both regimens. The risks of HT outweigh benefits for chronic disease prevention.

DOI: 10.1530/boneabs.3.D1.2

# Workshops

## Extracellular vesicles: from old to new frontiers

### W1.1

**Matrix vesicles and mineralisation**

Alexander Kapustin
London, UK.

Vascular calcification is a ubiquitous pathology in the aged and diseased vasculature. Recently we have focused on the earliest events in the calcification process and have shown that calcification is initiated in small membrane-bound bodies, or matrix vesicles, derived from both apoptotic and stressed VSMCs. Under normal conditions, these vesicles are loaded with potent inhibitors of mineralization, including matrix Gla protein (MGP) and fetuin-A, which act to block or control crystal nucleation and growth. However, if these proteins are lacking or dysfunctional, or vesicle release is overwhelming calcification ensues. Importantly, using vessels obtained from children with chronic kidney disease (CKD) we have shown that apoptosis and vesicle release, together with dysfunctional inhibitors, are fundamental events in the calcification process *in vivo*. More recent work has focussed on the mechanisms via which matrix vesicles calcify and we have identified annexins and phosphatidyl serine as important in forming a nucleation complex for the initiation of mineralization. We have also made progress in identifying the sub-cellular origin of VSMC-derived matrix vesicles.
Learning objectives
Vascular calcification is a regulated cell mediated process.
Matrix vesicles are the nucleating factors in vascular calcification.
Extracellular calcium acts to change the mineralization capacity of matrix vesicles.
Matrix vesicles are of endosomal origin and are released as exosomes.

DOI: 10.1530/boneabs.3.W1.1

### W1.2

**Tumour derived vesicles in bone metastasis**

Yibin Kang
New Jersey, USA.

Bone metastasis is a frequent occurrence in cancer patients, with severe complications such as fracture, bone pain, and hypercalcemia. The pathogenesis of osteolyitc bone metastasis depends on cross-communications between tumor cells and various stromal cells residing in the bone microenvironment. Many of these interactions are mediated by tumor derived growth factors, cytokines, proteases, and other secreted and exosomal proteins. Using gene expression profiling and proteomic analysis, we identified several key protein components of metastatic tumor secretome and exosomes that were uniquely associated with bone metastasis. We then incorporated bioinformatic analyses of large clinical metastasis datasets and functional studies in animal models to identify novel bone metastasis proteins. Several selected and exosomal proteins are found to strongly promote *in vivo* bone metastasis, including i) exosomal c-MET, ii) soluble ICAM1 and VCAM1; iii) matrix metalloprotease MMP1 and ADAMTS1, iv) the salivary cystatins CST1, CST2, and CST4; and v) the plasminogen activators PLAT and PLAU; or vi) the collagen functionality proteins PLOD2 and COL6A1. These findings underscore the importance secreted and exosomal proteins in mediating tumor-stromal interactions that drive osteoclastogenesis and bone destruction during metastasis.

DOI: 10.1530/boneabs.3.W1.2

### W1.3

**Extracellular RNAs and cell to cell communication**

Esther Nolte-'t Hoen
The Netherlands.

Release of RNA into the extracellular space is a newly identified means of intercellular communication operating in many organisms. Such extracellular RNAs can be released via 50–1000 nm vesicles Almost all cell types produce such extracellular vesicles (EV) and the secretion and proteins/lipids per RNA content is regulated by the producing cell. Besides release of vesicle-enclosed genetic material, cells can also release RNA in association with macromolecular protein complexes. Upon transfer to target cells, the (EV-associate) RNA can modify the function of targeted cells by regulation of gene expression. Moreover, extracellular RNA in body fluids can serve as biomarkers for disease.
In-depth identification of the molecular composition of EV is crucial for evaluating their function. Recently, we developed a high-resolution flow cytometric method enabling quantification and characterization of individual nano-sized vesicles. We have used this technique to analyze EV subsets produced during interactions of immune cells. Furthermore, we applied deep sequencing for a screen of small ($<70$ nt) RNAs in vesicles released during these cellular interactions. A selective set of small RNAs was released by cells into the extracellular space. microRNAs formed only a minority of EV-associated small RNA species. In contrast, other small noncoding RNAs with regulatory capacity were highly abundant. These included fragments of RNA repeat sequences and of known non-coding transcripts that could regulate gene expression similar to microRNAs. In addition, the cell-derived vesicles were highly enriched in a set of noncoding structural RNAs.
Major questions in the field are i) which mechanisms are underlying the selective sorting of RNAs into EV or other macromolecular complexes released by cells and ii) how the extracellular RNA can enter target cells and modify their behavior.

DOI: 10.1530/boneabs.3.W1.3

## Genetics of bone disease

### W2.1

**GWAS: what comes next?**

Fernando Rivadeneira
Rotterdam, The Netherlands.

Abstract unavailable.

DOI: 10.1530/boneabs.3.W2.1

### W2.2

**Epigenetics**

Jose Riancho

Epigenetic mechanisms regulate gene expression and are heritable through cell divisions even though they do not involve modifications in the gene sequence. They include methylation and other chemical modifications of cytosine nucleotides, histone posttranslational modifications, microRNAs and other non-coding RNAs. Most cytosines in the human genome are methylated, particularly in the inactive, tightly packed, heterochromatin of autosomes, and the inactivated X-chromosome in females. This is thought to contribute to DNA stability. However, the promoters and enhancers of many genes contain CpG-rich regions that may remain unmethylated. The methylation of cytosines in CpG dinucleotides in gene promoters tends, in association with specific histone modifications, to repress gene transcription. This allows body cells to display a whole variety of phenotypes despite them all having the same gene sequence. Thus, the methylation/demethylation of specific cytosines plays a central role in embryogenesis, and in cell differentiation needed for normal tissue homeostasis and turnover, as well as in cancer and other diseases. It also represents a pathway by which environmental influences, either in the uterus or after birth, impact on the phenotype of the individual. The role of DNA methylation in the differentiation of cells of the osteoblastic lineage has been well demonstrated. Thus, the expression of genes playing a major role in bone homeostasis (such as those encoding alkaline phosphatase, os teoprotegerin, RANKL or sclerostin) is associated with the demethylation of CpG-rich regions in their promoters. Modern technologies permit the use of genome-wide approaches to study DNA methylation. Investigators comparing the methylation patterns in patients with osteoporosis and other skeletal disorders have found several differentially methylated regions. However, the interpretation of these studies has inherent complexities because, unlike the genome, the epigenome of the individual is tissue and cell-specific and may change with time.

DOI: 10.1530/boneabs.3.W2.2

## W2.3

**Pharmacogenetics**
Munir Pirmohammed
Liverpool, UK.

Abstract unavailable.

DOI: 10.1530/boneabs.3.W2.3

# Blood supply in pathophysiology of bone

## W3.1

**Functional role of blood vessels in bone**
Marie-Helene Lafage Proust
Saint-Etienne, France.

Blood vessels and bone display spatial and functional relationships. While the coupling of angiogenesis to bone formation during modeling situations is well documented, the vessels roles in bone remodeling, although widely acknowledged, remain poorly explored. In order to image and quantify the bone vessel network in rodents, we contrasted the vascular bed with barium sulfate, followed by histology or either conventional or synchrotron radiation micro-computed tomography. We showed that treadmill running induces angiogenesis in the rat while unloading decreases bone vessel density. Further, blocking vessel development with anti-VEGF-antibody prevented physical exercise-induced bone gain. Conversely, intermittent PTH1-84 (iPTH) administration, although osteoanabolic, was not associated with bone angiogenesis. However, image quantitative analysis revealed that PTH affects the smallest vessels by relocating them closer to bone-forming sites. At that point, our next aim was to analyse bone vessels at both structural and functional levels and we turned to the mouse model. We found that tibia perfusion, evaluated by Laser Doppler, as well as vessel density, declined after ovariectomy prior to bone loss. We then compared iPTH to continuous PTH at the same daily doses, which both stimulate bone formation regardless of administration mode. iPTH increased vessel size and bone perfusion while cPTH did not. Thus, angiogenesis and bone formation in anabolic situations are not always coupled and challenge-induced variations of vessel density and bone perfusion do not necessarily point in the same direction. We hypothesize that a post-angiogenesis process may be involved in the bone-vessel crosstalk whose deciphering will need further technical developments

DOI: 10.1530/boneabs.3.W3.1

## W3.2

**Vessel–tissue interactions in the skeletal system**
Ralf Adams
Muenster, Germany.

Angiogenesis is the main process mediating the expansion of the blood vessel network during development, tissue regeneration or in pathological conditions such as cancer. The formation of new endothelial sprouts, a key step in the angiogenic growth program, involves the selection of endothelial tip cells, which lead new sprouts. Angiogenic sprouting is induced by tissue-derived signals such as vascular endothelial growth factor (VEGF), which activates signaling by endothelial receptor tyrosine kinases. However, this response is strongly modulated by intrinsic signaling interactions between endothelial cells, which involve the Notch pathway.
More recently, we found that blood vessel growth in bone involves a specialized, tissue-specific form of angiogenesis that is distinct from other organ systems. Notch signaling promotes endothelial cell proliferation and vascular growth in postnatal long bone, which is the opposite of the well-established function of Notch and its ligand Dll4 in the endothelium of other organs and tumors. We also found that Notch controls the release of angiocrine signals from the bone endothelium and thereby controls perivascular osteoprogenitor cells. Using a combination of inducible, cell type-specific mouse genetics and pharmacological approaches, we also discovered that endothelial hypoxia-inducible factor 1 (H1F1$\alpha$) promotes angiogenesis in the postnatal skeletal system and thereby bone formation. The sum of these findings establish a molecular framework coupling angiogenesis, angiocrine signals, and osteogenesis through endothelial Notch and HIF signaling, which may prove significant for the development of future therapeutic applications.

DOI: 10.1530/boneabs.3.W3.2

## W3.3

**Skeletal blood flow in bone repair**
Ryan Tomlinson
Washington, USA.

All biological tissues, including bone, require vascular support to survive. In fact, bone is a highly vascularized tissue, although this aspect of bone is often overlooked. Extensive work has demonstrated that the blood vessels in bone are necessary for nearly all skeletal functions, including development, homeostasis, and repair. In addition, blood vessels lost due to trauma regenerate, and new bone tissue formed in response to injury is aggressively vascularized. As a consequence of this environment, the blood vessels in bone are highly active, not simply a passive source for the delivery of nutrients. In this session, the mechanisms of blood flow in bone repair and regeneration will be reviewed. First, the skeletal vascular anatomy will be reviewed, with an emphasis on the long bones. Next, the distinct mechanisms for vascularizing bone tissue as well as methods for remodeling existing vasculature will be reviewed. In addition, techniques for quantifying bone blood flow will be briefly summarized. Finally, the body of experimental work that demonstrates the role of bone blood flow in fracture healing, distraction osteogenesis, osteoporosis, disuse osteopenia, and bone grafting will be reviewed.

DOI: 10.1530/boneabs.3.W3.3

# Endocrine regulation of bone

## W4.1

**Hyponatremia, sodium metabolism and bone**
Joseph Verbalis
Pittsburgh, Pennsylvania, USA.

Several independent international studies have shown increased bone fracture rates in patients with hyponatremia (HN). A likely major contributor to this finding is gait instability and increased falls in HN patients. Studies in experimental animals have also demonstrated HN-induced bone loss, and analysis of human subjects in NHANES III showed a significantly increased odds ratio for osteoporosis by hip DXA in the HN subjects in this database. *In vitro* studies of the mechanisms underlying HN-induced osteoporosis have implicated a resorptive osteoporosis due to osteoclast sensing of serum and extracellular fluid sodium concentration ($Na^+$) with subsequent stimulation of osteoclast proliferation and resorptive activity; this pathophysiology occurs independently of vasopressin levels. In considering the teleological basis for osteoclast activation by low extracellular ($Na^+$), it is notable that bone mineral is rich in sodium, upto 40% of which is exchangeable with circulating sodium within a relatively short time. We therefore hypothesize that bone acts as a sodium storage reservoir that can be mobilized to maintain the sodium content of the ECF at levels adequate to maintain blood volume, blood pressure and tissue perfusion during times of sodium deficiency, similar to the release of bone calcium to maintain calcium homeostasis during calcium deficiency. However, this evolutionarily adaptive mechanism to maintain sodium homeostasis during times of environmental sodium deprivation also has adverse consequences by negatively impacting bone quality and increasing fracture risk. This talk will review current data regarding the pathophysiology of HN-induced osteoporosis, discuss the role of bone in maintaining sodium homeostasis, and present new data regarding the prevalence and potential impact of HN on osteoporosis and fragility fractures that support our hypothesis that HN is a significant and clinically important risk factor for osteoporosis and bone fractures.

DOI: 10.1530/boneabs.3.W4.1

## W4.2

**Bone sparing glucocorticoids**
Mark Cooper
Sydney, New South Wales, Australia.

Therapeutic glucocorticoids are still widely used for their anti-inflammatory effects. There are however significant side effects associated with their use. These include effects on bone leading to osteoporosis and fracture but also on muscle (increasing risk of falls) and systemic fuel metabolism (leading to diabetes and increased cardiovascular risk). Research over many decades has tried to develop agents which retain the anti-inflammatory effects of glucocorticoids but have reduced impact on bone, muscle, and glucose metabolism. The main approach to

developing these agents was based on a model in which the glucocorticoid receptor (GR) regulated separate pathways for the anti-inflammatory and non-inflammation related effects of glucocorticoids (transrepression and transactivation respectively). However, recent work characterising the action of the GR suggests that these pathways overlap considerably and are very difficult to 'disassociate'. A more recent approach has been to examine compounds that appear to be bone sparing and then work out which molecular properties make them useful. Using this approach interesting compounds have been identified although they do not appear safe enough for use in humans. Recent research has also highlighted how little we understand about how glucocorticoids have their effects in various diseases. In the future it is likely that anti-inflammatory bone sparing agents that work through the GR will be developed that have to be carefully matched to the underlying illness being targeted.

DOI: 10.1530/boneabs.3.W4.2

## W4.3

**Thyroid hormones and bone**

Graham Williams
London, UK.

Hypothyroidism delays bone development and linear growth, whereas thyrotoxicosis accelerates skeletal development and is an important risk factor for osteoporosis in adulthood. Even sub-clinical thyrotoxicosis is associated with fracture, and treatment with thyroxine at doses that suppress TSH results in increased bone turnover and low BMD in postmenopausal women.

To investigate the mechanism of $T_3$ action in the skeleton, we characterized mice with mutation or deletion of $T_3$ receptor (TR) $\alpha$ and TR$\beta$ in several genetic backgrounds. Delayed ossification was accompanied by growth retardation in TR$\alpha$ mutant mice, whereas juvenile TR$\beta$ mutant mice had advanced ossification but persistent short stature due to early growth plate quiescence. Adult TR$\alpha$ mutants had skeletal dysplasia and osteosclerosis, whereas TR$\beta$ mutants were osteoporotic. $T_3$ target gene expression was reduced in osteoblasts and growth plate chondrocytes in TR$\alpha$ mutant mice but increased in TR$\beta$ mutants, indicating impaired skeletal $T_3$ action in TR$\alpha$ mutants but enhanced signalling in TR$\beta$ mutants. TR$\alpha$ was expressed at 15-fold higher levels than TR$\beta$ in bone, whereas TR$\beta$ predominates in hypothalamus and pituitary, where it controls feedback regulation of TSH secretion. Accordingly, TR$\alpha$ mutant mice were euthyroid, whereas TR$\beta$ mutants had elevated circulating thyroid hormone concentrations with pituitary resistance to thyroid hormone. These data demonstrate that TR$\alpha$ is the major functional TR in bone, whereas skeletal responses to disruption of TR$\beta$ result from effects on the hypothalamic–pituitary–thyroid axis resulting in elevated circulating thyroid hormone concentrations.

Recently, four individuals with dominant-negative mutations of TR$\alpha$ have been identified. These children exhibit skeletal dysplasia characterised by delayed ossification, growth retardation, epiphyseal dysgenesis, patent fontanelles, and macrocephaly, and thus demonstrate the critical role for TR$\alpha$ in bone formation and maturation in humans.

DOI: 10.1530/boneabs.3.W4.3

# The interactive extracellular matrix

## W5.1

**The non-collageous matrix network in bone and cartilage**

Francesco Ramirez
New York, USA.

Abstract unavailable.

DOI: 10.1530/boneabs.3.W5.1

## W5.2

**Collagenous matrix and cell behaviour**

Karl Kadler
Manchester, UK.

During the last 12 months we have worked on 2 projects that I will talk about at the ECTS 2014 conference. The first is our work on collagen fibril formation at the cell surface, using tendon as a model tissue. We have shown that the transport of newly-formed fibrils at the plasma membrane requires non-muscle myosin II. The second is that tendon is a peripheral clock tissue. We have shown that the tendons in mice lacking circadian rhythm have abnormal collagen fibril assembly and calcific deposits. Importantly, we show that the tendon clock diminishes with age in wild-type mice that also develop age-related calcific tendinopathy. This phenotype is identical to what is seen in human age-related and injury-related calcific tendinopathy. My talk will be divided equally between these two topics.

DOI: 10.1530/boneabs.3.W5.2

## W5.3

**Skeletal genetic diseases involving matrix proteins**

Michael Briggs
Newcastle, UK.

Skeletal diseases are a large and diverse group of rare monogenic phenotypes and there are more than 450 unique and well-characterised chondrodysplasia phenotypes that range in severity from relatively mild to severe and lethal forms. Studying these genetically tractable chondrodysplasia phenotypes provides insight into disease pathways that may be relevant to the more common and polygenic forms of OA.

Pseudoachondroplasia (PSACH) and multiple epiphyseal dysplasia (MED) present with varying degrees of joint pain and stiffness, short stature and early onset osteoarthritis that often requires joint replacement within the 2nd or 3rd decade of life. PSACH and the severe forms of MED result from mutations in cartilage oligomeric matrix protein (COMP), whilst the more moderate and mild forms of MED result from mutations in matrilin-3 and type IX collagen respectively. Genetic variations in the genes encoding all three proteins have also been associated with OA through both family and/or genetic association studies. To determine PSACH-MED disease mechanisms in vivo and provide knowledge on potential disease pathways in OA we generated a series of knock-in mouse models of PSACH-MED. Mutant mice develop mild to moderate short stature and display a growth plate dysplasia that is characterized by varying degrees of disrupted chondrocyte alignment, reduced chondrocyte proliferation, increased and/or spatially dysregulated apoptosis. Expression of mutant COMP and matrilin-3 induced either canonical or novel ER stress pathways. To relate the expression of mutant gene products to OA pathology we performed sequential protein extractions on the cartilage from COMP and matrilin-3 mice. We identified quantitative changes in the extraction of structural and non-structural ECM proteins, including proteins with roles in cellular processes such as protein folding and trafficking. In particular, genotype-specific differences in the extraction of collagens XIV and XII and tenascins C and X were identified. In summary the mutations of matrilin-3 and COMP lead to changes in the interactions of other cartilage proteins and our proteomic analyses revealed both common and discrete disease signatures that provide novel insight into mechanisms of skeletal disease and cartilage degradation.

DOI: 10.1530/boneabs.3.W5.3

# Osteoimmunology

## W6.1

**Osteochondral alterations in osteoarthritis**

Martine Cohen-Solal
Paris, France.

Abstract unavailable.

DOI: 10.1530/boneabs.3.W6.1

## W6.2

**Inflammation, bone destruction and osteoproliferation in spondylarthritis**

Georg Schett
Erlangen, Germany.

Abstract unavailable.

DOI: 10.1530/boneabs.3.W6.2

## W6.3

**TRAP mutations and immunology**

Tracey Briggs
Manchester, UK.

Background
Biallelic pathogenic variants in *ACP5*, encoding tartrate-resistant acid phosphatase (TRAP), result in the immuno-osseous disorder spondyloenchondrodysplasia (SPENCD), characterized by metaphyseal dysplasia and a variety of autoimmune phenotypes, particularly systemic lupus erythematosus (SLE). Importantly, patients with SPENCD demonstrate an upregulation of type 1 interferon (IFN) stimulated genes (ISGs) similar to that observed in SLE. Since very little is known about the function of TRAP in immune cells, our objectives were: i) to determine the consequences of TRAP deficiency in human immune cells, ii) to identify substrates of TRAP, and iii) to establish whether *ACP5* mutations occur in 'idiopathic' SLE.
Results
i) We knocked down TRAP expression in plasmacytoid dendritic cells (pDCs), the sentinel IFN producing immune cell, and observed increased expression of ISGs. ii) TRAP interacted or co-localised with the osteo-immune molecule Osteopontin (OPN) as determined by Yeast 2 Hybrid, confocal microscopy and by IP-western in human immune cells. Furthermore, mass spectometry demonstrated that TRAP dephosphorylated OPN at two serine residues in vitro. iii) Sequencing of *ACP5* in 856 SLE patients suggested an excess of heterozygous, possibly pathogenic missense, *ACP5* variants, when compared to controls. Transient transfection of several mutants and SLE patient serum assays revealed a reduction in TRAP enzyme activity.
Conclusions
We have shown that TRAP deficiency in pDCs leads to increased IFN production, partially explaining why *ACP5* mutations cause lupus in the context of SPENCD. Furthermore, TRAP and OPN co-localise and OPN is a substrate for TRAP in immune cells, which is of particular interest as OPN has a role in IFN production in pDCs. Detection of *ACP5* missense variants in lupus patients suggests that impaired function of TRAP may play a role increasing susceptibility to adult-onset idiopathic lupus. We hope that our ongoing studies of the interaction between TRAP, IFN and OPN will facilitate the use 'smart medicines' in SPENCD, and perhaps other IFN driven autoimmune disorders.

DOI: 10.1530/boneabs.3.W6.3

# Educational Symposium

## Educational Symposium 1

### ES1.1

Abstract unavailable.

DOI: 10.1530/boneabs.3.ES1.1

### ES1.2

Abstract unavailable.

DOI: 10.1530/boneabs.3.ES1.2

### ES1.3

Abstract unavailable.

DOI: 10.1530/boneabs.3.ES1.3

## Educational Symposium 2

### ES2.1

**Hypophosphatasia**

Nick Bishop
Sheffield, UK.

Hypophosphatasia (HPP) occurs when there is lack of the activity of the alkaline phosphatase protein (ALP). The principal clinical manifestations are seen in the skeleton, although some infants can develop convulsions due to the failure of pyridoxine, cleaved from pyridoxal-5-phosphate by ALP, to enter brain cells.
HPP has traditionally been classified by age at presentation, with severe forms being diagnosed *in utero* or at birth because of fractures, short limbs and respiratory distress, or in the early months of life because of failure to thrive and increasing respiratory difficulty. Biochemical abnormalities include hypercalcaemia with hypercaliuria leading to diuresis and nephrocalcinosis. Craniosynostosis is seen in both treated and untreated individuals and may lead to raised intracranial pressure, necessitating craniectomy. A benign form with suggestive features *in utero* but post-natal resolution has been described. Homozygous or compound heterozygous mutations are responsible for the severe forms; heterozygous mutations affecting a single gene copy result in presentation in older individuals with early loss of primary dentition with intact tooth roots, bone pain, rachitic-like lesions, low bone mass, and recurrent, poorly healing fractures. Some cases are misdiagnosed as chronic recurrent multifocal osteomyelitis in older children, or as osteoporosis or ankylosing spondylitis in adults.
The fundamental defect is the loss of alkaline phosphatase activity; the clinical manifestations clearly speak to the function of ALP at a tissue level, which is its removal of factors that can inhibit mineralisation. These include pyrophosphate (PPi), generated from ADP and ATP by the action of ENPP-1, pyridoxal-5-phosphate (PLP), otherwise known as vitamin B6, and phosphoethanolamine PEA). Of these, PEA is commonly measured in the urine and PLP in serum. PPi is only available in the context of research studies.
The human phenotype is accurately recapitulated in the murine model of HPP, the Alpl−/− mouse. The mice die young with multiple fractures and intractable seizures. Mice treated with a recombinant human ALP protein linked to an Fc-fragment and deca-aspartate tail (initially ENB-0040, now asfotase alfa) showed clear resolution of the skeletal and neurological phenotypes. Further work demonstrated the value of the bone-targeting deca-aspartate motif with significantly increased residence time of the recombinant protein in bone allowing s.c. dosing on an intermittent basis.
There is a single published peer-reviewed paper describing the effects of asfotase alfa on the skeleton in young human subjects, showing significant improvement in the X-ray appearances of bone tissue and concomitant clinical improvement in respiratory function, motor development and calcium homeostasis. Children entered into the original study have now received more than 3 years of treatment. Some children have developed craniosynostosis requiring neurosurgical intervention, but it is unclear whether this is a treatment related effect, since some untreated individuals have similar problems.
The broader application of this form of intervention requires further study. It is unclear at present whether PTH or other forms of osteoblast-stimulating treatments have lasting benefit in this context. The use of bisphosphonates is certainly contraindicated and likely to worsen clinical outcomes, so it is important to consider HPP when starting such therapy in older patients with a low ALP at presentation.
Learning points
Variability in presentation with age.
Insights into the function of alkaline phosphatase at a tissue level.
Options for therapeutic intervention.

DOI: 10.1530/boneabs.3.ES2.1

### ES2.2

**Gaucher's disease**

Tim Cox
London, UK.

DOI: 10.1530/boneabs.3.ES2.2

### ES2.3

**Updated biology of vitamin D: from the blood vessel to the bone**

Craig Langman
Chicago, USA.

Classical vitamin D biology relates the active hormone, 1,25-dihydroxvitamin $D_3$, to promotion of calcium homeostasis through actions on the intestine and bone, and regulation of parathyroid hormone production and release. Added to this classical action of vitamin D is the up-regulation of FGF23 production and release by osteocytes as a means of regulation of circulating levels of vitamin D by reducing kidney synthesis of 1,25-dihydroxvitamin $D_3$ and increasing catabolism peripherally of substrate 25-hydroxyvitamin $D_3$ and of 1,25-dihydroxvitamin $D_3$. A review of mechanism of action in producing rickets related to altered classical pathway vitamin D metabolism will be presented, including FGF23 disorders.
However, non-canonical pathways of vitamin D actions are recognized increasingly, and involve both substrate 25-hydroxyvitamin D, as well as active hormone. An important pathway in this regard includes control of the vasculature and the cardiovascular system. Vitamin D insufficiency is recognized as being associated with myocardial infarction, incident hypertension, cerebrovascular accidents, and peripheral arterial disease. Further, as vitamin D alters inflammation, and insufficiency is recognized as being associated with elevations of highly-sensitive C-reactive protein. Further, vitamin D replenishment may improve the deleterious effects on the microvasculature as studied in humans. Thus, vitamin D becomes an important regulator of the endothelium. The pathogenesis of vitamin D related endothelial dysfunction will be presented and reviewed for human disease.
A newer area of vitamin D biology relates to definitions of normal levels, especially as parsed by patient ancestry/ethnicity. Currently, adequacy of vitamin D are defined by blood levels of total 25-hydroxyvitamin $D_3$, while only 'free' (unbound) hormone is felt to be biologically active. Data will be reviewed for the meanings of the algorithms for free vitamin D and newer studies that challenge them.

DOI: 10.1530/boneabs.3.ES2.3

# Oral Communications

## Phosphate metabolism, fracture repair and osteoarthritis

### OC1.1

**Type 2 cannabinoid receptor protects against osteoarthritis in mice**

Antonia Sophocleous, Anna E Börjesson, Donald M Salter & Stuart H Ralston
University of Edinburgh, Edinburgh, UK.

Background
Cannabinoid receptors are expressed in synovial joints but their role in joint disease is unknown. Here we examined the role of the type 2 cannabinoid receptor (CB2) in the pathogenesis of age-related osteoarthritis and osteoarthritis caused by destabilisation of the medial meniscus (DMM) in WT and CB2-deficient mice ($Cnr2^{-/-}$ mice).
Methods
The severity of arthritis was graded histologically according to standard techniques and differences between genotypes were compared by Student's *t*-test
Results
Cartilage degeneration was (mean $\pm$S.E.M.) 34% $\pm$10 more severe at the medial compartment of operated joints of $Cnr2^{-/-}$ mice compared with WT littermates ($P=0.009$) and specifically 40% $\pm$13 more severe at the tibial plateau ($P=0.012$). There were no significant differences between $Cnr2^{-/-}$ mice and WT in the microarchitecture of subchondral bone. Similar analyses of aged mice that developed spontaneous osteoarthritis revealed that arthritis in the medial compartment of $Cnr2^{-/-}$ mice was 60% $\pm$14 more severe than WT littermates ($P=0.004$). Furthermore, treatment of young mice that underwent DMM and were administered with the CB2-selective agonist HU308 significantly inhibited progression of arthritis in the medial compartment of operated joints in WT mice with 32% less damage at the tibial plateau ($P=0.019$) and 18% less damage at the femoral condyle ($P=0.029$) compared with mice administered with vehicle. In contrast treatment with HU308 had no significant protective effect in $Cnr2^{-/-}$ mice, indicating a CB2-mediated effect.
Conclusion
We conclude that CB2 receptor deficiency predisposes to age-related and surgically-induced osteoarthritis in mice and that CB2 agonists have protective effects. These findings suggest that further studies on the role of CB2 pathway in humans with osteoarthritis are warranted.

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### OC1.2

**Study of *Hyp* or *Phex* null male fetuses reveals that eightfold increased FGF23 does not alter fetal–placental phosphorus homeostasis or prenatal bone formation and mineralization**

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Fibroblast growth factor-23 (FGF23) controls serum phosphorus by acting on the kidneys to excrete phosphorus and reduce calcitriol. These actions are well established in adults and children, but whether FGF23 regulates fetal phosphorus metabolism is unknown. We used X-linked *Hyp* or *Phex* null male fetuses to study the effect of excess FGF23 on fetal phosphorus metabolism. $Phex^{+/-}$ females and WT males were mated to generate WT, $Phex^{+/-}$ female, and *Phex* null male fetuses. Reverse matings of WT female to *Phex* null males were also done. All studies were approved by the local ethics committee.
$Phex^{+/-}$ mothers had low serum phosphorus compared to WT mothers, but maternal hypophosphatemia did not affect fetal serum phosphorus levels. $Phex^{+/-}$ female and Phex null male fetuses had 7.8-fold higher serum FGF23 levels than WT littermates. Increased FGF23 did not affect fetal serum calcium and phosphorus, urine (amniotic fluid) calcium and phosphorus, parathyroid hormone, skeletal morphology or limb lengths, tibial growth plate morphology, skeletal ash weight, and skeletal calcium and phosphorus. Serum calcitriol was reduced in Phex null male fetuses (WT 52.0 $\pm$5.8, Phex null 34.7 $\pm$2.6 pmol/l, $P<0.013$). We administered $^{32}P/^{51}Cr$-EDTA by intracardiac injection to pregnant mothers and found no difference among genotypes in placental transport of $^{32}P$ after 5 min. WT placentas and fetal kidneys abundantly expressed FGF23 target genes, including Cyp27b1, Cyp24a1, Klotho, NaPi2a, NaPi2b, NaPi2c, and FGF receptors 1–4. Of these, Cyp24a1 was significantly increased in Phex null placentas and fetal kidneys, while Klotho was significantly reduced in Phex null kidneys. However, these changes in gene expression did not disturb fetal phosphorus parameters.
In conclusion, FGF23 is not an important regulator of fetal phosphorous metabolism. The active delivery of phosphorus across the placenta does not require FGF23 and overrides any effect that excess FGF23 might otherwise have on phosphate handling by fetal kidneys.

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### OC1.3

**Klotho deficiency impairs mineralization by vitamin D hormone-driven upregulation of osteopontin and pyrophosphate in bone**

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Lack of Klotho, the co-receptor for the bone-derived hormone fibroblast growth factor-23 (FGF23), leads to a severe impairment in bone mineralization in mice. However, the mechanisms underlying the Klotho deficiency-associated defects in bone mineralization are still poorly understood. Here, we sought to elucidate the vitamin D independent role of Klotho in bone mineralization. To ablate increased vitamin D signaling in Klotho knockout mice ($Kl^{-/-}$), we crossed $Kl^{-/-}$ mice with mice expressing a non-functioning vitamin D receptor ($VDR^{\Delta/\Delta}$). As expected, $Kl^{-/-}$ mice were characterized by increased serum 1,25-dihydroxy-vitamin $D_3$ (1,25 $(OH)_2D_3$) and Fgf23, and impaired bone mineralization as evidenced by μCT, quantitative backscattered electron imaging, and histomorphometric analysis. The mineralization defect in $Kl^{-/-}$ mice was associated with increased bone mRNA expression of *ANK* (progressive ankylosis), *ENPP1* (ectonucleotide pyrophosphatase phosphodiesterase 1), *ENPP3*, and osteopontin as compared to WT and $VDR^{\Delta/\Delta}$ mice. In addition, we found increased pyrophosphate levels and osteopontin protein expression in bones of $Kl^{-/-}$ mice. However, ablation of vitamin D signaling in $Kl^{-/-}/VDR^{\Delta/\Delta}$ compound mutants normalized serum Fgf23 levels, bone mineralization, bone pyrophosphate levels, and bony expression of ANK, ENPP1, ENPP3, and osteopontin. Treatment of differentiated primary osteoblasts isolated from WT mice with $1,25(OH)_2D_3$, but not with recombinant FGF23, increased *ANK*, *ENPP1*, *ENPP3* and osteopontin mRNA expression. Moreover, primary differentiated osteoblasts isolated from $Kl^{-/-}$ did not show cell autonomous changes in mRNA expression of *ANK*, *ENPP1*, *ENPP3*, or osteopontin as compared to WT cells. Our data suggest that Klotho lacks a vitamin D independent role in bone mineralization, and that the mineralization defect observed in Klotho deficient mice is entirely due to $1,25(OH)_2D_3$-driven upregulation of the mineralization inhibitor osteopontin, and of the pyrophosphate-regulating factors ANK, ENPP1, ENPP3.

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### OC1.4

**Long-term Fgf23 deficiency induces renal and skeletal PTH resistance in vitamin D receptor-ablated mice**

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Fibroblast growth factor-23 (FGF23) is a hormone originating from osteocytes with suppressive actions on renal phosphate reabsorption and vitamin D synthesis. We recently identified FGF23 as a calcium-conserving hormone, regulating the membrane transport of the epithelial calcium channel TRPV5 in distal renal tubules. Here, we analyzed the long term sequelae of Fgf23 deficiency and renal calcium wasting on bone and mineral metabolism in 9-month-old mice lacking both *Fgf23* and a functioning vitamin D receptor (VDR). To prevent secondary hyperparathyroidism (sHPT) in VDR deficient mice, the mice were kept on a so-called rescue diet enriched with calcium, phosphate, and lactose. VDR mutants were normocalcemic and normophosphatemic, and had normal tibial bone mineral density (BMD), but slightly increased serum intact PTH. Relative to VDR mutants, compound mutants had unchanged serum calcium, but were characterized by hyperphosphatemia and very high serum PTH. sHPT in compound mutants was associated with only moderate cortical bone loss at the tibial shaft. Despite ~13-fold higher serum PTH levels in compound mutants, urinary excretion of phosphate, calcium, and deoxypyridinoline remained unchanged relative to VDR mutants. The increase in plasma cAMP 10 min after injection of hPTH(1–34) was similar in WT, VDR and *Fgf23*/VDR mutant mice. However, a 5-day infusion of hPTH(1–34) *via* osmotic minipumps resulted in reduced phosphorylation of extracellular signal-regulated kinase 1 and 2 (ERK1/2) in bone and kidney protein homogenates of *Fgf23*/VDR compound mutants, relative to VDR and WT controls. Similarly, the PTH-mediated ERK1/2 phosphorylation was blunted in primary osteoblasts isolated from *Fgf23*/VDR mutant mice, but was completely restored by concomitant treatment with recombinant FGF23. Collectively, our data indicate that the phosphaturic, calcium-conserving, and bone resorption-stimulating actions of PTH are blunted by Fgf23 deficiency. Hence, FGF23 may be an important modulator of PTH signaling in bone and kidney.

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## OC1.5

**The role of alarmins in fracture repair**

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Fractures are very common and affect 2% of the population per annum. Fragility fractures represent the greatest unmet need and are associated with high rates of morbidity and mortality. Currently, there is no approved therapy for enhancing healing of fragility fractures. We previously reported that upregulation of the early inflammatory response following skeletal injury can promote fracture repair (Glass et al PNAS 2011). Inflammation represents the earliest response following trauma and initiates a cascade of downstream events crucial for wound healing. However, the mechanism by which this occurs remains poorly defined. The earliest event following injury is the release of endogenous danger signals, or alarmins, by injured or necrotic cells. These initiate the innate inflammatory response by stimulating proinflammatory cytokine production and recruitment of leukocytes. HMGB1 and S100A8 are the best characterized alarmins. We set out to establish the role of HMGB1 and S100A8/A9 in fracture repair. By generating fracture supernatants using human patient samples, we found that HMGB1 and S100A8/A9 are released locally during skeletal injury and that their levels correlated with their osteogenic activity on primary human mesenchymal stromal cells. In our murine fracture model, we found that HMGB1 and S100A8/9 are released locally and systemically immediately following injury. Furthermore, murine fracture supernatants stimulated alternatively activated macrophages and dendritic cells to express KC and IL-6, key neutrophil chemoattractants. Finally, local addition of rHMGB1 at the fracture site enhanced fracture healing by day 28 in our murine fracture model. A detailed understanding of how the upstream events initiate fracture repair is a necessary step in the development of therapeutics to enhance this process. Interventions targeting these events would allow effective clinical translation as the therapeutic would be administered at the time of fracture reduction and surgical fixation.

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## OC1.6

**The role of neuropeptide Y $Y_1$ receptor signalling in fracture healing**

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Recent studies have demonstrated that the global or osteoblast-specific deletion of neuropeptide Y $Y_1$ receptor ($Y_1R$), as well as the pharmacological blockade of $Y_1R$, leads to pronounced anabolic effects in bone metabolism. This suggests that anti-$Y_1R$ drug therapy might have clinical applications for the prevention/recovery of bone loss occurring in osteoporosis. Given the high fracture incidence in this target population, it remained important to evaluate $Y_1R$ role's in bone regeneration/healing.

Therefore, we investigated the effects of germline ($Y_1R^{-/-}$) or osteoblast-specific ($Y1^{lox/lox;Cre/+}$) deletion of $Y_1R$ in bone fracture healing, using a murine model of tibial fracture. Closed fractures stabilized by intramedullary fixation were generated in 11-week-old mice, and the process of fracture repair was monitored by X-ray, micro-CT and histomorphometric analyses.

At 3-weeks post-fracture, $Y_1R^{-/-}$ mice already exhibited a smaller callus when compared to their WT littermates. In fact, $Y_1R^{-/-}$ fracture calluses were still not bridged, in comparison to the 89% of bridged fractures in WT ($P=0.002$). Structural analysis detected a 27% decrease in callus tissue volume ($P<0.001$) and a 9% reduction in bone volume density (BV/TV; $P<0.001$), which together resulted in a decrease of polar moment of inertia (callus strength; $P<0.05$). Moreover, histologically $Y_1R^{-/-}$ fracture callus presented a reduced vascular area ($P<0.05$) and a trend towards increased cartilage content ($\approx 5\%$; $P=0.07$). Importantly, by 6-weeks post-fracture all $Y_1R^{-/-}$ fracture calluses were radiologically bridged. Interestingly at 6-weeks $Y_1R^{-/-}$ calluses exhibited an increase of 31% in BV/TV ($P<0.05$). Importantly, no structural or histological changes were detected on fracture calluses from $Y1^{lox/lox;Cre/+}$ and WT at 3-weeks post-fracture.

In conclusion, our findings suggest that $Y_1R$ global deletion delays the early stages of bone fracture bridging, without inhibiting the completion of healing to union. This delay was independent from osteoblast-specific $Y_1R$. The knowledge acquired is important for the design of new $Y_1R$-based therapeutic approaches.

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# Osteoporosis epidemiology

## OC2.1

**Low serum thyrotropin level and duration of suppression as a predictor of major osteoporotic fractures – the openthyro register cohort**

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Objective
To assess the relationship between thyrotoxicosis and osteoporotic fractures in men and women.
Design
Register-based cohort study in patients with a TSH measurement in the region of Funen 1996–2010. All determinations were done in the same lab serving all hospitals and GP practices. Persons with raised TSH or a history of thyroid/pituitary disease were not included.
Results
The study population consisted of 222 138 (96%) persons with normal and 9217 (4%) with low TSH. During a median follow-up of 7.5 years, 13.5% of the low TSH group and 6.9% of the normal TSH group sustained major osteoporotic fractures (MOF), $P<0.01$. A single, low TSH at baseline was associated with increased risk of hip fractures (Table 1) but less strongly with MOF (HR 1.06, 95% CI 0.99–1.12, $P=0.058$). There was a significant association also with duration of thyrotoxicosis. In euthyroid patients, the risk of hip fractures (HR 1.45, 95% CI 1.22–1.71, $P<0.001$) and MOFs (HR 1.32, 95% CI 1.19–1.46, $P<0.001$) increased with each SD unit of TSH decrease.

| | Single, low TSH measurement | Per 6 mo of low TSH |
|---|---|---|
| All | 1.16 (1.07–1.26); $P<0.01$ | 1.07(1.04–1.10); $P<0.01$ |
| Women | 1.17 (1.06–1.28); $P<0.01$ | 1.07 (1.04–1.10); $P<0.01$ |
| Men | 1.17 (0.95–1.42); $P=0.1$ | 1.07 (0.99–1.14); $P=0.05$ |

Conclusion
In a population-based cohort, a single, first measurement of decreased TSH in a patient without known thyroid disease was associated with an increased long term risk of hip fracture, which remained significant in women but not in men after adjusting for confounders. Moreover, the risk of both hip fracture and MOF increased exponentially by the length of time during which TSH had remained low.

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## OC2.2

**Effect of daily vitamin B12 and folic acid supplementation on fracture incidence in elderly with an elevated plasma homocysteine level: B-PROOF, a randomized controlled trial**

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Background
Elevated plasma homocysteine levels are a risk factor for osteoporotic fractures. Supplementation with vitamin B12/folic acid lowers homocysteine levels. This study aimed to determine whether vitamin B12/folic acid supplementation reduces osteoporotic fracture incidence in hyperhomocysteinemic elderly.

Methods
B-PROOF is a double-blind, randomized, placebo-controlled trial including 2 919 participants aged ≧65 years with elevated homocysteine levels (12–50 μmol/l). Participants were assigned to daily 500 μg vitamin B12 and 400 μg folic acid or placebo supplementation for 2 years. Both tablets also contained 600 IU vitamin D3. The study was approved by the medical ethical committees of the 3 participating centers. Primary endpoint was time-to-first osteoporotic fracture. Stratified analyses were conducted if pre-specified covariates interacted significantly with treatment. Data were analyzed according to intention-to-treat and per-protocol principles.
Results
Osteoporotic fractures occurred in 61 persons (4.2%) in the intervention group compared with 75 (5.1%) in placebo. Osteoporotic fracture risk was not significantly different between groups in the intention-to-treat analyses (Hazard Ratio (HR) = 0.84, 95%CI 0.58–1.22) or per-protocol analyses (HR = 0.82, 95%CI 0.55–1.22). For persons > 80 years, in per-protocol analyses, osteoporotic fracture risk was 72% lower in the intervention group compared with placebo (HR = 0.28, 95%CI 0.10–0.74). Mortality did not differ between groups. Sixty-three *vs* 42 participants in the intervention and placebo group, respectively, reported incident cancer (HR = 1.55, 95%CI 1.04–2.30).
Conclusion
Combined vitamin B12/folic acid supplementation had no effect on osteoporotic fracture incidence in this elderly population. Stratified analyses suggested a beneficial effect on osteoporotic fracture prevention in compliant persons > 80 years. However, treatment was also associated with increased cancer risk, although this possible adverse effect should be interpreted with caution. In conclusion, vitamin B12/folic acid supplementation cannot be recommended at present for fracture prevention in elderly people.

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## OC2.3

### Hip fractures in Sweden and Denmark 1987–2010 – period and cohort effects

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Objective
The reasons for the recent decrease in hip fracture rates remain unclear. While current antiosteoporosis efforts are important also factors earlier in life seem essential and we examined age-period-cohort (APC) effects in hip fracture incidence in the Sweden (SE) and Denmark (DK).
Material and Methods
We studied the entire populations aged ≧50 years from 1987 to 2010 in SE and DK and ascertained acute hip fractures in nationwide discharge registers using diagnosis and surgical procedure codes for proximal femoral fracture. APC effects were evaluated country specific by log-likelihood estimates in Poisson regression models (with adjustment for sex and a scale parameter included to account for overdispersion). Results are presented as Incidence Rate Ratios (IRR) compared to the most recent 3-year period (2008–2010) or 6 year birth cohort (1953–60).
Results
During the examined years there were 399 596 hip fractures in SE and 207 304 in DK. The combined period and cohort effects were generally stronger in SE than DK and in women than men. IRR ranged from 1.05–1.30 in SE women, 1.04–1.18 in SE men, 1.21 –1.11 in DK women and 0.95–1.11 in DK men per period. The corresponding IRR per birth cohort ranged from 1.15–3.13 in SE women, 1.07–1.78 in SE men, 1.07–1.67 in DK women and 0.85–1.14 in DK men. Relative period effects increased with successive period for men and women in SE and described a convex curve for both men and women in Denmark with higher than expected risk in the periods in the middle of the examination years. Relative cohort effects were increasing with successive birth cohort for both genders in both countries but with markedly lower risks for DK women born 1925–44 and DK men born 1929–52 and a lower risk for SE women born 1933–44.
Conclusion
Cohort and period effects were different in SE and DK. This may in part be referred to differences in general health as evident in differences in life expectancy and to differences in exposure to war and famine as well as differences in use of osteoporosis drugs.

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## OC2.4

### Are the high hip fracture rates among Norwegian women explained by impaired bone material properties?

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Hip+ fracture rate in Norway is the highest registered in World, and more than double that of Spanish women. Previous studies were unable to demonstrate significant differences between the two populations with respect to bone mass or calcium metabolism. In order to test, whether the difference in fracture propensity between both populations could be explained by differences in bone material quality we assessed bone material strength using microindentation in 41 Norwegian and 46 Spanish women with normal BMD-values (*T*-score > −1 and < +2,0), without clinical or morphometric vertebral fractures, no clinical or laboratory signs of secondary osteoporosis and without use of drugs with known influence on bone metabolism. Bone material properties were assessed by microindentation of the thick cortex of the mid tibia following local anesthesia of the area using the Osteoprobe® device (Active Life Scientific, Sta Barbara, CA). Indentation distance was standardized against a calibration phantom of methylmethacrylate and results, as percentage of this reference value, expressed as BMS (Bone Material Strength) units.
We found that the bone material properties reflected in the BMS value of Norwegian women was significantly inferior when compared to Spanish women (77.0 ± 7.1% *vs* 80.7 ± 7.8%, $P = 0.02$). Total hip BMD was significantly higher in Norwegian women (1,218 g/cm$^2$ *vs* 0.938 g/cm$^2$ $P = < 0.001$, but regression analysis revealed that indentation values did not vary with BMD ($r = 0.03$, $P = 0.12$) or age ($r = 0.04$, $P = 0.42$).
In conclusion Norwegian women show impaired bone material properties, when compared to Spanish women. This is the first demonstration of ethnical differences in bone material properties and could partly explain the much higher propensity for fracture in Norwegian women than in Spanish women.

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## OC2.5

### IGFBP1 as a predictor of hip fractures

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Insulin-like growth factor 1 (IGF-1) is known to be a predictor of future osteoporotic fractures. Insulin-like growth factor binding protein 1 (IGFBP1) regulates the bioavailability of IGF-1 and thus in reason IGFBP1 also could be a fracture predictor. To our knowledge there are no previous studies published on the relation between the serum concentration of IGFBP1 and fractures.
This is a population-based prospective cohort study on 351 Swedish women aged 69–79 years at inclusion. These women were tested for serum IGFBP-1 at inclusion between 1999 and 2001. Follow up was performed 10 years later through Swedish medical records. The main outcome was a hip fracture.
The aims of this study were to evaluate whether IGFBP1 could predict hip fractures and further to compare predictive ability of IGFBP1 with the standard method FRAX. The data was analyzed with Cox regression. No participant was lost to follow up. The relation between IGFBP1 and the risk of a hip fracture was found to be positively linear. The age-adjusted risk of a hip fracture increased significantly by 2% for every unit increase in IGFBP1 (Hazard ratio (HR) 1.020, 95% CI 1.004–1.036). Surprisingly, this HR was not significantly altered by further adjusting for either BMI, the serum concentration of IGF-1 or for BMD of the femoral neck. The age-adjusted IGFBP1 had a Harrell's c of 0.63 compared to 0.59 for FRAX which indicates that IGFBP1 has a predictive ability similar to that of FRAX. Harrell's c is the equivalent to the area under curve (AUC) used in logistic regression, but designed for Cox regression models. For a model combining IBFBP1 with FRAX, Harrell's c was 0.64.
In conclusion, IGFBP1 could predict hip fractures as good as FRAX and unexpectedly, the relation was not explained by differences in BMI, IGF-1 or BMD of the femoral neck.

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## OC2.6

### Absolute fracture and mortality risk in patients with a recent non-vertebral fracture: the contribution of secondary osteoporosis or other metabolic bone disease (SECOB)

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Objective
To investigate whether patients with secondary osteoporosis or other metabolic bone disease (SECOB) have a higher re-fracture or mortality risk.
Method
Patients with a recent non-vertebral fracture who visited the Fracture Liaison Service (FLS) of a hospital were prospectively followed for 2 years. Pearson Chi-square, Fisher's Exact test, independent samples T-test, and Cox regression models were used.
Results
In total, 713 patients were invited to attend the FLS, and 510 attended (78.4% women). Of the 510 patients, 179 (35.1%) had osteoporosis, 251 (49.2%) were osteopenic, and 80 (15.7%) had normal bone mineral density (BMD). In total, 215 patients (42.2%) had known or newly diagnosed SECOB (77.7% women). Patients with SECOB were significantly older (68.9 *vs* 64.4 years, $P<0.001$) had lower BMD (*T*-score: −2.3 *vs* −1.9, $P<0.001$), were prescribed more bisphosphonates or PTH (53.6 *vs* 46.4%, $P<0.001$) and sustained more initial hip fractures (57.4% *vs* 42.6%, $P=0.042$) than patients without SECOB. Gender (women: 41.8 *vs* 43.6%, $P=0.723$) was not significantly different.
In total, 37 patients (7.3%) re-fractured and 15 (3.0%) died within 2 years of follow-up. The absolute re-fracture risk was not significantly different between patients with or without SECOB (8.8 *vs* 6.1%, $P=0.240$; relative risk (RR): 1.2). However, absolute mortality risk was significantly different (6.1 *vs* 0.70%, $P=0.001$; RR: 8.9). Although the numbers of events are small (<10%), Cox models showed similar results: no significant differences for re-fracture risk, but age-adjusted mortality risk was significantly higher for patients with SECOB (hazard ratio: 7.0, 1.6–31.8).
Conclusion
Two out of five patients, who attend the FLS because of a recent fracture, had SECOB. Absolute re-fracture risk is similar between patients with or without SECOB, but mortality risk is substantially higher in patients with SECOB.

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# Osteoclasts, gastric hormones and HIF

## OC3.1

### HIF prolyl hydroxylase 2 (PHD2) controls bone homeostasis through HIF2α – a novel player in osteohematology

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Prolyl hydroxylase 2 (PHD2) regulates hypoxia-inducible factor α (HIFα) transcription factors and thus, erythropoietin (EPO) production. Under normoxic conditions, HIFα is constantly inactivated through hydroxylation by PHD2. Due to the embryonic lethality of PHD2 knock-out mice, its precise role in erythropoiesis and tissue homeostasis has long remained unknown. Recently, we generated a conditional knock-out (cKO) mouse lacking PHD2 in EPO-producing cells. These mice have high levels of EPO and an increased hematocrit, which is dependent on HIF2α. In this study, we determined the role of PHD2 in bone.
Total, trabecular, and cortical bone density at the femur was significantly decreased by 12, 20, and 5% in cKO as compared to WT mice. Results were confirmed using histomorphometry, showing a 38% decrease in bone volume in the tibia, accompanied by microarchitectural changes including fewer trabeculae (−33%) and an increased trabecular spacing (+59%) in cKO mice. Bone resorption was not affected, as determined using serum levels of C-terminal telopeptide of type I collagen and tartrate-resistant acid phosphatase, and the histological evaluation of osteoclasts. In contrast, osteoblasts function was severely impaired. Serum levels of procollagen type I N-terminal propeptide and osteocalcin were decreased by 30–40% in cKO mice. In line with that, the mineralized surface, the mineral apposition rate, the bone formation rate, and the osteoblast surface were reduced by 35–50%. To further pinpoint which downstream signals are involved, we used PHD2/HIF1α (P2/H1) and PHD2/HIF2α (P2H2) double-knock-out (DKO) mice. While P2/H1 DKO mice, which also have high EPO levels, showed a similar bone phenotype as cKO, including decreased bone density and bone formation, bone density was restored in P2/H2 DKO mice, which have normal EPO levels.
Thus, these data identify PHD2 as a novel regulator of osteohematology, by controlling erythropoiesis and bone homeostasis.

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## OC3.2

### Activation of the $P2Y_2$ receptor enhances osteoclast function by stimulating the release of ATP, a pro-resorptive extracellular nucleotide

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Extracellular nucleotides, signalling through P2 receptors, play a significant role in bone biology. ATP and ADP act *via* the $P2Y_1$ or $P2Y_{12}$ receptors to promote osteoclast formation and activity. Bone cells express the $P2Y_2$ receptor and, in osteoblasts, it plays a role in regulating bone mineralisation. This investigation examined the role of the $P2Y_2$ receptor in osteoclasts. Primary osteoclasts were isolated from the bone marrow of 8-week WT or $P2Y_2$ receptor knockout mice ($P2Y_2R^{-/-}$) and cultured on dentine discs for 9 days in the presence of 150 ng/ml M-CSF and 2 ng/ml RANKL. UTP (≧0.1 μM), the principal $P2Y_2$ receptor agonist, increased bone resorption by 40% ($P\leq0.05$). $P2Y_2R^{-/-}$ osteoclasts displayed a 65% reduction in resorptive activity ($P\leq0.001$); osteoclast number was unchanged. Osteoclasts constitutively release ATP into the extracellular environment where it acts locally to stimulate resorption. We found that ATP release was reduced 60% in $P2Y_2R^{-/-}$ osteoclasts ($P\leq0.001$). To investigate whether decreased levels of extracellular ATP were causing the reduction in osteoclast function, $P2Y_2R^{-/-}$ cells were cultured with exogenous ATP (1–10 μM). Addition of ATP to the culture medium fully rescued the bone resorption defect in $P2Y_2R^{-/-}$ osteoclasts. We found that UTP, (≧10 μM), stimulated ATP release from osteoclasts by up to fourfold ($P\leq0.001$). The enhanced ATP release was evident from 10 min after UTP treatment and was sustained for up to 90 min. The stimulatory action of UTP on ATP release was mediated *via* the $P2Y_2$ receptor, since UTP failed to induce ATP release in $P2Y_2R^{-/-}$ osteoclasts. MicroCT analysis of $P2Y_2R^{-/-}$ mice demonstrated an increased trabecular bone volume (20%, $P<0.001$) and trabecular number (25%, $P<0.01$) in the long bones. Taken together, these data suggest the $P2Y_2$ receptor regulates osteoclast function indirectly by promoting ATP release. Once released, ATP and its breakdown product, ADP, can act *via* other P2Y receptors to increase bone resorption.

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## OC3.3

### Thyroid hormones stimulate osteoclastogenesis via TRα-dependent actions in osteoblasts

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Thyrotoxicosis results in osteoporosis, and thyroid hormone (T3) stimulates osteoclastic bone resorption by unknown mechanisms. We previously demonstrated that knockout mice lacking thyroid hormone receptor α ($TR\alpha^{0/0}$) are euthyroid but have high bone mass, whereas mice lacking TRβ ($TR\beta^{-/-}$) are thyrotoxic and osteoporotic. Tartrate resistant acid phosphatase (TRAcP) staining revealed osteoclast numbers were reduced by 13% ($P<0.05$) in $TR\alpha^{0/0}$ mice, but increased by 20% ($P<0.05$) in $TR\beta^{-/-}$ mice, suggesting T3 acts *via* TRα to stimulate osteoclastogenesis and bone resorption.
To investigate this hypothesis, we treated WT, $TR\alpha^{0/0}$ and $TR\beta^{-/-}$ bone marrow (BM) with M-CSF (25 ng/ml) and RANKL (10 ng/ml) in the absence or presence of T3 (100 nM). T3 treatment had no effect on the number, size or survival of osteoclasts from any genotype, indicating T3 does not exert direct actions in

osteoclasts. Nevertheless, threefold more osteoclasts formed in TRα$^{0/0}$ BM cultures compared to WT or TRβ$^{-/-}$ ($P<0.001$), suggesting that osteoclast precursor cell differentiation is impaired *in vivo* in TRα$^{0/0}$ mice. We then examined interactions between osteoblasts and osteoclasts by co-culturing WT osteoblasts with WT, TRα$^{0/0}$ or TRβ$^{-/-}$ BM in the absence and presence of T3. In T3-treated cultures there was an increase in osteoclast number (40–90%, $P<0.05$), TRAcP activity (76–91%, $P<0.05$) and resorption (73–80%, $P<0.05$) irrespective of BM genotype, thus indicating that the impaired osteoclastogenesis in TRα$^{0/0}$ mice is not due to an osteoclast defect. We next co-cultured WT BM with WT, TRα$^{0/0}$ or TRβ$^{-/-}$ osteoblasts in the absence or presence of T3. T3 treatment increased osteoclast number (45–66%, $P<0.05$) and TRAcP activity (21–80%, $P<0.05$) in co-cultures containing either WT or TRβ$^{-/-}$ osteoblasts. By contrast, almost no osteoclasts formed in co-cultures containing TRα$^{0/0}$ osteoblasts and WT BM in either the absence or presence of T3.

Overall, these data demonstrate that T3 stimulates osteoclastic bone resorption indirectly via TRα-dependent actions in osteoblasts.

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## OC3.4

### RANKL enhances TNF-induced osteoclast formation by degrading TRAF3 in osteoclast precursors independent of TRAF6

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TNF receptor-associated factors (TRAFs) −6 and −3 regulate RANKL and TNF signaling in osteoclast precursors (OCPs), but they can have opposing effects, and it is not known if their functions are inter-dependent. For example, TRAF6 is required for RANKL/RANK-induced osteoclastogenesis, while TRAF3 limits both RANKL- and TNF-induced osteoclastogenesis through proteasomal degradation of NF-κB-inducing kinase; and inhibition of autophagic degradation of TRAF3 by chloroquine prevents ovariectomy-induced osteoporosis. TRAF6−/− and TRAF3−/− mice die before or within ∼2 weeks of birth, limiting *in vivo* studies. To further examine the relationship between these cytokines and TRAFs, we treated TRAF6−/− OCPs with RANKL, TNF, or RANKL+TNF on plastic. As expected, RANKL did not induce osteoclastogenesis, but TNF induced small numbers of osteoclasts (51±6/well), while RANKL significantly increased TNF-induced osteoclastogenesis (276±18/well). Surprisingly, RANKL induced functional osteoclasts from TRAF6−/− OCPs when they were cultured on bone slices (186±25/slice), which was inhibited by TNFR:Fc, but not by IL-1R antagonist or TGFβ1 neutralizing antibody. WT or TRAF6−/− OCPs cultured on bone slices secreted similar concentrations of TNF (169±43 and 151±17 pg/ml), which were significantly higher than those from cells cultured on plastic (26±4 and 19±2 pg/ml). TNF increased TRAF3 protein levels in TRAF6−/− OCPs, and addition of RANKL degraded TRAF3, while over-expression of TRAF3 inhibited osteoclastogenesis induced by RANKL+TNF. Finally, over-expression of NFATc1 significantly increased osteoclastogenesis induced by TNF in WT OCPs, but not by RANKL or by TNF in TRAF6−/− OCPs; however, it induced similar numbers of osteoclasts from TRAF6−/− and WT OCPs treated with RANKL+TNF (174±14 and 196±21/well). We conclude that interaction with bone matrix increases OCP expression of TNF, and that RANKL enhances TNF-induced osteoclastogenesis by promoting degradation of TRAF3 through a TRAF6-independent mechanism. Thus, strategies to increase TRAF3 levels in OCPs should inhibit RANKL- and TNF-induced osteoclastogenesis.

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## OC3.5

### Double incretin receptor knock-out (DIRKO) mice present with alterations of trabecular and cortical microarchitectures and bone strength.

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Objectives
A role for the gut hormones glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) in controlling bone mass and strength has previously been reported. However, lack of one gut hormone is compensated by an elevated sensitivity to the other in single receptor knockout mice. As such the exact role of GIP and GLP-1 in bone is unclear. The aims of the present study were to assess bone mass and strength in mice with functional deletion of both the GIP and GLP-1 receptors.
Materials and methods
Eight (26 week-old) mice with deletion of the GIP and GLP-1 receptors (DIRKO mice) were age- and sex-matched with 10 WT mice. Trabecular and cortical bone microarchitecture was studied by high-resolution microCT at the femur. Bone strength and intrinsic material properties were studied respectively by three-point bending and nanoindentation. Bone mineral and collagen properties were assessed by quantitative backscattered electron imaging and Fourier-transformed infrared microscopy. Non-parametric Mann–Whitney *U*-test was used to compare differences between groups.
Results
As compared with control mice, DIRKO animals exhibited significant increases in BV/TV (210%, $P=0.003$) and trabecular number (207%, $P=0.012$). Cortical microarchitecture was affected in DIRKO mice as demonstrated by a significant reduction in bone outer diameter (−9%, $P=0.028$) with unchanged marrow diameter. Cortical thickness and cortical area were significantly reduced by 17% and 14% respectively. Investigation of bone strength by 3-point bending revealed significant reductions in yield stress (−48%, $P=0.032$), ultimate stress (−44%, $P=0.034$) and post-yield work to fracture (−31%, $P=0.034$) in DIRKO mice. Alterations of intrinsic material properties were evident in DIRKO animals with significant decreases in maximum force (−21%, $P=0.027$), hardness (−29%, $P=0.014$) and dissipated energy (−25%, $P=0.014$). Interestingly, bone mineral density distribution was not affected by in DIRKO mice, but the ratio of mature/immature collagen cross-links was significantly reduced by 9% ($P=0.011$).
Conclusions
Overall, these data demonstrate the role of gut hormones in bone physiology. This is important regarding the use of GLP-1 mimetics in type 2 diabetes.

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## OC3.6

### Skeletal effects of the gastrin receptor antagonist netazepide in H+/K+ ATPase beta-subunit deficient mice

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Epidemiological studies suggest that patients using proton pump inhibitors (PPIs) have increased fracture risk. We have previously shown that H+/K+ATPase beta-subunit knockout (KO) mice have reduced BMD, BMC and mechanical bone strength compared to WT. Like users of PPIs, these mice have elevated serum gastrin levels due to high gastric pH. We wanted to study whether elevated gastrin influences bone quality in these mice.
Female KO and WT mice aged 6 weeks were randomly assigned to subcutaneous injection of either a gastrin receptor antagonist (netazepide 40 mg/kg per 2 weeks) or vehicle (polyethylene glycol) for 1 year. Whole body BMD and BMC were measured by DXA at initiation and before termination. Blood drawn at termination was analyzed for gastrin, RANKL, OPG, osteocalcin, leptin and sclerostin. Right femurs were examined with μCT and 3-point bending tests. The study was approved by the local Animal Welfare Committee.
KO mice had significantly ($P<0.5$) lower BMD (0.080 *vs* 0.070 g/cm$^2$) and BMC (0.82 *vs* 0.61 g) at termination, while no significant differences between KO mice receiving netazepide and vehicle were observed. Cortical thickness, cortical area fraction, trabecular thickness and trabecular BMD were significantly higher in WT compared to KO mice, and significantly higher in KO mice receiving netazepide than in the vehicle group. Stiffness was significantly higher in WT

compared to KO, and tended to be higher in the KO group receiving netazepide ($P=0.06$). Breaking force tended to be higher in WT than KO mice ($P=0.08$), but no differences were seen between the netazepide and vehicle groups. Serological bone markers did not differ significantly between any of the groups. The gastrin receptor antagonist netazeptide partly prevents bone loss and deterioration of bone quality in $H^+/K^+$ATPase KO mice. Hypergastrinemia therefore, could play a part in the osteoporotic process in mice lacking the gastric proton pump.

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## Genetics of bone disease

### OC4.1

**PLS3 mutations in X-linked osteoporosis with fractures**

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Background
We identified a family with early onset X-linked osteoporosis and fractures
Methods
We performed whole exome sequencing of the X chromosome in three affected members. After discovering a putative pathogenic variant we performed Sanger sequencing of all exons of this gene in other members of this family and in 95 unrelated men suspected of OI type I without COL1A1/2 mutations. We also genotyped a SNP in this gene (minor allele frequency 0.02) in elderly subjects in three cohorts of a prospective population-based study for association analyses with BMD and incident fractures. We performed functional studies in zebrafish after pls3 knock-down and expression analysis of PLS3 in human differentiating mesenchymal stem cells (MSCs) and in differentiating human osteoclasts.
Results
We discovered five pathogenic variants in PLS3 in five families with osteoporosis and osteoporotic fractures. Furthermore, a rare SNP in PLS3 (rs140121121) was associated with a twofold increased fracture risk in heterozygous postmenopausal women in a population-based study and with decreased BMD, although increased fracture risk was not fully explained by BMD. PLS3 is expressed during osteogenic and decreased during adipogenic differentiation of MSCs and its expression increased during differentiation and activation of osteoclasts. Knock-down experiments in zebrafish showed malformations of the developing craniofacial bone structure, body axis and tail that could be rescued dose-dependently by human PLS3 mRNA, supporting the concept that PLS3 is a bone regulatory protein.
Conclusion
We identified loss-of-function variants in PLS3 as a monogenetic cause of X-linked osteoporosis and osteoporotic fractures and found a rare SNP in this gene associated with a twofold increased fracture risk and decreased BMD in heterozygous women in a population-based study, indicating genetic variation in PLS3 as a novel factor involved in common, multifactorial osteoporosis.

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### OC4.2

**Variants in the *LRP4* gene are associated with bone mineral density in males and females**

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Background
*LRP4* gene encodes a member of the LDL receptor gene family, expressed by osteoblasts, which negatively regulates Wnt/β-catenin signalling by potentiating the inhibitory effect of sclerostin on *LRP5* signalling. Previously, missense mutations of *LRP4* have been described in patients with the phenotype of sclerosteosis, a disease associated with high bone mass and bone overgrowth. Here we investigated the hypothesis the *LRP4* mutations might also be involved in regulating bone mass in general population.
Methods
We conducted mutation screening of *LRP4* in subjects with bone mineral density (BMD) values above the 98th centile in the Orkney Complex Disease Study (ORCADES) cohort, a population isolated from the UK, and conducted an association study of *LRP4* variants in relation to BMD in the whole study population of 1567 subjects (599 males and 968 females).
Results
We found a novel mutation in *LRP4*, 316+1G>A, and identified several known polymorphisms in *LRP4* that cause amino acid substitutions (rs118009068, rs72897663, rs6485702, rs2306033, rs2306029 and rs3816614) and a silent change, rs61746928. Mutation 316+1G>A had an allele frequency of 0.0053 and showed an association with whole body BMD ($P=0.045$) in females. We also reported, for the first time, an association with rs2306033 (p.Ala1203Val) and spine BMD in females (wt=0.98±10.15; heterozygous=0.94±90.16; homozygous=0.955±0.18; $P=0.042$). Surprisingly, rs61746928 was strongly associated with spine BMD ($P=0.004$), and whole body BMD ($P=0.05$) in males after correcting for age, BMI, smoking, alcohol and calcium intake. The effect size in the seven heterozygous carriers of the p.Arg1273Arg allele was large, amounting to approximately 0.163 g/cm$^2$ or 0.673 *T*-score units.
Conclusions
We have confirmed that *LRP4* is a candidate gene for regulating BMD and have identified a silent variant with large effect size that is associated with increased spine BMD in males, although further functional analysis should be performed to address its role in regulating BMD.

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## OC4.3

### Exome-chip meta-analysis identifies novel associations of coding variants in BSN and GLRA4 with lumbar spine BMD in 27 339 adults of European descent

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In previous work we identified 63 common variants (MAF > 5%) from 56 loci associated with BMD fully comprising non-coding regions of the genome. We hypothesized that genes may harbour both common and rare variants in the protein-coding regions may influence BMD variation. The availability of the 'exome-chip' with 235 933 protein-coding variants (non-synonymous, splice sites and stop-altering) provides a feasible way to identify low-frequency variants in exomes.

We conducted an exome-chip meta-analysis for BMD to scrutinize coding variants for association with BMD. Up to 27 339 participants from 16 studies were genotyped using the exome-chip. BMD at the lumbar spine (LS) and femoral neck (FN) was measured by DXA. We performed regression analyses using an additive genetic-effect model in each study adjusting for age, age$^2$, sex, weight and ancestral genetic background. Gender-specific analyses were done for markers on the X-chromosome. An inverse-variance fixed-effect meta-analysis of 79 982 polymorphic variants (minor allele count $\geq 3$) was conducted. Exome-chip significance threshold was set at $2.1 \times 10^{-7}$.

The most significant association was found for a non-synonymous SNP V667M mapping to the *LRP5* gene (MAF = 5.3%, $P = 2 \times 10^{-10}$ with LS BMD) among 61 other significant variants in known loci. Also two novel associations of non-synonymous variants with LS-BMD included: 3p21.31 harbouring A3863T in the *BSN* gene, MAF = 46%, $P_{meta\_all} = 3.8 \times 10^{-8}$ and Xq22.2 harbouring P335S in the *GLRA4* gene, MAF = 0.6%, $P_{meta\_males} = 1.9 \times 10^{-7}$. The bassoon presynaptic cytomatrix protein (*BSN*) has unknown relation to bone biology. Males carrying the P335S variant in the glycine receptor, alpha 4 (*GLRA4*) had in average 0.5 standard deviations higher BMD at the LS compared to non-carriers in our studies.

In summary, we identified two novel loci carrying both common and rare non-synonymous variants associated with BMD. Better understanding of the mechanisms by which P335S *GLRA4* increases BMD could lead into novel therapies for the treatment of osteoporosis in males.

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## OC4.4

### Variants in RIN3 predispose to Paget's disease of bone

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Background

Paget's disease of Bone (PDB) has a strong genetic component and a candidate locus for the disease has been identified on chromosome 14q32, tagged by rs10498635 located within RIN3 (Albagha et al, Nat Genet 2011). RIN3 encodes a protein that acts as a guanine nucleotide exchange factor for Rab5b and Rab31. Here we investigated the candidacy of RIN3 as a predisposing gene for PDB.

Methods

We studied expression of *RIN3* by quantitative PCR, western blotting and immunohistochemistry in bone and bone derived cells and conducted DNA sequencing of the exons, intron–exon boundaries and promoter in 196 PDB cases where *Q1STM1* mutations had been excluded. We then conducted an association study of the variants identified in a further study of 475 PDB cases and 475 controls.

Results

The mRNA for *RIN3* was strongly expressed in lung, bone and liver and at lower levels in kidney, brain and muscle. Levels of *RIN3* mRNA expression were higher in osteoclasts ($P = 0.02$) but lower in bone marrow macrophages ($P = 0.006$) than whole bone marrow. However, western blotting showed that RIN3 protein expression was highest in whole bone marrow, lower in bone marrow macrophages, and lower still in osteoclasts. The mutation screening identified 19 missense variants in *RIN3* including 3 novel mutations that were not present in controls or publicly available databases including 1000 Genomes. Additionally, two common SNPs in the *RIN3* promoter and 3'UTR were strongly associated with the disease ($P = 2.5 \times 10^{-7}$ and $5.8 \times 10^{-6}$ respectively).

Conclusions

We conclude that RIN3 is expressed in the bone microenvironment and that the levels of expression vary during osteoclast differentiation. The mutation screening experiments raise the possibility that susceptibility to PDB may be influenced by rare protein coding mutations as well as more common variants in the gene promoter and 3′ UTR.

According to densitometry criteria 22% of the patients have osteoporosis and 22% are in the range of osteopenia.

1 Pathological fracture was registered (vertebral).

Conclusions

ERT prevents progression of bone abnormalities in GD. Vitamin D insufficiency is frequent in GD and almost half of the patients have decreased bone mass.

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## OC4.5

### Bone microarchitecture, geometry and volumetric BMD assessed using HR-pQCT in adult patients with hypophosphatemic rickets

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Although X-linked hypophosphatemic rickets (XLH) is characterized by hypophosphatemia leading to a generalized mineralization defect with rickets (and/or osteomalacia), densitometric studies point towards a tendency towards an elevated bone mineral density (BMD). The aim of this cross-sectional *in vivo* study was to assess bone geometry, volumetric BMD (vBMD), microarchitecture and estimated bone strength using high-resolution peripheral quantitative computed tomography (HR-pQCT) in patients with XLH. After receiving the Regional Ethical Committee approval, 28 patients with genetically proven XLH aged $45.5 \pm 16.2$ years (21 females, 7 males) were matched with respect to age and gender with 31 healthy subjects aged $46.6 \pm 16.4$ years. Eleven patients were currently receiving therapy with calcitriol and phosphorus with a median duration of treatment of 29.1 years (12.0 to 43.0 years). Due to the disproportionate short-stature in XLH, HR-pQCT images at the distal radius and tibia were obtained at a region of the bone in a constant proportion to the entire length of the bone, in both, patients and healthy volunteers. In age and weight adjusted models, XLH patients had a significantly enlarged mean total bone cross-sectional areas (radius 33%, tibia 18%; both $P < 0.001$) with significantly higher trabecular bone areas (radius 39%, tibia 18%; both $P < 0.001$). There was a reduction in total vBMD (radius −13%, tibia −12%; both $P < 0.01$), cortical vBMD (radius −5%, $P < 0.01$), trabecular number (radius −14%, tibia −16%; both $P < 0.01$) and cortical thickness (radius −16%; $P < 0.01$), while the trabecular thickness (radius 13%, $P = 0.05$) and trabecular spacing (radius 21%, tibia 23%; $P < 0.01$) were increased. Estimated bone strength was similar between the groups. In conclusion, the negative impact of a reduction in volumetric density and trabecular number on bone strength, was compensated by an overall increase in bone size and thickness of the individual trabeculae, resulting in patients with XLH having similar estimates of bone strength as healthy subjects.

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## OC4.6

### Gout is associated with an excess risk of osteoporotic fracture: findings from a Danish registry

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Objective
Although metabolic syndrome is common in gout patients, recent reports that bone mineral density may actually be reduced (and falls common) in this group have led researchers to hypothesise that osteoporotic fracture may be more common in subjects with gout than in healthy controls. We tested this hypothesis in a national Danish registry.
Material and Methods
We identified subjects as new users of allopurinol, a proxy for gout, for the years 1996–2010. Each incident user was assigned up to 10 age- and gender matched controls. We used propensity score matching to identify a highly matched control population. Patients with a diagnosis of malignancy in the year prior to the first allopurinol prescription were excluded. The final propensity score model included hospital diagnoses since 1994; Charlson index components; and prior osteoporotic fractures; use of drugs (including osteoporosis medication, prednisolone and HRT) in the last year. Conditional Cox regression modelling was undertaken.
Results
We studied 86 129 patients and the same number of controls (58 129 men and 28 000 women). Thirteen thousand and ninety one cases and 12 188 controls sustained any osteoporotic fracture; the number of major osteoporotic fractures was 5574 in the cases and 4893 in the control group. We found a modest adjusted effect of allopurinol prescription on major osteoporotic fractures; an association with hip fractures just failed to attain statistical significance.
Among patients who were incident allopurinol users and who also had at least one hospital contact with a gout diagnosis (about 20% of allopurinol users, median number of allopurinol rx 12 *vs* 6 in non-hospital group), we found stronger associations.

| | Major osteoporotic fracture | Hip fracture |
|---|---|---|
| Allopurinol | 1.075 (1.031–1.121) $P<0.001$ | 1.060 (0.990–1.134) $P=0.093$ |
| Allopurinol + gout diagnosis | 1.211 (1.093–1.343) $P<0.001$ | 1.188 (1.007–1.403) $P=0.04$ |

Conclusion
These data suggest that gout requiring allopurinol prescription is a risk factor for osteoporotic fracture.

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# Important pathways in bone biology and cancer

## OC5.1

### *In vivo* efficacy of a pharmacological inhibitor of TNAP to prevent arterial calcification and its associated cardiac hypertrophy and mortality

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Medial vascular calcification (MVC) is a pathological condition common to a variety of diseases, including chronic kidney disease, diabetes, obesity, generalized arterial calcification of infancy, arterial calcification due to deficiency of CD73, and Keutel syndrome. These diseases share the common feature of tissue-nonspecific alkaline phosphatase (TNAP) upregulation in the vasculature. We developed a mouse model that overexpresses human TNAP in vascular smooth muscle cells, in an X-linked manner. As early as 7 days of age, mice showed strong TNAP expression in the aorta and, by 14 days, distinct aortic calcification was visible by X-ray. Hemizygous overexpressor male mice ($Tagln^{Cre/WT}$; $HprtALPL^{flox/Y}$) exhibit severe cardiac hypertrophy and have a median age of death of 44 days, whereas the hypertrophy is less pronounced and life expectancy is normal in heterozygous females ($Tagln^{Cre/WT}$; $HprtALPL^{flox/WT}$). Gene expression analysis showed upregulation of classical markers of MVC (*Bmp2*, *Mgp* and *Spp1*) and osteoblast (*Runx2* and *Col1a1*) and chondrocyte (*Acan* and *Sox9*) markers and a decrease in expression of the smooth muscle marker *Tagln* indicating that altering the local Pi/PPi balance is sufficient to initiate the transdifferentiation of smooth muscle cells, a hallmark of MVC and that TNAP overexpression is sufficient to cause MVC. Through medicinal chemistry efforts, we developed inhibitors of TNAP with drug-like characteristics. Overexpressor mice were injected i.p. once daily with 10 mg/kg of the TNAP inhibitor SBI-425 or vehicle from day 7 to day 30 and day 60 respectively. Heart weight to body weight ratios were significantly lower in both male and female treated mice, indicating improvement in cardiac hypertrophy. SBI-425 treatment also significantly increased the median life expectancy of affected male mice from 44 to 68 days. No secondary effects were observed in the skeleton of treated mice. These results indicate that SBI-425 can effectively reach and inhibit TNAP in the vasculature reducing the calcium loads, preventing the cardiovascular consequences of MVC and increasing the survival of overexpressor mice. Pharmacological inhibition of TNAP appears to be a viable treatment option for MVC.

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## OC5.2

### Regulation of bone metastasis by the IKKβ/FoxO3a axis

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IκB Kinase β (IKKβ), a key component of NFκB signaling, plays an important role in bone disease and cancer. Genetic and pharmacological inhibition of IKKβ/NFκB signaling increase bone mass and protect against ovariectomy- and inflammation-induced bone loss. Here, we describe a previously unknown role of the IKKβ/FoxO3a axis in bone metastasis associated with breast cancer. We observed that IKKβ expression is prevalent in invasive breast cancer carcinoma and significantly elevated in bone metastasis specimens from the same breast cancer patients. Overexpression of IKKβ (ninefold increase) in the human breast cancer cells MDA-MB-231 (MDA-231) significantly worsened cachexia (40% increase) and mortality (50% increase) and exacerbated bone metastasis (25% increase) after intracardiac injection, enhanced tumour volume (60% increase) after orthotopic injection and provoked osteolysis (30% loss of bone volume) after supracalvarial injection of conditioned medium from these cells. Conversely, pharmacological inhibition and selective knockdown of IKKβ in MDA-231 cells inhibited cell migration (69% reduction), significantly reduced the ability of MDA-231 cells to induce osteoclast formation (87% reduction), to enhance osteoblasts support for osteoclastogenesis (75% reduction) and to reduce osteoblast differentiation *in vitro* (29% increase), and to cause osteolysis *in vivo* (27% gain in bone volume). An integrative analytic approach that utilizes data from co-culture systems, western blot analysis and gene expression microarrays revealed that disruption of IKKβ interaction with the pro-apoptotic transcription factor FoxO3a, but not NFκB, increases FoxO3a nuclear localisation (10% increase) that alter the balance of tumour derived osteolytic (IL8, VEGF, ADM and CXCL16) and pro-osteoblastic (BMP4 and WNT7B) secreted factors, thereby favouring osteoblast differentiation over osteoclast formation. In conclusion, we demonstrate that the IKKβ/FoxO3a axis serves as a common target that regulate cancer cell – osteoblast – osteoclast paracrine crosstalk in bone metastatic environment, and inhibition of IKKβ-FoxO3a interaction protects against bone metastasis and encourages bone formation.

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## OC5.3

### Beta haemoglobin (hbb) is a novel marker of breast cancer progression

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Breast cancer (BrCa) patients with metastases restricted to bone (BO) show a longer overall survival compared to BrCa patients developing bone and visceral

metastases (BV). To unveil the molecular mechanisms that segregate these two BrCa patient groups, we performed microarray analyses on bone metastasis samples from BO and BV patients, finding in the latter a set of up-regulated genes involved in oxygen metabolism. We focused on Hemoglobin B (HBB) and evaluated its expression in human BrCa samples by immunohistochemistry. In ductal infiltrating carcinoma the percentage of HBB positive cells was significantly higher in the invasive lesions than in the *in situ* counterpart. A higher expression of HBB was also observed in ductal infiltrating carcinoma vs the lobular invasive histotype, while benign lesions, the *in situ* counterpart of lobular carcinoma and normal tissue were negative. We also observed a positive correlation between HBB expression and the Ki67 proliferation marker. We next compared the expression of HBB between poorly aggressive (MCF7, HCC1954) and highly aggressive (MDA-MB231) BrCa cells, finding a higher transcriptional and protein expression in the latter. MDA-MB231 cells overexpressing HBB (MDA-HBB) showed an increased ability to migrate and invade *in vitro* compared to control cells (MDA-empty). Orthotopic injection in nude mice revealed a greater ability of MDA-HBB cells to grow in the primary site compared to MDA-empty-injected mice, while histology showed less fibrosis in MDA-HBB tumour sections. Moreover, local recurrence and visceral metastases were observed in three and two over five MDA-HBB injected mice, respectively, while MDA-empty tumour relapse did not occur over the same timeframe. Our results demonstrate a positive correlation between HBB expression and BrCa cell aggressiveness, paving the way for a possible use of HBB as a novel marker for BrCa progression.

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## OC5.4

### WNT5A has anti-prostate cancer effects and protects against bone metastases

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Prostate cancer (PCa) is the most common cancer type in older men and often metastasizes to bone in advanced stages. Wnt proteins are implicated in carcinogenesis and especially WNT5A has been discussed to influence the clinical outcome of various cancer types, including PCa. In addition, WNT5A stimulates osteogenic differentiation and may thus not only be involved in PCa development, but also in the formation of subsequent skeletal metastasis. Here, we determined the role of WNT5A in PCa *in vitro* and *in vivo*.
A tissue microarray was created using a cohort of 397 mainly high-risk PCa patients and stained for WNT5A. Effects of WNT5A knock-down or over-expression on proliferation and apoptosis of PCa cell lines (PC3, C42B, MDA-PCa-2b) were examined *in vitro*. *In vivo,* WNT5A was overexpressed in luciferase-labeled PC3 cells (PC3-Luc) and injected subcutaneously, intratibially or intracardially into nude mice to determine tumor growth.
Expression of WNT5A was higher in PCa patients as compared to patients with benign prostatic hyperplasia ($P<0.05$). Patients with high WNT5A levels had a better overall survival than those with low WNT5A expression ($P<0.05$). *In vitro*, WNT5A overexpression reduced proliferation by 39% in PC3 cells and simultaneously induced apoptosis twofold (as determined by caspase 3/7 activation, annexin V/PI-positive cells, PARP cleavage, and DNA fragmentation). Knock-down of WNT5A yielded opposite results. Similar effects were seen in C42B and MDA-PCa-2b. *In vivo,* subcutaneous tumor growth and tumor growth within the bone microenvironment was inhibited in WNT5A-over-expressing PC3-Luc cells as compared to PC3-Luc cells ($-90\%$ and $-85\%$, $P<0.05$). Moreover, while 80% of the mice receiving PC3-Luc cells developed bone metastasis and bone lesions, overexpression of WNT5A abolished this process. These data indicate that WNT5A may act as a tumor suppressor in PCa and suggest that WNT5A may emerge as a novel therapeutic target for prostate cancer and bone metastases.

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## OC5.5

### Rs55710688 in the Kozak sequence of *WNT16* increases translation efficiency and is associated with osteoporosis related parameters

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Recent studies confirmed the role of *WNT16* in bone mineral density (BMD), bone strength and fracture risk. These findings made *WNT16* interesting for further genetic and functional studies to clarify its effect on osteoporosis related parameters.
A *WNT16* candidate gene association study was performed in men from the Odense Androgen Study (OAS). Five tagSNPs and one multimarker test were selected for genotyping to cover most of the common genetic variation in and around *WNT16*. This study confirmed previously reported associations for rs3801387 and rs2707466 and additionally showed a strong association between rs2908007 and BMD at several sites. Next, re-sequencing of *WNT16* was performed on two cohorts selected from the young OAS cohort, based on extreme BMD values. Rs55710688 showed a significant difference in genotype frequencies between the two BMD cohorts. This variant was selected for an *in vitro* translation experiment since it is located in the Kozak sequence of the WNT16a transcript. We observed an increased translation efficiency and thus a higher amount of WNT16a generated from the Kozak sequence that was significantly more prevalent in the high BMD cohort. This observation is in line with the results of the *Wnt16*$^{-/-}$ mice. Finally, a WNT luciferase reporter assay was performed in HEK293T cells and unexpectedly showed no activation of canonical WNT signaling by Wnt16. Moreover, a dose-dependent inhibitory effect of Wnt16 on WNT1 activation of this pathway was detected. This is in contrast with the known activating effect of canonical WNT signaling on bone formation. Therefore, the assay was performed in Saos-2 cells, a human osteosarcoma cell line. Preliminary results do show an significant activation of canonical signaling by Wnt16 in Saos-2 cells.
Our results strongly suggest that WNT16 is most likely able to increase BMD, by stimulation of canonical WNT signaling in bone forming cells. More research, including *in vivo* studies, is required to verify this hypothesis.

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## OC5.6

### Enhanced load adaptation in long bone of cathepsin K-deficient mice

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Gene deletion or treatments with a cathepsin K (CatK) inhibitor in mature preclinical models result not only result in lower bone resorption but also in higher bone formation (BF) on both remodeling and modeling surfaces. Although increased production of clastokines and matrix-derived growth factors may explain the increased BF at remodeling surfaces, the mechanisms for greater BF at modeling surfaces, including the periosteum, remain unexplained. We hypothesized that the absence of the CatK gene (*Ctsk*) enhances the skeletal response to mechanical loading. To test this hypothesis, *in vivo* cyclic axial compression (40 cycles, 7 min, 3day/week, for 2 weeks) was applied to the left tibia of 12 weeks old *Ctsk*$^{-/-}$ mice and their WT littermates ($n=6$ per groups), while the non-loaded (NL) tibia served as the comparator. Since *Ctsk*$^{-/-}$ mice have higher bone mass *vs* WT, the compression force was adjusted to 16N and 12N, respectively, in order to achieve 1700–1800 microstrain in both types of mice. Loading stimulated tibia BMD gain in *Ctsk*$^{-/-}$ ($+24.2\pm3.2$ *vs* $+0.2\pm0.3$ mg/cm$^2$ in NL) more than that in WT ($+9.0\pm1.4$ *vs* $+0.9\pm1.9$ mg/cm$^2$, $P<0.01$ for genotype x load inter.; $P<0.001$ *Ctsk*$^{-/-}$ *vs* WT). At the tibia midshaft, periosteal (Ps) MAR and BFR, as well as endocortical MAR, MPm/BPm and BFR were all significantly increased by loading in *Ctsk*$^{-/-}$, whereas only Ps.MAR was modestly increased in WT ($+60\%$ *vs* 566% in *Ctsk*$^{-/-}$, $P<0.05$). As a result, loading increased cortical bone volume fraction (Ct.BV/TV, $+19\%$) and thickness (CtTh, $+35\%$), in *Cstk*$^{-/-}$ (both $P<0.001$), but not in WT. Furthermore, when the same compression force (16N) was applied to both *Ctsk*$^{-/-}$ and WT mice, i.e. a lower strain in *Ctsk*$^{-/-}$, Ps.BFR and CtTh still increased more in *Ctsk*$^{-/-}$ than WT ($+1765\%$ *vs* $+494\%$ and $+35\%$ *vs* $+15\%$, respectively, both $P<0.05$). In conclusion, deletion of CatK enhances

the long bone adaptation to load at both Ec and Ps surfaces. While endocortical BF in *Cstk*$^{-/-}$ mice may result from increased production of growth factors within the BMUs, the results from this study suggest the presence of novel CatK-related molecular targets at periosteal surfaces.

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## Osteoporosis treatment and the effects of physical activity

### OC6.1

**Sex differences in bone acquisition of pre-pubertal children are consequence of differential responsiveness to mechanical loading**

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Aim
Lean mass is a strong determinant of bone structure, particularly during growth and development. We sought to determine if there are sex differences in bone acquisition in pre-pubertal children and the potential relation with skeletal loading.
Methods
This study included 3602 children (50.7% girls) whose total body BMD and hip scans were measured on the same iDXA devise (GE-Lunar) at 6 years of age. Hip scans underwent hip structural analysis (HSA) of which shaft parameters were analysed. Sex-differences across bone parameters were assessed by multivariate regression models (comparing least-squares means), adjusted for age, height, ethnicity, fat mass and with/without total-body lean mass. We also examined the relationship between bone strength and skeletal loading calculated as an index of mechanosensitivity (ratio of bone strength divided by height (moment arm) to total body lean mass (TB-LM).
Results
TB-BMD (less head) was 0.77% lower in girls than in boys ($P=0.01$) after adjustment for age, height, fat mass and ethnicity. Additional adjustment for TB-LM resulted in girls having 1.78% higher TB-BMD than boys ($P=1.9E-14$). Similarly, HSA models showed that girls had significant (all $P<0.01$) −5.8% thinner cortices (CT), 0.6% greater width (W), −4.7% axial (CSA) and −4.6% bending (SM) strength than boys. After TB-LM correction, girls had −1.4%, CT, 2.2% W, 1.3% CSA and 3.7% SM. The shaft mechanosensitivity index was 2.9% lower in boys than in girls after adjustment for age and ethnicity ($P=4.76E-11$).
Conclusion
At 6 years of age girls have higher bone mass and strength relative to muscle load than boys. These results are in line with previous studies reporting higher mechanosensitivity in women, attributing these differences to (post) pubertal hormonal changes. Our results show that when considering the relation with lean mass small but significant sex differences in bone density, structure, strength and mechanosensing are already present at pre-pubertal ages.

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### OC6.2

**Gender-specific associations between physical functioning, bone quality, and fracture risk in older people**

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Introduction
Measures of physical function may be related to osteoporosis and fractures in older individuals and may thus be used in the identification of individuals at high fracture risk.
Aim
The aim of this study was to investigate which measures of physical functioning are associated with bone quality and fracture incidence and whether gender-specific differences exist within these associations.
Methods
We studied 1486 participants of the Longitudinal Aging Study Amsterdam. As measures of physical functioning, handgrip strength, physical performance, and level of physical activity were assessed. To assess bone quality, broadband ultrasound attenuation (BUA) and speed of sound (SOS) were measured at baseline using quantitative ultrasound and bone mineral density (BMD) at baseline and after 3 years by dual-energy X-ray absorptiometry. In addition, fracture incidence over 6 years was assessed.
Results
After adjustment for confounders (age, serum 25(OH)D, smoking and body weight), in men physical performance was positively related to BUA, SOS, and BMD cross-sectionally and to BMD longitudinally. Using Cox proportional hazards model, in men higher handgrip strength and physical performance were associated with reduced fracture risk after adjustment for confounders (hazard ratio (HR) 0.96, 95% CI 0.92–0.99, and HR 0.89, 95% CI 0.80–0.98 respectively). In women a moderate level of physical activity was related to reduced fracture risk (HR 0.57, 95% CI 0.33–0.99).
Conclusion
In men higher handgrip strength and physical performance are related to higher bone quality and reduced fracture risk, whereas in women a moderate to high level of physical activity is associated with reduced fracture risk. These measurements may contribute to the identification of individuals at high fracture risk. Both causality of and explanations for gender-specific differences in these relationships remain subject to further studies.

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### OC6.3

**Vitamin D is low in obesity, and this is due to greater volume of distribution**

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Low circulating levels of total 25-hydroxyvitamin D (25OHD) have been reported in obese people of different ethnicities in several countries. Low total 25OHD in obesity could be due to lower binding proteins (with normal free 25OHD), lower dietary intake or sunlight exposure, greater volume of distribution (pool size) or more rapid metabolic clearance.
The aims of this study were to determine if free 25OHD and 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) are also low in obese people, and the cause of low vitamin D in obesity.
We assessed 216 normal weight (BMI 18.5–25), overweight (BMI 25–30) and obese (BMI > 30) adults ages 25–40 and 55–75 in autumn/spring, with vitamin D measurements and questionnaires for sunlight exposure and dietary vitamin D. Metabolic clearance of 25OHD was assessed by half-life of an orally administered stable isotope in 112 participants in winter. In autumn/spring, total 25OHD (immunoassay and LC-MS/MS), free 25OHD (measured and calculated) and total $1,25(OH)_2D$ (all adjusted for date of sample collection, age group and gender) were lower in obese people (ANOVA $P<0.002$), and negatively correlated with BMI ($R^2$ total 25OHD 0.248; free 25OHD 0.296; $1,25(OH)_2D$ 0.055, all $P<0.05$). The difference in 25OHD between normal weight and obese groups was greater in autumn/spring than in winter. Dietary vitamin D, sunlight exposure and 25OHD half-life did not differ by BMI group. We conclude that total and free 25OHD and $1,25(OH)_2D$ are lower in obesity, and this is likely to be due to greater volume of distribution for vitamin D.

| | Total 25OHD, nmol/l | Free 25OHD, pmol/l | Total $1,25(OH)_2D$, pmol/l |
|---|---|---|---|
| Normal | 53.6 | 10.63 | 95.0 |
| $n=75$ | (46.7, 61.4) | (9.43, 11.98) | (87.1, 103.7) |
| Overweight | 40.6 | 7.49 | 79.4 |
| $n=61$ | (35.4, 46.6) | (6.54, 8.59) | (72.3, 87.1) |
| Obese | 38.5 | 7.79 | 78.5 |
| $n=80$ | (34.0, 43.7) | (6.91, 8.76) | (72.3, 85.3) |

Measurements by immunoassay in autumn/springGeometric mean (95% CI), ANOVA group differences all $P<0.002$

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## OC6.4

**Long-term denosumab therapy further reduces the rate of non-vertebral fractures in women with persisting low hip BMD after 3 years**

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Objective
Evidence for further reduction of nonvertebral fracture (NVFX) beyond 3 years of antiresorptive therapy is limited. Since long-term denosumab (DMAb) treatment is associated with continuous increases in BMD and sustained fracture reduction, we analyzed the influence of femoral neck (FN) BMD after 3 years on NVFX rates.
Methods
Long-term subjects received 7 continuous years of DMAb; cross-over subjects received 3 years of placebo (FREEDOM) and 4 years of DMAb (extension). NVFX rates for years 1–3 *vs* the 4th year of DMAb were compared in each group separately and combined, and for subgroups defined by FN BMD T-score after 3 years of DMAb treatment. Adjusted rate ratios (RR) (95% CIs) between observational periods were computed by GEE Poisson regression.
Results
For long-term subjects, the NVFX rate was 1.98/100 subject-years during years 1–3 of DMAb treatment *vs* 1.43 (RR(95%CI)=0.73(0.50–1.06)) during year 4 and 1.45 (RR(95%CI)=0.74(0.59–0.95)) during years 4–7. For cross-over subjects, the NVFX rate was 2.20 during years 1–3 of DMAb *vs* 1.03 (RR(95%CI)=0.48(0.29–0.79)) in year 4. In the combined groups ($n=4073$) the rate was 2.08 during years 1–3 of DMAb and 1.27(RR(95%CI)=0.62(0.46–0.83)) in year 4. Compared with the first 3 years of treatment, the greatest reduction in NVFX in year 4 was observed in subjects with FN *T*-scores $\leq -2.5$ ($n=778$; RR(95%CI)=0.39(0.19–0.80)). A further reduction in NVFX was also present, but less pronounced, in subjects with *T*-scores $> -2.5$ and $< -1.0$ ($n=2407$; RR(95%CI)=0.69(0.47–1.01)), but not in subjects with *T*-scores $\geqq -1.0$.
Conclusion
Continued DMAb treatment beyond 3 years was associated with further reductions in NVFX rates, particularly in women whose BMD remained low after 3 years of treatment. These data suggest it may be possible to identify a BMD value below which ongoing treatment will have maximum fracture reduction benefit.

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## OC6.5

**The effect of bisphosphonate treatment on osteoclast precursor cells in postmenopausal women with rsteoporosis: The TRIO study**

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Bisphosphonates are used to treat bone disease characterised by increased bone resorption by inhibiting the activity of mature osteoclasts, resulting in decreased bone turnover. Bisphosphonates may reduce the population of osteoclast precursor cells (OPCs). Our aims were to investigate the effect of bisphosphonates on i) OPCs and ii) bone turnover in postmenopausal women with osteoporosis compared with healthy premenopausal women. Participants were 62 postmenopausal women (mean age 66) from a parallel group trial of bisphosphonates. They received ibandronate 150 mg/month ($n=22$), alendronate 70 mg/week ($n=19$) or risedronate 35 mg/week ($n=19$). Fasting blood was collected at baseline, weeks 1 and 48. 25 healthy premenopausal women (mean age 37) were recruited, blood was collected at baseline. Peripheral blood mononuclear cells were extracted and stained for CD14, M-CSFR, CD11b and TNFRII receptors. Flow cytometry was used to identify monocytes (CD14+) and OPCs (CD14+ and CD11b+ or M-CSF+ or TNFRII+). CTX was measured using the iSYS-IDS analyser (UK).
After 48 weeks of treatment, there was a decrease in the percentage of OPCs expressing MCSFR and CD11b receptors by 53 and 49% respectively ($P<0.01$). CTX decreased by 62% after 1 week and by 83% after 48 weeks. OPCs expressing M-CSFR and CD11b were decreased with ibandronate and risedronate after 48 weeks (table) to the lower part of the premenopausal limits.
Significance from baseline tested using non-parametric Wilcoxon signed test. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$

| | Baseline | | | Week 1 | | | Week 48 | | |
|---|---|---|---|---|---|---|---|---|---|
| | Iban | Alen | Rise | Iban | Alen | Rise | Iban | Alen | Rise |
| %M-CSFR | 2.0 | 1.5 | 1.8 | 1.2 | 1.7 | 1.7 | 0.7* | 1.0 | 0.7** |
| %CD11b | 2.5 | 2.6 | 3.5 | 2.7 | 3.2 | 3.1 | 1.5 | 2.0 | 1.6** |
| %TNFRII | 2.6 | 2.2 | 2.1 | 2.7 | 2.8 | 2.1 | 1.8 | 1.5 | 1.8 |

Bisphosphonates inhibit bone resorption in the short-term by direct action on mature osteoclasts. There is also a later effect mediated by a reduction in the population of circulating osteoclast precursors.

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## OC6.6

**External auditory canal and middle ear diseases in bisphosphonate-treated osteoporosis patients: A Danish national register based cohort study**

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Background and aim
Eight cases of bisphosphonate-associated osteonecrosis of the external auditory canal have been reported in case-reports. Our aim was to describe the incidence of external auditory canal and middle ear diseases in Danish patients exposed to bisphosphonates in the treatment of osteoporosis.
Methods
The study was a retrospective, nationwide cohort study, within the Danish population of approximately 5.6 million individuals and based on Danish national registers.
From 2003–2010, 131 794 patients had bisphosphonates prescribed for treatment of osteoporosis. These cases were matched 3:1 for age and gender with a total of 395 382 persons unexposed to bisphosphonates. Primary outcome was disease in the external auditory canal and middle ear, defined as first occurrence of an ICD10 hospital diagnosis code of destruction of bones in the ear (H74.3B), cholesteatoma of the external auditory ear canal (H60.4) or cholesteatoma of the middle ear (H71.9). The primary explanatory variable was bisphosphonate exposure.
Results
The overall incidence of cholesteatoma in the ear was low. Only 350 events were seen in 527 176 cases and controls. One hundred and nineteen events of cholesteatoma in the ear were recorded after initiation of bisphosphonate therapy, 34 in the external auditory canal and 85 in the middle ear. Cholesteatoma in the external auditory canal was significantly higher in the exposed than in the unexposed group ($P<0.0001$). We found a significant dose-event relationship between incidence of cholesteatoma and dose of alendronate ($P<0.0001$) and etidronate ($P<0.0001$) Furthermore, we found an association between duration of treatment and risk of cholesteatoma in the external auditory ear canal (log rank, $P=0.002$).
No cases of bone destruction were observed in cases or controls.
Conclusion
Use of oral bisphosphonates is associated with an increased risk of cholesteatoma of the external auditory canal. The risk is small and associated with duration and dosage of bisphosphonate.

DOI: 10.1530/boneabs.3.OC6.6

# Hot Topic Oral Communications

## HT1

### Atypical femoral fracture and bisphosphonate use: association with duration, cessation, gender, and type of bisphosphonate: a population-based study of 172 cases.

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Background
Bisphosphonate use is associated with an increased risk of atypical femoral fractures in women. The risk in men and the risk pattern dependent on treatment duration, recent use and type of bisphosphonate however remains unclear.
Methods
All 5715 Swedish men and women, 55 years or older, with a fracture of the femoral shaft in the three-year period 2008–2010 were identified by the national patient registry. Radiographs were reviewed and we found 160 female and 12 male patients with atypical fracture. Medical background information was also obtained from register data. The relative and absolute risk of atypical fractures associated with bisphosphonate use was estimated in a nationwide cohort analysis. The 172 case patients were compared with 952 control patients with ordinary shaft fractures.
Results
The age-adjusted relative risk (RR) of atypical fracture in the cohort analysis was 55 (95% CI 39 to 79) in women and 54 (CI 15-192) in men. Among bisphosphonate users, women had a three-fold higher risk (RR 3.08; CI 1.13–8.42) compared to men. The average absolute risk with bisphosphonate use was 5 per 10 000 (CI 4 to 6) person-years of use in women and 2 per 10 000 (CI 0 to 3) in men. The relative risk increased for every year of use and reached 126 (CI 55 to 288) after 4 or more years. The multivariable-adjusted odds ratio of sustaining an atypical fracture after 4–5 years of bisphosphonate use was 116 (CI 58–234). The risk diminished by 70% for each year after cessation of treatment. The odds with alendronate use was 3 times higher than with risedronate use.
Conclusion
Women have a higher risk of atypical fracture than men. The risk-benefit ratio for bisphosphonate deteriorates with long-term treatment. The rapid decrease in risk after cessation suggests that the pathophysiological mechanism is related to targeted remodeling.

DOI: 10.1530/boneabs.3.HT1

## HT2

### Osteoblast-specific ablation of *p38α* blunts the bone anabolic activity of parathyroid hormone

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Intermittent PTH administration (iPTH) increases bone mass in humans and animals. PTH exerts its effects by binding to PTH type 1 receptor (PTHR1) predominantly expressed in osteoblasts, resulting in activation of multiple downstream signaling pathways. *In vitro* investigations have suggested that p38 mitogen-activated protein kinase (MAPK) signaling is an important mediator of PTH-induced osteoblast functions.
To evaluate the contribution of p38alpha MAPK signaling in iPTH-induced bone anabolism, 3-month-old male mice lacking *p38α* in osteoblasts (*Ocn-Cre;p38α$^{f/f}$*) and their control littermates (*p38α$^{f/f}$*) were treated with daily subcutaneous injections of either 40 µg/kg PTH (1–34) or its vehicle. After 4 weeks of treatment, bone phenotypes were assessed by dexa, microCT, histomorphometry and quantitative gene expression analyses (n=6 per group). Data were analyzed by two-way ANOVA and post hoc analyses were performed using the Holm-Sidak method.
iPTH treatment increased total bone mineral density (+8.5%, $P=0.003$ vs Veh), femoral cortical thickness (+10.9%, $P=0.005$), femoral cancellous bone volume (+35.4%, $P=0.007$) and trabecular thickness (+21.9%, $P=0.008$) in control mice but did not induce significant changes of those parameters in *Ocn-Cre;p38α$^{f/f}$* mice. Consistent with this, iPTH significantly stimulated trabecular mineralizing surfaces (1.5-fold), mineral apposition rate (1.9-fold) and bone formation rate (2.8-fold) in control animals, whereas it only enhanced mineralizing surfaces (1.5-fold) in *Ocn-Cre;p38α$^{f/f}$* mice, indicating a functional defect of *p38α*-deficient osteoblasts in response to iPTH. Furthermore, iPTH significantly increased osteoblast marker gene expressions (*Col1a1*, *Alp*, *BspII* and *Ocn*) in control mice but not in *Ocn-Cre;p38α$^{f/f}$* mice. Finally, *p38α*-deficient osteoblasts exhibited normal *Pthr1* gene expression in comparison to control osteoblasts, but did not display elevations of *Alp*, *BspII* and *Ocn* expressions and matrix mineralization in response to PTH *in vitro*.
Our findings indicate that the *p38α* MAPK in osteoblasts plays an important role in PTH-induced bone anabolism in mice.

DOI: 10.1530/boneabs.3.HT2

## HT3

### Consistent, marked and rapid increases in hip and spine BMD with the $PTHrP_{1-34}$ analog, abaloparatide (BA058), compared to placebo and teriparatide

John Yates[1], Peter Alexandersen[2], Annesofie Krogsaa[2], Bettina Nedergaard[2], Marcie Clarkin[1], Gary Hattersley[1], Morten Karsdal[2] & Claus Christiansen[2]
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Background
Treatments that result in greater increases in bone mass of normal quality by increasing bone formation rather than decreasing resorption are needed. Abaloparatide is a synthetic analog of $PTHrP_{1-34}$ that has shown strong efficacy to increase bone mass and bone strength in animals. We conducted two phase 2 placebo-controlled studies both of which included abaloparatide 80 µg sc daily (ABL) in postmenopausal women with osteoporosis. Study 1 also included teriparatide (Forteo) 20 µg sc daily (TER). Together, these studies included 95, 94 and 45 women treated with placebo, ABL and TER respectively.
Results
Relative to placebo, ABL increased spine BMD at 24 weeks by 5.1 and 5.8% and increased total hip BMD by 2.2 and 2.7% in Studies 1 and 2 respectively (all $P<0.005$). TER increased spine BMD by 3.9% ($P<0.001$), but had no significant effect on total hip BMD relative to placebo (0.1%). The increase in CTX was substantially higher with TER, reflecting a greater increase in bone resorption. In a subset extension of Study 1, mean increases in BMD at 48 weeks relative to placebo were 12.2 and 7.9% for ABL and TER at the spine and 2.7, and 1.3 at the total hip respectively. Both ABL and TER were generally well tolerated.
Comments
ABL induces consistent, rapid, substantial increases in BMD over up to 48 weeks, which are greater than those seen with either TER or, historically, with any other currently-approved treatment for osteoporosis. The greater BMD efficacy is most likely due to greater selectivity of ABL to increase bone formation with a less marked increase in bone resorption relative to TER. ABL is currently in a >2400-patient, phase 3, 18-month, placebo- and TER-controlled, fracture study which will complete this year. ABL is a promising new therapy for treatment of osteoporosis.

DOI: 10.1530/boneabs.3.HT3

## HT4

### Sclerostin prevents mice from osteoarthritis despite high subchondral bone accretion

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INSERM U1132 and University Paris-7, Paris, France.

Purpose
Sclerostin, a Wnt inhibitor produced by osteocytes that regulate bone remodelling, might be involved in cartilage metabolism. Therefore, we assessed the effect of sclerostin in osteoarthritis using SOST-deficient mice.
Methods
SOST-KO and wild type (WT) mice underwent partial meniscectomie (Mnx). Mice were sacrificed at 4, 6 or 9 weeks after Mnx to analyze i) bone volume (BV/TV) at the femoral condyle, ii) osteophyte volume (microCT), iii) cartilage damage (OA score) and iv) expression of matrix proteins. Primary murine chondrocyte were cultured with Wnt3a and sclerostin to analyze metabolic markers (RT-qPCR, WB). Proteoglycan content and GAGs accumulation was quantified. We next investigated the role of canonical and non-canonical Wnt pathways.
Results
Sclerostin was expressed in the calcified cartilage and enhanced in OA joint. At any time, cartilage was preserved in SOST-KO mice despite a markedly high BV/TV.
Mnx induced a higher OA score in SOST-KO mice than in WT at Week 4 ($6.66\pm0.57$ vs $3.25\pm0.95$, $P<0.05$) and Week 6 ($11\pm1$ vs $7\pm0.81$, $P<0.05$).

Osteophyte volume was not affected by the lack of sclerostin. Enhanced chondrocyte catabolism was observed with increased type X collagen and Adamts-4 expressions. In primary chondrocytes, Wnt reduced the proteoglycan release but was rescued by sclerostin. Wnt increased the expression of Adamts, MMPs and type X collagen, while this effect was totally abolished by sclerostin through the activation of the canonical pathway. Furthermore, sclerostin inhibited the Wnt-induced phosphorylation of JNK, rescued the accumulation of GAGs and the expression of the anabolic genes when JNK pathway was inhibited.
Conclusions
Lack of sclerostin increases the subchondral bone accretion and accelerates cartilage damage in OA. Sclerostin maintains the chondrocyte metabolism by inhibiting Wnt canonical and non-canonical JNK pathways. These data suggest that sclerostin contribute to cartilage integrity in OA.

DOI: 10.1530/boneabs.3.HT4

## HT5

**Skin inflammation causes bone loss with reduced bone formation through systemic IL-17A release**

Ozge Uluckan[1], Johannes Keller[2], Susanne Karbach[3], Andrew Croxford[3], Stephanie Finzel[4], Marije Koenders[5], Wim Van Den Berg[5], Michael Amling[2], Ari Waisman[3], Georg Schett[4] & Erwin Wagner[1]
[1]Spanish National Cancer Center, Madrid, Spain; [2]University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [3]University of Mainz, Mainz, Germany; [4]University of Erlangen-Nuremberg, Erlangen, Germany; [5]Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands.

Patients with chronic inflammatory diseases such as psoriasis are at high risk for developing osteoporosis. Psoriatic arthritis patients exhibit bone loss caused by increased bone resorption through activation of osteoclasts. However, it is not clear whether psoriasis can lead to bone loss in the absence of arthritis. Using mouse models with skin inflammation as well as psoriasis patient samples, we show that increased circulating IL-17A from the inflamed skin triggers bone loss through inhibition of bone formation. Osteocalcin (OCN) levels as well as bone formation rates are decreased in mice with an epithelial(Keratin5)-specific deletion of JunB ($JunB^{\Delta ep}$). Furthermore, transgenic mice expressing IL-17A in keratinocytes and mice injected with Adenoviruses expressing IL-17A exhibit decreased OCN levels. We show for the first time that, together with the previously-described γδT-cells, keratinocytes of epithelial origin also express IL-17A, which is transcriptionally controlled by JunB/AP-1. The inhibition of bone formation by IL-17A is independent of its expression by T/B-cells, since $JunB^{\Delta ep}$ mice on a Rag1-/- background still display decreased levels of OCN. Mechanistically, through RNAseq analyses in osteoblasts, we identified nitric oxide and lipocalin-2 as mediators of IL-17A-dependent osteoblast inhibition. Pharmacologic IL-17A blockade rescues OCN expression and bone formation rates in $JunB^{\Delta ep}$ mice. Importantly, psoriasis patients without arthritis develop bone loss with decreased OCN levels and increased serum IL-17A levels. Therefore, this study suggests that IL-17A, upregulated in inflammatory and autoimmune diseases, provides a risk for bone loss and its blockade should be considered in such diseases to prevent the adverse consequences on the skeleton.

DOI: 10.1530/boneabs.3.HT5

# Clinical Case Oral Communications

## CC1

### Molecular diagnosis of osteopetrotic patients with atypical presentations using traditional approaches and exome sequencing

Eleonora Palagano[1,2], Alessandra Pangrazio[1,2], Dario Strina[1,2], Alessandro Puddu[3,4], Manuela Oppo[3], Maria Valentini[3], Paolo Vezzoni[1,2], Anna Villa[1,2] & Cristina Sobacchi[1,2]
[1]CNR-IRGB, Milan Unit, Milan, Italy; [2]Istituto Clinico Humanitas IRCCS, Rozzano, Italy; [3]CRS4 Bioinformatics Laboratory, Parco Scientifico e Tecnologico POLARIS, Pula, Italy; [4]CNR-IRGB, Cittadella Universitaria di Monserrato, Cagliari, Italy.

Autosomal Recessive Osteopetrosis (ARO) presents early in life with extreme sclerosis of the skeleton, reduction of bone marrow spaces, hepatosplenomegaly, cranial nerves compression and severe growth failure. ARO is often lethal and at present the only therapy is HSCT, which should be performed as soon as possible in order to obtain a major benefit. ARO is genetically heterogeneous and delays in clinical diagnosis sometimes occur, due to its rareness and to the presence of complex phenotypes which may be misinterpreted. Therefore, the molecular classification of the disease is essential to promptly establishing the appropriate treatment.

We report the use of exome sequencing in the molecular diagnosis of two siblings initially thought to be affected by 'intermediate osteopetrosis', which identified a homozygous mutation in the CTSK gene. Prompted by this finding, we investigated additional patients addressed to us for recessive osteopetrosis and found CTSK mutations in four of them, whose clinical and radiographic features were retrospectively found to be compatible with, but not typical for, Pycnodysostosis. So, we recommend that CTSK gene be included in the molecular diagnosis of intermediate forms of human ARO and, in general, of high-density bone conditions.

We also describe the first patient with mild osteopetrosis due to recessive TCIRG1 mutations causing an incomplete splicing defect, allowing for the production of the small amount of protein sufficient for dampening the clinical outcome. This finding widens the clinical spectrum that may arise from recessive mutations in TCIRG1, demonstrating that extremely rare, mild forms of TCIRG1-dependent ARO exist, so this gene should also be included in the molecular work-up of intermediate cases.

Overall, we underline the difficulties of differential diagnosis in patients whose clinical appearance does not fit the classical malignant or benign picture, and confirm the role of exome sequencing in the molecular classification of genetically heterogeneous diseases.

DOI: 10.1530/boneabs.3.CC1

## CC2

### Hyperphosphatemic familial tumoral calcinosis and hyperphosphatemic hyperostosis syndrome caused by a novel *GALNT3* mutation; Long-term clinical outcome and phenotypic variability.

Silje Rafaelsen[1], Stefan Johansson[1,2], Helge Ræder[1,3] & Robert Bjerknes[1]
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Background

Hyperphosphatemic Familial Tumoral Calcinosis (HFTC) and Hyperphosphatemic Hyperostosis Syndrome (HHS) are two phenotypes of a disease associated with autosomal recessive mutations in *FGF23*, *GALNT3* and *KL*, leading to reduced levels and clinical effects of fibroblast growth factor 23 (FGF23). We describe a consanguineous family with two affected individuals with HFTC and HHS caused by a novel homozygous mutation in *GALNT3*. We also review the literature on *GALNT3*-associated HFTC and HHS.

Results

Calcific tumours appeared at three weeks of age in the sister and at 14 years of age in the brother. The sister displayed episodic diaphysitis from age 9 years. Abnormal dental roots, tooth loss and musculoskeletal complaints were present in both from their mid-twenties. The sister had calcifications in the placenta, iliac vessels and thyroid cartilage, and developed band keratopathy. New calcific tumours appeared more than 20 years after the initial episodes, delaying diagnosis and treatment. Both subjects had elevated serum phosphate levels, inappropriately elevated tubular maximum phosphate reabsorption per unit glomerular filtration rate (TmP/GFR), reduced levels of intact FGF23 and increased levels of c-terminal FGF23. Mutation analysis identified a novel homozygous G-to T substitution in exon 3 of *GALNT3* (c.767G>T; p.G256V). A review of all 52 previously published cases of *GALNT3, FGF23*, and *KL* associated HFTC and HHS, showed that more subjects than previously recognized have a combined phenotype of HFTC and HHS.

Conclusion

We have described HFTC and HHS in a consanguineous Caucasian family with a novel *GALNT3* mutation, demonstrating great phenotypic variability and long asymptomatic intervals, with HFTC from infancy and later symptoms of HHS in the sister, and classic HFTC in the brother. HHS and HFTC are probably two distinct phenotypes in a spectrum of *GALNT3* and *FGF23* mutation related calcification disorders, where additional factors determining the phenotypic expression, are yet to be clarified.

DOI: 10.1530/boneabs.3.CC2

## CC3

### Absence of ER cation channel *TMEM38B*/TRIC-B causes recessive osteogenesis imperfecta by dysregulation of collagen post-translational modification

Wayne Cabral[1], Elena Makareeva[2], Masaki Ishikawa[3], Aileen Barnes[1], MaryAnn Weis[4], Felicitas Lacbawan[5], David Eyre[4], Yoshihiko Yamada[3], Sergey Leikin[2] & Joan Marini[1]
[1]Bone and Extracellular Matrix Branch, NICH, NIH, Bethesda, Maryland, USA; [2]Section on Physical Biochemistry, NICHD, NIH, Bethesda, Maryland, USA; [3]Molecular Biology Section, NIDCR, NIH, Bethesda, Maryland, USA; [4]Department of Orthopaedics and Sports Medicine, University of Washington, Seattle, Washington, USA; [5]Department of Medical Genetics, Children's National Medical Center, Washington, District of Columbia, USA.

Recessive osteogenesis imperfecta (OI) is caused by mutations in genes encoding proteins involved in post-translational interactions with type I collagen. A founder mutation in a new gene responsible for recessive OI has recently been reported in Bedouins from Israel and Saudi Arabia, who have a homozygous deletion of *TMEM38B* exon 4 and surrounding intronic sequence. *TMEM38B* encodes TRIC-B, an integral ER membrane monovalent cation channel involved in $Ca^{++}$ release from intracellular stores. However, the molecular mechanisms through which this mutation causes an OI phenotype are unknown. We identified a 20 month-old girl with moderately severe OI, born to consanguineous parents from Saudi Arabia, who is homozygous for the *TMEM38B* founder mutation. *TMEM38B* transcripts are 25% of control level, and include six alternatively spliced forms of the transcript, of which one in-frame transcript deletes the central transmembrane domain and putative ion pore. The complete absence of TRIC-B protein was confirmed by Western blot and resulted in decreased intracellular $Ca^{++}$ concentration and ATP-induced $Ca^{++}$ flux from the ER. Surprisingly, SDS-Urea PAGE demonstrated increased electrophoretic migration of collagen alpha chains, suggesting altered post-translational modification. Although LH1 transcripts and protein were increased, proband collagen revealed a 30% reduction in helical lysine hydroxylation. Furthermore, the detection of lower stability collagen species (30–40% of total collagen) on differential scanning calorimetry, increased intracellular PDI and GRP/BiP, and decreased procollagen pericellular processing, imply that proband procollagen conformation is abnormal. FKBP65, which is stabilized by $Ca^{++}$ and required for collagen telopeptidyl hydroxylation and crosslinking, was decreased in proband fibroblasts. Matrix deposited in culture by proband fibroblasts has 30% reduction of immaturely and maturely crosslinked collagen. We propose that these data support a role for TRIC-B in intracellular $Ca^{++}$ mobilization, and that absence of TRIC-B causes OI by dysregulation of multiple $Ca^{++}$-regulated collagen-specific chaperones and modifying enzymes in the ER.

DOI: 10.1530/boneabs.3.CC3

## CC4

### Two novel compound heterozygous mutations in *LRP5* cause osteoporosis pseudoglioma syndrome

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[1]Rheumatic Diseases Unit, MRC Institute of Genetics and Molecular Medicine, Centre for Genomic and Experimental Medicine, University of Edinburgh, Edinburgh, UK; [2]MRC Human Genetics Unit, MRC Institute of Genetics and Molecular Medicine, Centre for Genomic and Experimental Medicine, University of Edinburgh, Edinburgh, UK; [3]Department of Rheumatology, Derby Hospitals NHS Foundations Trust, Derby, UK; [4]Musculoskeletal Research Unit, School of Clinical Sciences, University of Bristol, Bristol, UK.

Osteoporosis pseudoglioma syndrome (OPPGS) is a rare autosomal recessive disorder characterised by congenital or juvenile-onset blindness, severe juvenile-onset osteoporosis, and skeletal fragility. OPPGS is caused by loss-of-function mutations in the *LRP5* gene, a member of the LDL receptor family. It activates the canonical Wnt/β-catenin pathway, regulating osteoblastic bone formation. We investigated a 40-year-old Caucasian male presenting with congenital blindness and osteoporosis, with multiple fractures before the age of ten; and his 57-year-old mother, showing a milder bone-specific phenotype, with low bone mineral density (BMD) (spine *T*-score −2.7; femoral neck *T*-score −1.7) and no adult fractures, within a non-consanguineous family. We sequenced all 23 exons of *LRP5* in the index case and his mother and found a heterozygous missense mutation, g.C2254T, p.R752W, in both. The index case carried another heterozygous missense mutation, g.T235C, p.W79R, not found in his mother. Both these missense mutations are novel. To assess pathogenicity, we created and validated three-dimensional homology models for the four extracellular YWTD β-propeller/EGF-like domains (E1–E4) of LRP5. Mutation W79R is located in the second β-strand of blade 2 (E1 domain), within the highly conserved 'YWTD' signature motif and deeply buried in the protein core; energy stability calculations using FoldX predict this mutation to severely destabilise structure (mean $\Delta\Delta G = \sim 5$ kcal/mol; $> 1.6$ kcal/mol is considered destabilising). The R752W mutation is located in blade 3 of E3 domain, near the interface with E4, but is also predicted to destabilise structure, albeit to a lesser extent (mean $\Delta\Delta G = \sim 2$ kcal/mol). In conclusion, the novel mutation R752W is associated with low BMD, as seen in the mother, but the combination of this mutation with the novel W79R, causes a severe case of OPPGS due to destabilisation of the β-propeller motifs of the LRP5 protein which are required for protein and ligand binding.

DOI: 10.1530/boneabs.3.CC4

# Oral Posters

## Clinical

### OP1

**A transdermal patch delivering the $PTHrP_{1–34}$ analog, abaloparatide (BA058), dose-dependently increases spine and hip bmd compared to placebo**

John Yates, Peter Alexandersen, Annesofie Krogsaa, Bettina Nedergaard, Marcie Clarkin, Gary Hattersley, Kris Hansen, Morten Karsdal & Claus Christiansen

see PP351.

DOI: 10.1530/boneabs.3.PP351

### OP2

**Abaloparatide (BA058), a novel human PTHrP analog, restores bone mass and strength in the aged osteopenic ovariectomized cynomolgus monkey**

Gary Hattersley, Nancy Doyle, Aurore Varela, Robert E Guldberg & Susan Y Smith

see PP352.

DOI: 10.1530/boneabs.3.PP352

### OP3

**PTH treatment induces WNT10b expression in humans lymphoid cells**

Patrizia D'Amelio, Francesca Sassi, Ilaria Buondonno, Elena Spertino, Lucia D'Amico, Ilaria Roato & Giovanni Carlo Isaia

see PP353.

DOI: 10.1530/boneabs.3.PP353

### OP4

**Denosumab treatment in women with osteoporosis reduces hip cortical porosity**

Roger M Zebaze, Cesar Libanati, Michael R McClung, Jose R Zanchetta, David L Kendler, Arne Høiseth, Andrea Wang, Ali Ghasem-Zadeh & Ego Seeman

see PP354.

DOI: 10.1530/boneabs.3.PP354

### OP5

**Continuous modelling-based bone formation could explain sustained increases in hip bone mineral density with denosumab treatment**

Michael S Ominsky, Cesar Libanati, Rogely Boyce, Paul J Kostenuik, Roland Baron, Rachel B Wagman & David W Dempster

see PP355.

DOI: 10.1530/boneabs.3.PP355

### OP6

**Changes in lumbar spine QCT, DXA and TBS with denosumab, alendronate or placebo in postmenopausal women with low bone mass**

Thierry Thomas, Angela M Cheung, Elizabeth Shane, Jose R Zanchetta, Ann Kearns, Didier Hans, Celia J F Lin, Matthew Austin & Cesar Libanati

see PP356.

DOI: 10.1530/boneabs.3.PP356

### OP7

**In postmenopausal women previously treated with an oral bisphosphonate and at higher risk of fracture, denosumab significantly increases bone mineral density compared with ibandronate and risedronate**

Jacques P Brown, Michael A Bolognese, Pei-Ran Ho, Christian Roux, Henry G Bone, Sydney L Bonnick, Joop van den Bergh, Irene Ferreira, Prayashi Ghelani, Paula Dakin, Rachel B Wagman & Christopher Recknor

see PP357.

DOI: 10.1530/boneabs.3.PP357

### OP8

**Effects of up to 15 years of recombinant human GH replacement therapy on the skeleton in adult GH deficiency: the Leiden Cohort Study**

Natasha Appelman-Dijkstra, Kim Claessen, Neveen Hamdy, Alberto Pereira & Nienke Biermasz

see PP404.

DOI: 10.1530/boneabs.3.PP404

### OP9

**Zoledronate prevents lactation induced loss of bone strength and micro-architecture**

Mette Høegh Wendelboe, Jesper Skovhus Thomsen & Annemarie Brüel

see PP89.

DOI: 10.1530/boneabs.3.PP89

### OP10

**Zoledronate reverses bone marrow adiposity in disuse osteopenic rats**

Michael Vinkel Jensen, Annemarie Brüel & Jesper Skovhus Thomsen

see PP358.

DOI: 10.1530/boneabs.3.PP358

## OP11

**A randomized, double-blind, placebo-controlled, ascending, single-dose study of a human monoclonal anti-FGF23 antibody (KRN23) in X-linked hypophosphatemia**

Thomas Carpenter, Erik Imel, Mary Ruppe, Thomas Weber, Mark Klausner, Margaret Wooddell, Tetsuyoshi Kawakami, Takahiro Ito, Xiaoping Zhang, Jeffrey Humphrey, Karl Insogna & Munro Peacock

see PP90.

DOI: 10.1530/boneabs.3.PP90

## OP12

**Pharmacokinetics and pharmacodynamics of a human monoclonal anti-FGF23 antibody (KRN23) after ascending single-dose administration in patients with X-linked hypophosphatemia**

Xiaoping Zhang[1], Thomas Carpenter[2], Erik Imel[3], Mary Ruppe[4], Thomas Weber[5], Mark Klausner[1], Tetsuyoshi Kawakami[1], Takahiro Ito[1], Jeffrey Humphrey[1], Karl Insogna[2] & Munro Peacock[3]

see PP91.

DOI: 10.1530/boneabs.3.PP91

## OP13

**Decrease in expression of MMP3 in osteoblast protects against bone loss**

Mylene Zarka-Prost-Dumont, Frederic Jehan, Agnes Ostertag, Marie-Christine de Vernejoul & Valerie Geoffroy

see PP288.

DOI: 10.1530/boneabs.3.PP288

## OP14

**Ten year alendronate use does not adversely affect bone quality compared to 5 years use: a human iliac crest biopsy study**

Norbert Hassler, Sonja Gamsjaeger, Birgit Hofstetter, Wolfgang Brozek, Barbara Misof, Paul Roschger, Klaus Klaushofer & Eleftherios Paschalis

see PP359.

DOI: 10.1530/boneabs.3.PP359

## OP15

**Predictors of second fracture while on treatment with oral bisphosphonates: a multinational retrospective cohort study**

Sam Hawley, Gemma Wallace, M Kassim Javaid, Katrine Rubin, Andrew Judge, Peter Vestergaard, Richard Eastell, Adolfo Diez-Perez, Nigel K Arden, Cyrus Cooper, Daniel Prieto-Alhambra & Bo Abrahamsen

see PP360.

DOI: 10.1530/boneabs.3.PP360

## OP16

**The impact of common comorbidities (as measured using the Charlson index) on hip fracture risk in elderly men: a population-based cohort study**

Carlen Reyes, Xavier Nogués, Cyrus Cooper, Adolfo Diez-Perez & Daniel Prieto-Alhambra

see PP289.

DOI: 10.1530/boneabs.3.PP289

## OP17

**Dynamic changes in bone marrow adiposity during the menstrual cycle**

Annegreet Veldhuis-Vlug, Eelkje Limonard, Laura van Dussen, Jurgen Runge, Michael Tanck, Erik Endert, Annemieke Heijboer, Eric Fliers, Carla Hollak, Erik Akkerman & Peter Bisschop

see PP255.

DOI: 10.1530/boneabs.3.PP255

## OP18

**Does bone density, bone strength, sarcopenia or dynapenia explain greater risk of fracture in obesity?**

Amy Evans, Richard Eastell & Jennifer Walsh

see PP209.

DOI: 10.1530/boneabs.3.PP209

## OP19

**Effect of subcutaneous recombinant human parathyroid hormone, rhPTH(1–84), on skeletal dynamics in hypoparathyroidism: findings from the 24-week replace and 8-week relay phase III clinical trials**

John P Bilezikian, Gerard Maruani, Jeffrey Rothman, Bart L Clarke, Michael Mannstadt, Tamara Vokes, Hjalmar Lagast & Dolores M Shoback

see PP92.

DOI: 10.1530/boneabs.3.PP92

## OP20

**No beneficial effects of vitamin D supplementation in muscle function or quality of life in primary hyperparathyroidism: results from a randomized controlled trial.**

Lars Rolighed, Lars Rejnmark, Tanja Sikjaer, Lene Heickendorff, Peter Vestergaard, Leif Mosekilde & Peer Christiansen

see PP210.

DOI: 10.1530/boneabs.3.PP210

## Pre-Clinical

### OP21

**Novel evidence that apolipoprotein A-I deficiency is implicated in the pathogenesis of osteoporosis in mice**
Eleni Kalyvioti, Kyriakos Kypreos, Nicholas Papachristou, Malvina Orkoula, Irene-Eva Triantaphyllidou, Harry Blair & Dionysios Papachristou

see PP290.

DOI: 10.1530/boneabs.3.PP290

### OP22

**Apolipoprotein-E deficiency predisposes to the development of osteoporosis following long-term exposure to western-type diet, in mice**
Nicholas Papachristou, Eleni Kalyvioti, Irene-Eva Triantaphyllidou, Harry Blair, Kyriakos Kypreos & Dionysios Papachristou

see PP291.

DOI: 10.1530/boneabs.3.PP291

### OP23

**The peroxisome proliferator-activated receptor alfa agonist fenofibrate improves the effect of high-impact exercise on bone and muscle mass in ovariectomized rats**
Mats Peder Mosti, Madelene Ericsson, Unni Syversen & Astrid Kamilla Stunes

see PP211.

DOI: 10.1530/boneabs.3.PP211

### OP24

**New insights in the bone-muscle axis: the novel myokine irisin is involved in skeletal metabolism**
Graziana Colaianni, Concetta Cuscito, Teresa Mongelli, Angela Oranger, Giorgio Mori, Giacomina Brunetti, Silvia Colucci, Saverio Cinti & Maria Grano

see PP212.

DOI: 10.1530/boneabs.3.PP212

### OP25

**Selective inhibition of BET bromodomains epigenetic signaling interferes with the bone-associated tumor vicious cycle**
Francois Lamoureux, Marc Baud'huin, Lidia Rodriguez Calleja, Camille Jacques, Françoise Redini, Fernando Lecanda, James E. Bradner, Dominique Heymann & Benjamin Ory

see PP103.

DOI: 10.1530/boneabs.3.PP103

### OP26

**Disturbed cartilages of the mandible in achondroplasia are associated with defective mandible shape and position**
Martin Biosse Duplan, Federico Di Rocco, Yann Heuzé, Emilie Gaudas, Davide Komla-Ebri, Nabil Kaci, Catherine Benoist-Lasselin & Laurence Legeai-Mallet

see PP185.

DOI: 10.1530/boneabs.3.PP185

### OP27

**Novel mechanisms of action and new target genes of the glucocorticoid receptor in inflammatory bone disease and bone loss**
Ulrike Baschant, Mubashir Ahmad, Mascha Koenen, Jeanette Knoll, Stephan Culemann, Sebastian Schauer, Kerstin Bauer, Stephanie Wittig-Blaich, Alexander Rauch, Gehrhard Krönke, Anne Dudeck, Jean-Pierre David, Martina Rauner, Markus Seibel, Aspasia Ploubidou, Hong Zhou, Lorenz Hofbauer & Jan Tuckermann

see PP416.

DOI: 10.1530/boneabs.3.PP416

### OP28

**Bone matrix mineralization after sclerostin antibody treatment in a mouse model of osteogenesis imperfecta**
Andreas Roschger, Paul Roschger, Michaela Kneissel & Frank Rauch

see PP37.

DOI: 10.1530/boneabs.3.PP37

### OP29

**Innovative cell-based strategy for systemic delivery of soluble RANKL in RANKL-deficient osteopetrotic mice**
Alfredo Cappariello, Riccardo Paone, Mattia Capulli, Nadia Rucci, Maurizio Muraca & Anna Teti

see PP405.

DOI: 10.1530/boneabs.3.PP405

### OP30

**Bone secreted dickkopf-related protein 1 ameliorates osteoarthritis in mice**
Thomas Funck-Brentano, Wafa Bouaziz, Caroline Marty, Valérie Geoffroy, Eric Hay & Martine Cohen-Solal

see PP15.

DOI: 10.1530/boneabs.3.PP15

## OP31

**Increased expression of PTX3 in non-hematopoietic periosteal cells during fracture healing**

Tomislav Kelava, Sanja Ivcevic, Vedran Katavic, Natasa Kovacic, Hrvoje Cvija, Katerina Zrinski Petrovic, Sania Kuzmac, Ivo Kalajzic, Barbara Bottazzi & Danka Grcevic

see PP68.

DOI: 10.1530/boneabs.3.PP68

## OP32

**Enhancement of fracture repair by upregulation of the innate immune response**

Ana Isabel Espirito Santo, James K Chan, Graeme E Glass, Adel Ersek, Andrew Freidin, Garry A Williams, Kate Gowers, Rosemary Jeffery, William R Otto, Richard Poulsom, Marc Feldmann, Sara M Rankin, Nicole J Horwood & Jagdeep Nanchahal

see PP69.

DOI: 10.1530/boneabs.3.PP69

## OP33

**CXCL8 and CCL20 enhance osteoblast-mediated osteoclastogenesis**

Janak L Pathak, Astrid D Bakker, Patrick Verschueren, Willem F Lems, Frank P Luyten, Jenneke Klein-Nulend & Nathalie Bravenboer

see PP292.

DOI: 10.1530/boneabs.3.PP292

## OP34

**Telomerase promotes osteoblast differentiation by modulating IGF-signaling pathway**

Hamid Saeed, Weimin Qiu, Chen Li, Allan Flyvbjerg, Basem Abdallah & Moustapha Kassem

see PP149.

DOI: 10.1530/boneabs.3.PP149

## OP35

**PRKG1: A novel regulator of human skeletal (mesenchymal) stem cell differentiation**

Abbas Jafari, Majken Siersbaek, Matthias Dobbelstein & Moustapha Kassen

see PP150.

DOI: 10.1530/boneabs.3.PP150

## OP36

**The roles of CDC42 in bone development**

Jirong Wang, Ying Gong & Chengyun Xu

see PP70.

DOI: 10.1530/boneabs.3.PP70

## OP37

**Inhibition of bone remodeling by bisphosphonate displaces the plasma cell niche into the spleen**

Stefan Teufel, Bettina Grötsch, Julia Luther, Thorsten Schinke, Michael Amling, Georg Schett, Dirk Mielenz & Jean-Pierre David

see PP173.

DOI: 10.1530/boneabs.3.PP173

## OP38

**Syndecan-2 is a new negative modulator of Wnt signaling within bone marrow**

Rafik Mansouri, Valérie Geoffroy, Pierre Marie & Dominique Modrowski

see PP151.

DOI: 10.1530/boneabs.3.PP151

## OP39

**Inhibition of PDE5 decreases bone mass through inhibiting canonical Wnt signaling**

Gong Ying

see PP293.

DOI: 10.1530/boneabs.3.PP293

## OP40

**Protein malnutrition attenuates bone anabolic response to PTH in female rats**

Cedric Lavet, Giovanna Zacchetti, Jürg Andreas Gasser, René Rizzoli & Patrick Ammann

see PP361.

DOI: 10.1530/boneabs.3.PP361

# Poster Presentations

## Arthritis and other joint diseases: translational and clinical

### PP1

**Mutual associations among diseases causing disability, such as musculoskeletal diseases, metabolic syndrome components, and mild cognitive impairment: a 3-year follow-up of the ROAD study**

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To assess the associations among diseases causing disability requiring support, including i) musculoskeletal diseases (knee osteoarthritis (KOA), lumbar spondylosis (LS), and osteoporosis (OP)); ii) metabolic risk factors for cardiovascular diseases, including overweight (OW), hypertension (HT), dyslipidaemia (DL), and impaired glucose tolerance (IGT); and iii) mild cognitive impairment (MCI).
Among 1690 participants (596 men and 1094 women) at the baseline, 1384 individuals (81.9%; 466 men and 918 women) repeated the first follow-up in 2008 and completed blood, mini-mental state, and radiographic examinations, and measurement of bone mineral density. Logistic regression analysis was performed using occurrence or non-occurrence of the above-mentioned risk factors as the objective variable, after adjusting for confounders and presence or absence of the above-mentioned risk factors as explanatory variables.
Risk of KOA occurrence increased significantly by presence of HT, IGT, and MCI ($P=0.018$, 0.042, and 0.043 respectively). Occurrence of OP of the femoral neck was inversely associated with OW ($P=0.007$). OW was influenced significantly by presence of KOA and MCI ($P=0.001$ for both). Risk of HT occurrence increased by presence of KOA and OW ($P=0.030$ and 0.021 respectively). IGT occurrence was influenced significantly by presence of OW ($P=0.009$). In contrast, occurrence of LS or DL was not influenced by factors of metabolic syndrome or musculoskeletal diseases. In addition, occurrence of MCI was not influenced by any other factor.
We concluded that some musculoskeletal diseases, metabolic risk factors, and cognitive impairment were clarified to be associated with each other.

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### PP2

**Bone marrow lesions are characterized by increased bone turnover and increased vascularity**

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Bone marrow lesions (BMLs), previously denoted bone marrow edema are detected as water signals on MR scans (low intensity on $T_1$ weighted images and high intensity on $T_2$ weighted scans). Previous histologic studies were unable to demonstrate any edematous changes at the tissue level, which led us to hypothesize that the water signal stems from increased vascularization accompanying a high turnover state in bone. To test this hypothesis we performed tetracycline labeling and MR scans of 28 patients planned for total hip replacement surgery. Among 14 femoral heads, eight revealed BMLs on MR, while six were negative. The latter acted as controls in the analysis. On the femoral heads we took out cylindrical biopsies guided by the MR scans. These biopsies were stored in 70% alcohol prior embedding in methyl methacrylate. Subsequently 7 μm sections were prepared and subjected to histomorphometric analysis of the cancellous bone envelope using a histomorphometry software (BioQuant Osteo). We chose to focus on mineralizing surface (MS/BS) and vascular area (sinusoids + blood vessels), but also evaluated mineral apposition rate (MAR), bone formation rate (BFR/BS), bone volume (BV/TV) and trabecular thickness (Tb.Th).
The analysis revealed that compared to controls bone tissue from patients with BMLs exhibited increased bone turnover: MS/BS (median (95% CI)) 8.1% (3.41–33.29) vs 0.3% (0.06–1.64) ($P< 0.0007$) and increased vascular area 0.29 $mm^2$ (0.17–1.14) vs 0.03 $mm^2$ (0.004–0.16) ($P< 0.03$). None of the other variables of interest were different between the two groups.
In conclusion, this study confirms that bone marrow lesions are characterized by increased bone turnover and increased vascularity in keeping with it being a reparatory process. Thus, the water signal, which is the hallmark of BMLs on MR, is most probably reflecting increased tissue vascularity accompanying increased remodeling activity.

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### PP3

**Bone loss in patients with early axial spondyloarthritis**

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Objective
To examine bone mineral density (BMD) at the femoral neck (FN) and lumbar spine (LS) in patients (pts) with early axial spondyloarthritis (axSpA).
Methods
73 pts (33 men and 40 women) with early axSpA were included in this study. Pts fulfilled ASAS criteria. Pts enrolled in the study had inflammatory back pain for $<5$ years: mean disease duration $19.9\pm14.4$ months. Mean age $28.3\pm6.4$ years, mean BASDAI $4.1\pm1.9$; mean ASDAS-CRP $2.7\pm1.3$; 66 (90.4%) pts were HLA-B27 positive. Clinical data were collected and BMD was measured using dual energy X-ray absorptiometry (DXA) of the FN and LS (L2–4). Low BMD was defined as $Z$-score $\leq -2$ (at one site at least). High disease activity was assumed in cases where BASDAI $\geq 4$, ASDAS-CRP $\geq 2.1$.
Results
In all 73 pts mean $Z$-score was $-0.9\pm0.75$ for the FN and $-0.9\pm0.95$ for the LS. Low BMD (at one site at least) was found in 13 (17.8%) pts. 11 (15.1%) pts had BMD reduction of the LS, five (6.8%) pts had BMD reduction of the FN, bone loss in both LS and FN was found in three (4.1%) pts. There has been found an association between low BMD (at one site at least) and disease duration. There was a statistically significant difference ($P=0.04$) between the two groups of pts (disease duration $18.3\pm14.2$ and $27.4\pm13.7$ months). No association has been found between low BMD and age, gender, high disease activity (BASDAI, ADSAS), ESR, CRP.
Conclusions
17.8% of young adults with early axSpA have low BMD. Bone loss was found to be associated with disease duration. This emphasizes the need for early intervention in axSpA.

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### PP4

**Phenotypic and functional characterization of osteoclast progenitors in circulatory and synovial compartments of patients with rheumatoid arthritis**

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Rheumatoid arthritis (RA) is marked by the persistent inflammation and osteodestruction, causing progressive disability. Osteoclast progenitors (OCPs) represent a subpopulation of peripheral blood monocytes and have been shown to be present in healthy subjects and arthritic patients. The aim of our study was to determine the phenotype, frequency and osteoclastogenic potential of OCPs in the peripheral blood and synovial fluid of RA patients in response to anti-TNF therapy.
Mononuclear cells were isolated from peripheral blood of healthy controls and RA patients, after obtaining approval from the Ethical Committee and informed consent from patients. In addition, peripheral blood and synovial fluid samples were collected from RA patients prior and in the follow-up of TNF-blockage treatment. The phenotype of monocyte subpopulations was determined within mononuclear cells by flow cytometry using the following markers: CD3, CD11b, CD11c, CD14, CD16, CD19, CD56, CD115 and selected chemokine receptors. Monocyte subpopulations were then sorted and cultured with the addition of M-CSF and RANKL to induce osteoclast differentiation. Results were correlated with clinical data, including inflammatory indicators and variables describing disease activity and severity.
We have verified that human peripheral blood osteoclastogenic population (expressing CD11b, CD14, CD115, CCR2) is significantly increased in RA, comprising three-times more (3–6%) of circulating mononuclear cells compared with healthy controls. The same population was found among synovial fluid monocytes. Both circulatory and synovial monocyte progenitors exhibit *in vitro* osteoclastogenic potential. Anti TNF-treatment was not able to significantly decrease the proportion of peripheral OCPs, and only transiently suppressed their differentiation potential in osteoclastogenic cultures.
We concluded that the peripheral blood osteoclastogenic monocyte population has been specifically induced in RA, and possibly attracted to the synovial compartment. Anti-TNF treatment only transiently suppressed osteoclastogenic

potential of peripheral OCPs, indicating that additional therapeutic modalities, besides TNF-blocking agents, should be considered for sustained antiresorptive effect.

DOI: 10.1530/boneabs.3.PP4

## PP5

**Regional differences in microstructural and mechanical properties of the distal femur in health and osteoarthritis**

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Purpose
The purpose of this study is to analyse regional differences in the microstructural and mechanical properties of the distal femur depending on osteoarthritic changes using micro-images based on finite element analysis.
Materials and methods
Distal femur specimens were obtained from ten donors composed of ten women with OA (mean age of 65 years, ranging from 53 to 79). As controls, the normal distal femur was sampled from age and gender matched donors consisting of ten women (mean age of 67 years, ranging from 58 to 81). The areas of interest were six regions of the condyles of the femur (lateral-anterior, middle, posterior; medial-anterior, middle, posterior). A total of 20 specimens were scanned using the micro-CT system. Micro-CT images were converted to micro-finite element model using the mesh technique, and micro-finite element analysis was then performed for assessment of the mechanical properties.
Results
Trabecular bones from the distal femur in control and OA groups exhibited different microstructural and mechanical properties in the same region. BV/TV, Tb.N, Tb.S and Yield strength were different between LA and MM significantly ($P=0.005$). In control group, the lateral anterior region of the distal femur reflected subchondral trabecular remodeling, while in advanced OA group, the medial middle region showed prominent changes in the microstructural and mechanical properties.
Conclusion
The authors concluded that with aging and the progress of primary OA, changes of patello-femoral reaction force induced subchondral trabecular changes of the anterolateral region initially, and then progressed to the medial middle and posterior region in advanced OA.

DOI: 10.1530/boneabs.3.PP5

## PP6

**MIV-711, a highly selective cathepsin K inhibitor: safety, pharmacokinetics and pharmacodynamics of multiple oral doses in healthy postmenopausal women**

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Introduction
Cathepsin K is necessary for bone matrix resorption. Excessive resorption associated with osteoporosis and osteoarthritis can be reduced by cathepsin K inhibition. MIV-711, a highly selective cathepsin K inhibitor, successfully attenuates both bone resorption and cartilage degradation in non-clinical studies.
Aim
To determine safety, tolerability, pharmacokinetics and pharmacodynamics of MIV-711 during multiple oral dosing to postmenopausal women.
Methods
This was a double-blind, placebo-controlled, randomized study in 12 healthy postmenopausal females (eight on active drug and four on placebo). A dose of 100 mg MIV-711 or placebo was administered once daily for 28 days and investigated for adverse events, clinical chemistry and haematology, vital signs, ECG parameters, and biomarkers. Data are expressed as means $\pm$ S.E.M.
Results
Compared with placebo there was a statistically significant sustained reduction in serum CTX-I levels, a biomarker of bone resorption, with day 28 trough levels $67\pm3\%$ lower than baseline. Urinary excretion of bone resorption markers CTX-I and NTX-I were significantly reduced with day 28 levels $98\pm1$ and $76\pm1\%$ lower than baseline, respectively. Urinary excretion of CTX-II, a biomarker of cartilage degradation, was $55\pm9\%$ lower than baseline on day 28. MIV-711 had minimal effects on biomarkers of bone formation. Other exploratory biomarkers were also measured. Pharmacokinetic results agreed with previous data in healthy volunteers. MIV-711 was well tolerated with no safety concerns. Adverse events included skin reactions at ECG electrode sites, headache and gastrointestinal symptoms with comparable incidence after active drug or placebo.
Conclusions
MIV-711 effectively suppresses serum levels of CTX-I and urinary excretion of CTX-I, NTX-I and CTX-II. MIV-711 once daily for 28 days was safe and well tolerated in healthy postmenopausal females. These results support further development of MIV-711 for bone and cartilage related disorders such as osteoarthritis with a potential clinical dose of MIV-711 between 50 and 100 mg once daily.

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## PP7

**Do osteophytes protect femoral neck against fracture in osteoarthritis?**

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Beside the bone mass, structural changes are important determinants of the bone strength. Patients with osteoarthritis (OA) seem protected against femoral neck (FN) fracture. When compared to osteoporosis, FN in OA are characterized by a higher bone mass and a better trabecular microarchitecture (Blain *et al*., 2008, Boutroy *et al*., 2011). The presence of microcracks is one of the determinants of the bone strength. The aim of this study was to evaluate the microcracks density (Cr.N/BV) and length (Cr.Le) in the FN in hip OA and its relation with microarchitecture. Osteophyte being common features in OA, the presence of microcracks was separately analyzed in the osteophyte area. FN samples were obtained during arthroplasty for hip OA in 18 postmenopausal women. Bulk-staining of the specimens was performed with calceïn. 3D measurements of the bone mass and microarchitectrure were assessed by HR-pQCT (Xtreme CT, Scanco Medical). Osteophytes were present in all samples, but microcracks were observed in only four. Cr.N/BV was significantly higher in trabecular than cortical bone ($0.020\pm0.010$ vs $0.005\pm0.008$ /mm$^2$ respectively, $P<0.001$) but the mean length tended to be higher in cortical than cancellous bone ($103.0\pm61.9$ vs $60.5\pm28.2$ µm respectively, $P=0.07$). Cr.N/BV was significantly lower in osteophytes than in the other part of FN ($0.01\pm0.016$ vs $0.019\pm0.015$ respectively, $P<0.04$). The bone mass and microarchitecture of the osteophytes were significantly different than the other parts of FN ($P<0.001$ for BV/TV, Tb.N, Tb.Sp). However, no correlation was found between Cr.N/BV and parameters of microarchitecture. In conclusion, these results showed that in FN from hip OA, the microcracks formation tended to be lower in the osteophytes but was not related to the microarchitecture. These results suggest that osteophytes may limit the microcracks formation.

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## PP8

**Comparison of trabecular bone composition between osteoarthritis and non-osteoarthritis of distal femur by Raman spectroscopy**

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Objectives
The purpose of this study is to determine the chemical information in trabecular bone by Raman spectroscopy in the osteoarthritic and non-osteoarthritic distal femur.
Materials and methods
Distal femur specimens were obtained from ten donors composed of ten womens with OA (mean age of 63 years, ranging from 50 to 76). As controls, the normal distal femur was sampled from age and gender matched donors consisting of ten womens (mean age of 53 years, ranging from 58 to 79). The subchondral trabeculae were obtained from the middle of medial articular surface of distal femurs. Cored trabecular bone specimens were analyzed Raman spectroscopy.
SERS spectra were recorded with a SENTERRA confocal Raman system (Bruker Optics, Inc., Billerica, MA, USA). A 785 nm diode laser with 10 mW power at the laser source was used for excitation. The diameter of the focal spot was $\sim$ 2.4 µm with a 20$\times$ objective lens (N. A. 0.4). Spectra were recorded over the range of 150 1800 per cm with spectral resolution of 3 per cm and integration time of 30s. Baseline correction was performed by the rubber-band method.

Results
Wavenumber showed that a similar trend in the OA and non-OA. But, There was significant difference in Raman intensity (phosphate, hydroxyproline, proline, carbonate, amide I and III, CH2 between two groups.
Conclusion
The result suggested that OA may affects the chemical compositions of trabecular bone. The Raman spectroscopy is useful for analysis of the chemical information of trabecular bone. Information of chemical composition may be helpful in the diagnosis and treatment of bone diseases.

DOI: 10.1530/boneabs.3.PP8

## PP9

### The characteristics of bone mineral density, erosive and destructive changes in joints of hands and feet, vertebral deformities in patients with rheumatoid arthritis

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There is discussed an interrelation of BMD, progressing of erosive and destructive changes in hands and feet with development of vertebral deformations in rheumatoid arthritis (RA).
The aim
To receive data on bone mineral density (BMD), erosive and destructive changes in hands and feet and vertebral deformations in patients with RA.
Materials/methods
In this research, it was included 106 women, with RA, age 23–69 years. In all patients BMD was assessed by dual energy X-ray absorptiometry (DXA) on one Hologic «DiscoveryA» at three sites (lumbar spine (L1–L4), hip neck (HN) and distal part of forearm (DF) of nondominant hand) and was performed an X-ray morphometric analysis of vertebral bodies deformations with Genant method. The assessment of radiological signs of RA progressing was used with Sharp/van der Heijde method in 67 years. The Statistica 6.0 was used in the statistic analysis.
Results
The mean age of patients included in this research was $52.2 \pm 10.3$ years, the mean age at the time of the beginning of RA was $36.9 \pm 12.1$ of years, the mean duration of RA was $13.3 \pm 11.2$ years. The positive rheumatoid factor was in 77 (73%) patients. 50 (47%) patients received glucocorticoids (GC). The index of erosions amount was $38.5 \pm 51.2$ points, the amount of narrowed cracks $88.6 \pm 41.1$ points, the total Sharp/van der Heijde score was $126.5 \pm 84.9$ points. Mean BMD at L1–L4 was $0.911 \pm 0.147$ g/cm$^2$, at HN – $0.705 \pm 0.117$ g/cm$^2$, at DF – $0.463 \pm 0.09$ g/cm$^2$. Osteoporosis (OP) at L1–L4 was revealed in 14 (15%), at HN in 10 (11%), at DF – in 32 (35%) patients. Osteopenia (Op) at L1–L4 was in 45 (48%), at HN – in 48 (53%), at DF – in 36 (39%) patients. Normal BMD was revealed at L1–L4 in 35 (37%), at HN – in 33 (36%), at DF – in 24 (26%) patients. The vertebral deformations were revealed in 24 (22%) patients. The index of vertebral deformations at thoracic site of spine was $0.77 \pm 0.08$, at lumbar site – $0.79 \pm 0.06$.
Conclusions
Despite the young age, patients included in research had a long duration of disease. OP at least in one department of a skeleton was revealed at a third of patients, vertebral deformations – at every fifth.

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## PP10

### The influence of the 24-month treatment with anti-CD20 antibodies (rituximab) on bone mineral density in patients with rheumatoid arthritis

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Background
The activity of rheumatoid arthritis (RA) is the one of the significant reasons of the increase of bone resorption and decrease of bone mineral density (BMD). The control of activity of inflammatory process with biologics (including rituximab – anti-CD20 antibodies) can be considered as possibility of decrease in bone resorption and BMD stabilization.
Objectives
To access the dynamics of BMD after 24 months of treatment with rituximab in RA patients RA.
Methods
Retrospective study of a cohort of RA patients (33 women and four men), mean (s.d.) age at the beginning of rituximab treatment $48.8 \pm 14.2$ years, mean duration of disease $9.1 \pm 7.1$ years. The high activity of RA (DAS-28) was in 34 (92%), moderate – 3 (8%) patients. BMD was assessed in all patients at the beginning of therapy with rituximab and 24 months after by dual energy X-ray absorptiometry (DXA) on one Hologic «DiscoveryA» at least in one of following sites: lumbar spine(L1-L4) ($n=26$), hip neck ($n=19$) and left forearm ($n=7$).
Results
Mean BMD L1-L4 before\after the treatment with rituximab was $0.947 \pm 0.164$ and $0.971 \pm 0.133$ g/cm$^2$, at hip neck $0.759 \pm 0.110$ and $0.725 \pm 0.119$ g/cm$^2$, at forearm $0.493 \pm 0.084$ and $0.449 \pm 0.090$ g/cm$^2$ respectively. Distinctions are not revealed. The analysis of BMD loss/increase in 24 months showed that the increase of BMD was occurred respectively: at L1-L4 in 15 patients ($0.947 \pm 0.164$ and $0.971 \pm 0.133$ g/cm$^2$), at hip neck – in eight ($0.765 \pm 0.098$ and $0.787 \pm 0.096$ g/cm$^2$). The loss of BMD respectively at L1-L4 was occurred in 11 patients ($1.027 \pm 0.150$ and $0.986 \pm 0.141$ g/cm$^2$), at hip neck – in 11 patients ($0.755 \pm 0.122$ and $0.680 \pm 0.117$ g/cm$^2$), at forearm in seven patients ($0.493 \pm 0.084$ and $0.449 \pm 0.090$ g/cm$^2$) – with not significant results.
Conclusions
It was obtained no significant evidence of BMD loss or increase in patients with high activity of RA, taking rituximab within 24 months, nevertheless the BMD remained stable. Studying of a contribution of other factors, which influence on BMD change, requires more careful analysis.

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## PP11

### Multinucleated giant cells in synovia from people with rheumatoid arthritis or osteoarthritis

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Aims
To describe different multinucleated giant cell phenotypes and their associations with synovitis in people with rheumatoid arthritis (RA) or osteoarthritis (OA), and in an experimental model of chronic arthritis.
Material and methods
A cross-sectional comparative study was performed on samples of knee synovia from age-matched patients with RA, OA, or non-arthritic post mortem (PM) controls. OA synovia were stratified by histological inflammation grade using index tissue sections. Synovia were also obtained from rabbits with antigen-induced arthritis. Synovitis was assessed by Krenn score. Histological studies employed specific antibodies against macrophage markers or cathepsin K, or TRAP enzymatic assays.
Results
More RA and inflamed OA synovia displayed multinucleated giant cells than did non-inflamed OA and PM synovia. Positive associations were found between synovitis and synovial MGC density, both in RA and OA ($r=0.580$, $P=0.018$; $r=0.603$, 0.038 respectively). TRAP negative/cathepsin K negative Langhans-like and TRAP positive/cathepsin K negative foreign body-like were the predominant giant cell phenotype in RA, whereas Langhans-like was the commonest multinucleated giant cell phenotype in OA. Giant cells were localized near fibrin deposits, engulfing hemosiderin and tissue debris. TRAP positive/cathepsin K positive osteoclast-like cells were only identified close to bone surfaces. Touton-like giant cells surrounded adipocyte structures. Findings from rabbits with chronic arthritis reflected those observed in RA human samples.
Conclusions
Multinucleated giant cells are associated with synovitis both in OA and in RA. Further research targeting multinucleated giant cells is required to elucidate their discrete contributions to RA and OA pathology and symptoms.

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## PP12

### Cartilage oligomeric matrix protein assay on the IDS-iSYS automated system

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Diseases affecting the musculoskeletal system are a major cost burden to society. Articular cartilage loss or damage in these diseases is detected by radiography and measuring decreases in joint space width (JSW). The early stages of the disease may remain latent and asymptomatic for many years. This forms the rationale for applying molecular marker technology (biomarkers) to identify patients prone to joint diseases. One such biomarker is cartilage oligomeric matrix protein (COMP, thrombospondin five). COMP can be used to monitor the progress of cartilage degradation in inflammatory joint diseases such as rheumatoid arthritis (RA) and osteoarthritis (OA). Clinical studies have shown that measurements of serum COMP can be used a valuable tool for identifying patients at high risk from rapid joint destruction and for monitoring treatment efficacy.
We report the results of an automated COMP assay on the IDS-iSYS.The COMP assay is based on the direct sandwich technique in which two specific monoclonal antibodies are directed against separate antigenic determinants on the COMP molecule.
Below are the preliminary analytical performance of the IDS-iSYS COMP assay.

| Performances | Results |
|---|---|
| Inter-assay precision | Five samples tested over 12 days gave %CV's between 4 and 9% on dose at concentrations between 10 and 46 μg/ml. |
| Sensitivity | Limit of blank: 0.011 μg/ml; limit of detection: 0.027 μg/ml; limit of quantitation: $<0.0625$ μg/ml |
| Linearity | Variation being within $\pm 10\%$ |
| Recovery | Eight tests gave a range of 102–112% |
| High dose hook effect | No hook effect observed up to 100 μg/ml |
| Method comparison against AnaMar COMP kit | iSYS COMP $=1.425\times$(AnaMar) $-0.36$ μg/ml $R^2=0.64$ |

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## PP13

### RANK expression is reduced in circulating monocytes from ankylosing spondylitis patients

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Rheumatoid arthritis (RA) and ankylosing spondylitis (AS) are systemic, immune-mediated diseases. RA's main targets are the peripheral joints while AS has the axial skeleton and enthesis as the principal affected areas. RA is characterized by bone erosions and impaired repair whilst AS is typified by bone overgrowth. The causes for these differences are not yet understood; however we hypothesize that AS patients' monocytes receive reduced osteoclastogenic stimuli and/or have abnormal capacity to respond to them.
The aim of this study was to characterize AS patients' bone remodeling and pro osteoclastogenesis inflammatory environment and monocyte phenotypes as compared to RA patients and healthy controls.
Untreated AS or RA patients with active disease and age and gender-matched healthy donors were recruited for this study. Blood was collected for flow cytometry measurements of RANKL expression and monocyte subpopulation characterization. Serum was collected for cytokine and bone remodeling factors quantification. This study was approved by the hospital's ethics committee.
We found that RANKL lymphocyte expression was higher in AS and RA patients when compared to healthy donors. However, both in classical and intermediate monocyte subpopulations RANK expression was lower in AS patients as compared to RA patients and healthy controls. In accordance, CTX-I serum level was also lower in AS patients than in healthy controls.
Conclusion
Despite comparable osteoclastogenic stimuli in AS and RA patients, RANK expression is reduced in AS circulating monocytes which may contribute to the bone forming phenotype observed in AS patients.

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## PP14

### Effect of risedronate on painful periprosthetic resorption of total hip arthroplasty: preliminary observational study

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Total hip arthroplasty (THA) is the treatment for severe hip osteoarthritis. It improves the quality of life, pain and patient autonomy. However, a periprosthetic resorption may occur, in 3–5% of patients after 10 years of the primary surgery requiring implant replacement. Bisphosphonates (BP), inhibitors of bone resorption, represent a potential candidate for modulating periprosthetic bone loss. Randomized controlled trials have also suggested that BP could prevent early periprosthetic bone loss after THA.
The aim of our study was to investigate the effect of a BP, risedronate on painful periprosthetic resorption in patients with THA, mainly on pain, radiographic bone loss, and periprosthetic density.
A monocentric, longitudinal, observational study, cohort type, included patients with painful aseptic loosening of their THA. All patients received Risedronate 75 mg, twice a month, during 2 years. Patients with mobility implant were excluded.
A 22 patients were included, mean age: 68 years. The mean period of first surgery of THA was 9.9 years. At the inclusion, the mean walking pain, measured with VAS, was 5.02. A statistically significant decrease of walking pain was observed 18 months after treatment (mean VAS: 2.5). The walking distance has increase significatively by 1.25 km after 18 months. Concerning pain at rest, risédronate appears to provide non significatively improvements During the study, eight patients required surgical replacement of their THA.
The analysis of X-rays showed a non significative decrease of periprosthetic bone resorption, predominant peripheral acetabular area, and in proximal femoral area, like increase periprosthetic density.
In this observational study, in patients with painful periprosthetic resorption of THA, risedronate seems to have an effect on pain, with a significant decrease of walking pain, and a significant increase of walking distance. Only 36% needed replacement of prosthetic implant. A randomized study will be conducted to confirm these results.

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## PP15

### Bone secreted dickkopf-related protein 1 ameliorates osteoarthritis in mice

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Objective
Cartilage loss and subchondral bone changes are hallmarks of osteoarthritis (OA). The Wnt family is involved in the regulation of bone and cartilage. We have shown that the Wnt/β-catenin pathway is activated in bone during OA, but the effect of its inhibition in bone in cartilage remodeling is unknown. We here investigated the impact of the bone-specific inhibition of Wnt during the development of OA.
Methods
Partial meniscectomy (Mnx) was performed in mice to promote OA. We assessed the bone and cartilage parameters in OA development using mice overexpressing

Dkk1 in bone (2.3 Col1-Dkk1Tg). The effects of Dkk-1 in chondrocyte and osteoblast metabolism were further assessed using supernatant transfer and MMP expression.
Results
At baseline, Dkk1-Tg mice had lower bone volume which was further reduced in MNX knees. This was accompanied by a reduction of the subchondral bone and osteophyte volume. In WT mice, the number of Dkk1 (+) chondrocytes was high at baseline (84.2±3.1%), decreased markedly during the course of OA from week 4 (14.4±3.8%) to week 6 (5.7±1.6%). Dkk1-Tg experienced a lower OA score than WT mice (5.1±0.63 vs 8.4±0.6, $P=0.002$) independently of the expression of Dkk1 in chondrocytes. However, addition of supernatant of osteoblasts derived from Dkk-1-Tg mice or *in vitro* addition of rhDkk1 in chondrocytes promoted the chondrocytic expression of MMPs while was decreased by the supernatant of pre-exposed osteoblasts with Dkk1. Because Dkk1-Tg osteoblasts produced low VEGF, we tested whether VEGF could mediate the anti-catabolic effect observed *in vivo*. Blocking VEGF in the supernatant of osteoblast cultures reversed the expression of MMPs by chondrocytes.
Conclusion
Inhibition of Wnt pathway in bone cells decreased OA severity by reducing VEGF production. Targeting bone could be a useful approach for the treatment of OA.

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## Bone biomechanics and quality

### PP16

**Inhibition of RANKL-mediated bone remodeling decreases bone damage and improves strength in response to fatigue loading**

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Antiresorptives consistently improve bone mass and structural strength in normally- and under-loaded bones, but concerns have been raised regarding potential effects on skeletal adaptation to fatigue loading, including damage accumulation and atypical fractures. We thus inhibited or activated osteoclasts with OPG-Fc or RANKL treatment, respectively, and evaluated bone damage and strength after fatigue in the early and later phases of repair. Adult male mice were treated with RANKL (2 mg/kg/day), OPG-Fc (5 mg/kg 2/wk) or PBS (Veh). Axial compression (14 N, 3 Hz, 20 min) was applied to the tibia two days after initiating OPG-Fc or RANKL, with treatment continuing until sacrifice 1 or 28 days later ($n=6$ per group). We assessed cortical damage, bone mass, microarchitecture, and axial compressive strength. RANKL and OPG induced the expected changes, with bone resorption (serum TRAP5b) rapidly increasing and decreasing respectively, resulting in lower and higher bone mass/structure/strength in non-fatigued tibiae after 28 days. Fatigue rapidly increased cracks and resulted in a reactive callus that was present at day 28. OPG and RANKL did not affect crack accumulation one day after fatigue nor periosteal BFR 28 days after fatigue. However after 28 days, OPG decreased crack density and increased bone mass and strength, while RANKL had the opposite effect. Regression analysis across all groups indicate that total aBMD, proximal TbBV/TV and midshaft CtBV were positively associated with peak load ($r^2=0.67$-$0.76$, all $P<0.0001$). In summary, OPG-Fc decreased cracks, likely due to increased bone volume and improved strength. Therefore, in this murine model of stress-induced damage, RANKL inhibition did not cause bone fragility or damage accumulation.

| 2d Tx | CtBV ($mm^3$) | | | CrN/BA ($1/mm^2$) | | |
|---|---|---|---|---|---|---|
| *$P<0.05$ vs Veh | Veh | OPG | RANKL | Veh | OPG | RANKL |
| +one day post-F | 0.42±0.01 | 0.41±0.01 | 0.42±0.01 | 140±8 | 138±8 | 130±6 |
| +28 day post-F | 0.44±0.01 | 0.49±0.01* | 0.35±0.01* | 158±19 | 78±17* | 265±26* |

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### PP17

**Bone fragility and matrix hypermineralization is rescued in homozygous OI Brtl mice mutants**

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Classical osteogenesis imperfecta (OI) is caused by mutations in the two genes encoding type I collagen. OI is associated with low bone mass and abnormally high bone matrix mineralization. The Brtl/+ OI mouse is a knock-in model caused by a glycine substitution in one *COL1A1* allele. Brtl/+ pups display 30% perinatal lethality; survivors have small size and brittle bone. Unexpectedly, homozygous Brtl/Brtl pups, producing only mutant collagen, have normal survival rates and a rescued phenotype with normal bone fragility. We investigated whether the rescued bone fragility is reflected in bone matrix mineral content.
To further examine the roles of matrix homogeneity vs insufficiency in matrix mineralization, we crossed Brtl/+ mice with Mov13, a murine model with a null *COL1A1* allele, to obtain Brtl/Mov compounds. To determine bone phenotype, we obtained cortical cross sectional area, whole bone four point bending data and bone mineralization density distributions (BMDD) by quantitative backscattered electron imaging, using 2-month-old heterozygous Brtl/+, homozygous Brtl/Brtl, Mov13 and Brtl/Mov mutants ($n=8$–12 per group).
Cross-sectional area and ultimate load were significantly lower in Brtl/+, similar in Brtl/Brtl, and significantly higher in Mov/+ and Brtl/Mov, compared to WT. Analysis of bone matrix mineralization showed that mean (CaMean) and most frequent (CaPeak) calcium concentrations were similar in Brtl/Brtl and WT but significantly higher in all other groups compared, indicating an OI phenotype of the bone material in Brtl/+, Mov/+ and Brtl/Mov mutants while in Brtl/Brtl bone matrix mineralization was normalized.
These results indicate that in Brtl/Brtl mice bone mechanical properties and hypermineralization of the matrix are rescued by matrix homogeneity with mutant collagen, while Brtl/Mov mutants have increased bone strength due to increased cross-sectional area compared to WT. However, the hypermineralization associated with severe matrix insufficiency is not normalized despite the bone size adaptation.

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### PP18

**Influence of PTH treatment on the bone tissue mechanics of rats with type 2 diabetes mellitus using mechanical tests and finite element modelling**

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Diabetes mellitus results in increased skeletal fragility through reduced bone mineral density and altered collagen structure. How these changes affect bone mechanics at the tissue level remains largely unclear. Anti-osteoporosis medications improve bone mass, but whether they can fully restore tissue strength in diabetic bone, where collagen quality is compromised, has not been fully elucidated. The objective of this study was to determine the effect of type 2 diabetes mellitus and bone-anabolic treatment on bone tissue mechanics in Zucker diabetic fatty (ZDF) rats. We hypothesized that diabetic rats have inferior tissue mechanics, which cannot be fully restored with anabolic treatment due to diabetic changes in the organic phase of the bone.
Ten-week old ZDF diabetic and non-diabetic rats were given 75 µg/kg PTH(1–84) or vehicle 5 days/week over 12 weeks (four groups, $n=7$ per group). Right femora and $L_4$ vertebrae were excised, micro-CT scanned, and tested to failure in three points bending and compression, respectively. The force-displacement data were converted into stress-strain using the micro-CT geometry of the specimen cross-sections. In a second approach using linear finite element models of the vertebrae, failure was set to occur when 2% of the elements reached a critical value of stress or strain. Ultimate stress and strain were determined through back-calculation by setting the failure load to that of the mechanical test.
Diabetic rats had significantly lower femoral mid-shaft yield stress (−36%), and significantly lower vertebral yield and ultimate strain (−17 and −22%

respectively), and modulus of resilience and toughness (−34 and −46% respectively). After accounting for tissue mineral density, all differences at the vertebra remained significant ($P<0.05$). PTH treatment increased vertebral ultimate strain (+19%) but not stress, and had no effect at the femur.
Diabetes reduced bone tissue strength and toughness in ZDF rats, independent of mineral density. PTH treatment resulted in limited improvement at the vertebra and none at the femur mid-shaft. These data support the hypothesis that type 2 diabetes mellitus reduces bone tissue mechanics through both collagen and mineral alterations, which cannot be fully restored with bone anabolic agents.

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## PP19

**Scanning acoustic microscopy reveals heterogeneity of mechanical properties due to collagen orientation in mice cortical bone**

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The local mechanical properties of bone are influenced not only by the material chemical composition but also by the spatial arrangement of the component's e.g. orientation of collagen matrix. However, not much is known about local elastic modulus variations in cortical bone. Our goal was to use acoustic imaging to map elastic properties of murine bone with a several microns resolution. Rodent long bones exhibit a permanent growth with endosteal/periosteal bone formation and early bone formation residual may remain.
The local microstructure was characterized with a new scanning acoustic microscopy method (SAM-TOF) using tibia from normal mice. Time-of-flight differences of ultrasound pulses across thin transversal cortical sections with known thickness (∼30 microns) were determined with 0.125 ns time resolution to obtain sound velocities maps (2 µm pixel resolution) using a 330 MHz lens (kibero Gmbh). Velocity maps were combined with density maps derived from calcium content obtained by quantitative backscattered electron imaging to extract dynamic elastic moduli maps. Based on polarized light microscopy, we distinguished bone areas of different collagen fibril arrangement/orientation: bone with predominantly longitudinal collagen orientation (dark) (LB), with transverse collagen orientation (bright)(TB) and poorly ordered bone (PB) found as an asymmetrically band in the middle of the cortical cross-section.
The mean material density did not differ significantly between the three areas. However the velocity was found significantly lower in TB (−14.5%) and PB (−12.4%) compared to LB (4343 m/s). The elastic modulus was found lower in TB (−26%) and PB (−22%) compared to LB (33 GPa). No difference between TB and PB was found. No correlation was found between density and velocity ($r^2=0.074$) or elastic modulus ($r^2=0.055$).
These results show that TOF scanning acoustic microscopy reveals elastic moduli depending on collagen orientation and mineral content and emphasizes the importance of collagen orientation in determining the local mechanical properties of bone.

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## PP20

**Subchondral bone sclerosis in the DMM model of murine OA is not associated with changes in either BMD or nanomechanical properties**

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It has long been known that osteoarthritis induces dramatic structural changes in subchondral bone. Studies on patients' biopsies have also shown that the new sclerotic bone is hypomineralized and has decreased elastic modulus. Due to their amenability for genetic studies, murine models of OA are particularly important for drug target discovery. However, whilst osteosclerosis has been reported also in murine OA models, little is known about the compositional and mechanical properties of mouse subchondral bone (SCB). In this study we assessed microstructure, mineralization and nanomechanical properties of SCB in the surgical destabilization of medial meniscus (DMM) model of OA.
Six groups of C57BL/6 mice ($n=6$) underwent DMM surgery and were euthanized 1, 2, 4, 8, 12 and 20 weeks post-surgery. Tibiae were imaged in a BMD calibrated microCT scanner (Skyscan 1172), embedded in methyl methacrylate blocks and cut in half coronally. The surface of the block halves was polished and subjected to nanoindentation test (TI700 UBI indenter, Hysitron, MN, USA) using a Berkovich probe. Nanomechanical properties were extracted from load-displacement curves using Oliver-Pharr method.
Structural analysis by microCT (5 µm/pixel resolution) revealed SCB plate sclerosis and trabecular bone remodelling from 2 weeks post-surgery in the medial condyle of the DMM-operated tibiae and no changes in the lateral condyle. However, volumetric BMD was unaltered in both SCB compartments. Initial nanoindentation data (on tibiae from 4 weeks post-DMM) showed no changes in either bone elastic modulus or hardness.
This suggests the existence of a different compositional and nanomechanical environment in mouse SCB compared to human SCB with the progression of OA. A different SCB environment might explain some of the differences in OA pathogenesis in the mouse compared to human and should be further investigated in order to address one potential limitation of the murine DMM model of OA.

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## PP21

**Are the high hip fracture rates among norwegian women explained by impaired bone material properties?**

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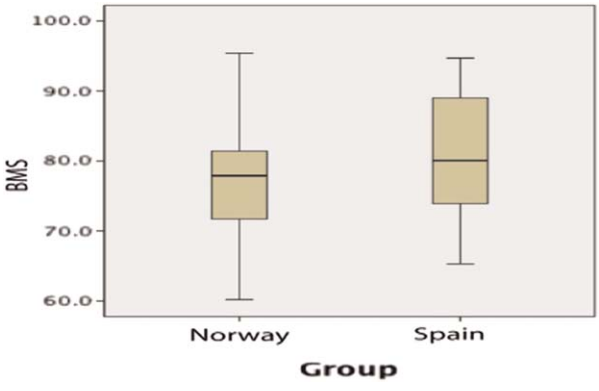
Hip fracture rate in Norway is the highest registered in World, and more than double that of Spanish women. Previous studies were unable to demonstrate significant differences between the two populations with respect to bone mass or calcium metabolism. In order to test, whether the difference in fracture propensity between both populations could be explained by differences in bone material quality we assessed bone material strength using microindentation in 41 Norwegian and 46 Spanish women with normal BMD-values ($T$-score $>-1$ and $<+2,0$), without clinical or morphometric vertebral fractures, no clinical or laboratory signs of secondary osteoporosis and without use of drugs with known influence on bone metabolism. Bone material properties were assessed by microindentation of the thick cortex of the mid tibia following local anesthesia of the area using the Osteoprobe® device (Active Life Scientific, Sta Barbara, CA). Indentation distance was standardized against a calibration phantom of methylmethacrylate and results, as percentage of this reference value, expressed as BMS (Bone Material Strength) units.
We found that the bone material properties reflected in the BMS value of Norwegian women was significantly inferior when compared to Spanish women (77.0±7.1% vs 80.7±7.8%, $P=0,02$). Total hip BMD was significantly higher in Norwegian women (1.218 g/cm$^2$ vs 0.938 g/cm$^2$ $P=<0.001$, but regression analysis revealed that indentation values did not vary with BMD ($r=0.03$, $P=0.12$) or age ($r=0.04$, $P=0.42$).
In conclusion Norwegian women show impaired bone material properties, when compared to Spanish women. This is the first demonstration of ethnical differences in bone material properties and could partly explain the much higher propensity for fracture in Norwegian women than in Spanish women.

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## PP22

### Bone matrix mineralization is preserved during perimenopausal stage in healthy women

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Menopause is accompanied by a decrease in bone mineral density which can be caused by a reduction in bone volume and/or degree of bone matrix mineralization. Both of them are suggested to contribute to the increased fracture risk in postmenopausal individuals. In the present work, we aimed for information whether a drop in bone matrix mineralization is occurring in the perimenopausal stage of women. For this purpose, we measured the bone mineralization density distribution (BMDD) by quantitative backscatter electron imaging (qBEI) in $n=17$ paired transiliac bone biopsy samples premenopausal baseline and 12 months after last menses (taken at the average ages of $49\pm2$ and $55\pm2$ years) from healthy perimenopausal women. Our study participants were a subgroup of a larger study where significant perimenopausal increases of about 80% in both activation frequency and bone formation rate have been reported.
In the current study, we found that none of the BMDD parameters of the postmenopausal BMDDs were significantly different compared to those of the premenopausal ones (signed rank tests, all $P>0.05$). Moreover, the average calcium concentration of cancellous bone was found to be in the normal reference range before as well as after menopause (22.11 (21.88; 22.75) wt%Ca vs 22.09 (21.79; 22.60) wt%Ca, median (25th, 75th percentiles) respectively, $P=0.255$). Our findings surprisingly revealed that the rise in histomorphometric bone turnover indices was not accompanied by changes in bone matrix mineralization. This might indicate that the substantial increase in bone turnover took place during the last months before the second biopsy (in line with the previously reported transmenopausal increases in osteocalcin) and the bone mineralization changes were lagging these bone turnover changes. We conclude that during the perimenopausal observation time BMDD measured by qBEI is preserved and does not contribute to decreases in bone mineral density.

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## PP23

### Influence of estrogen receptor and β-catenin signalling activation on mechanically induced bone formation in ovariectomized mice

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Dysfunctions of Wnt/β-catenin and estrogen receptor signalling resulted in impaired mechanotransduction and bone loss as in osteoporosis. Previous studies demonstrated the interaction of these pathways in mechanotransduction *in vitro* and *in vivo*. In this study, the influence of the activation of estrogen receptor and β-catenin signalling on mechanically induced bone formation was investigated in ovariectomized mice. 12-week-old mice were ovariectomized. 4 weeks later, an estrogen pellet was implanted and the right ulna was loaded for 2 weeks on five consecutive days. For β-catenin activation, the Gsk-3β inhibitor SB415286 was injected daily during the loading period. Endocortical and periosteal bone formation rates (EcBFR, PsBFR) were calculated and bone structure was analysed. Each treatment group included six mice. Data were analysed for significance (value $P<0.05$) using the Mann–Whitney $U$ test. All experimental procedures were approved by the National Ethics Committee. Loading induced bone formation. At the endocortical surface, estrogen induced bone formation and acted additively with loading. At the periosteal surface, both estrogen and SB415286 enhanced mechanically induced bone formation in ovariectomized mice. When estrogen and SB415286 were administered together, the sensitizing effect on mechanically induced bone formation of each activator alone was abolished in these mice.

| OVX | | Control | Estrogen | SB415286 | Estrogen + SB415286 |
|---|---|---|---|---|---|
| EcBFR | C | 0 | 1.2±0.3* | 0 | 1.2±0.5* |
| ($\mu m^3/\mu m^2$per day) | L | 1.1±0.4† | 2.4±0.3*† | 1.6±0.3*† | 2.3±0.5*† |
| PsBFR | C | 0.12±0.03 | 0.08±0.07 | 0.2±0.16* | 0.25±0.02* |
| ($\mu m^3/\mu m^2$per day) | L | 2.2±0.8† | 5.6±1.4*† | 6.6±1.8*† | 2.2±0.6† |

* $P<0.05$, treatment vs control group † $P<0.05$ loaded (L) vs unloaded (C) ulna.

The interaction of estrogen receptor and Wnt/β-catenin signalling might be one mechanism that is involved in regulating bone mass homeostasis in response to different loading conditions.

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## PP24

### Beneficial effects of a GIP mimetic on bone material properties

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Objectives
A role for glucose-dependent insulinotropic polypeptide (GIP) in controlling bone mass and strength has previously been reported. However, the rapid degradation of GIP in the bloodstream by the dipeptidyl peptidase-4 enzyme precludes therapeutic use. To circumvent this problem, a series of N-terminally modified GIP agonists have been developed. The aim of the present study was to investigate the effects of 28-day treatment with N-AcGIP on bone micro-architecture and strength in rats.
Materials and methods
Twelve copenhagen rats were randomly allocated to vehicle- or N-AcGIP-treated groups. All procedures were approved by the local animal care and use committee. Trabecular and cortical bone microarchitectures were studied by high resolution microCT whilst bone remodeling markers were assessed in plasma by ELISA. Intrinsic material properties were studied by nanoindentation in trabecular and cortical bone. Bone mineral and collagen properties were assessed by quantitative backscattered electron imaging and Fourier-transformed infrared microscopy. Non-parametric Mann–Whitney $U$ test was used to compare differences between groups.
Results
Compared to vehicle-treated animals, N-AcGIP treated rats did not exhibit modifications of trabecular or cortical microarchitecture. These results were further confirmed with no modification in the circulating levels of either CTx or osteocalcin between the two groups. Intrinsic material properties were improved only in the cortical bone of N-AcGIP-treated animals, with significant augmentations in maximum load (12%), hardness (14%), indentation modulus (13%) and dissipated energy (16%). Furthermore, the mineralization degree of the bone matrix was increased only in cortical bone with significant augmentation (9%) in Ca-peak and Ca-mean. Collagen maturity was also modified in cortical bone matrix with an augmentation of 13%.
Conclusions
Overall, 4 weeks treatment with the GIP mimetic (N-AcGIP) led to improved bone material properties in cortical bone of rats. The use of N-AcGIP might represent an alternative exciting treatment option for bone pathologies with decreased material properties, such as osteoporosis.

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## PP25

### Normal bone matrix mineralization in patients with chronic obstructive pulmonary disease

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Chronic obstructive pulmonary disease (COPD) has been found associated with low areal bone mineral density and an increase in fracture rate. Previous histomorphometric findings revealed abnormally low cancellous bone volume and thin cortices. In the present work, we studied the same transiliac bone biopsy samples from $n=19$ COPD patients for cancellous (Cn.) and cortical (Ct.) bone mineralization density distribution (BMDD) based on quantitative backscatter electron imaging (qBEI). The patients were postmenopausal women with an average age of $62.1\pm7.3$ years (mean ± s.d.). Eight of the patients had sustained at least one fragility fracture, 13 received treatment with inhaled glucocorticoids.

The BMDD outcomes from the patients were compared to reference BMDD data and were correlated with clinical and histomorphometric findings.
Comparison of Cn.BMDD findings to reference data revealed no significant difference, in particular the average degree of cancellous bone matrix mineralization Cn.CaMean was 22.10 (21.76; 22.51) wt%Ca, median (25th, 75th percentiles) which is in the normal range of 22.23 (21.84; 22.50) wt%Ca. There were no differences in Cn. or Ct.BMDD with respect to the occurrence of fragility fractures or to treatment with inhaled glucocorticoids. Correlation analyses with histomorphometric findings revealed highly significant negative correlations of Cn. and Ct.CaMean (both $R=-0.71$, $P<0.001$), of typical degree of mineralization Cn. and Ct.CaPeak (both $R=-0.65$, $P<0.01$), and of the percentage of highly mineralized bone areas Cn.CaHigh and Ct.CaHigh ($R=-0.65$ and $R=-0.69$ respectively, both $P<0.01$) with endocortical bone formation rate.
The lack of deviations in BMDD from the patients is consistent with the normal histomorphometric bone formation rates reported previously. Although within the normal range, bone mineralization indices were sensitive to variations in bone turnover rates as observed by strong negative correlations. Further, our findings suggest that the occurrence of fragility fractures was not associated with differences in bone matrix mineralization in this cohort with COPD.

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## PP26

### Structural analysis of tooth and jawbone in a type 2 diabetes mouse model

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In type 2 diabetes mellitus (T2DM) patients, an increased fracture risk is observed, although the bone mineral density is even higher than in non-diabetic patients, which raises the question of the quality of the organic and inorganic matrix in bone[1,2]. T2DM is also known to favor inflammation of the gingiva and paradontosis in general. However, little is known about the mineral nano-architecture in the mandible and about the possible influence of diabetes. Using synchrotron small-angle X-ray scattering (SAXS), we investigated the nanostructure of the mandible and tooth of a model of obese diabetes mice (KKAy) in comparison to controls (C57BL/6). Parameters determined were bone mineral particle size (thickness T and length L) and the alignment (rho-parameter) of the particles in the organic matrix[3]. The samples were embedded cross-sections of the jaw bone centered around the first molar of 15-week-old animals. Line scans with 30 μm resolutions (beam- and stepsize) were performed from the lingual to the buccal side of the mandible, including root dentin. Significant differences in the nanostructure of the mineral particles between the lingual and buccal side were found. In particular, the mineral platelets are thicker and more aligned on the buccal side compared to the lingual side in the control mice. In the diabetes model, the measurements indicate an even stronger structural difference between both sides of the tooth. We speculate that this high asymmetry in the bone microarchitecture might be due to asymmetric loads during mastication between the buccal and the lingual side of the tooth.
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## PP27

### Long term treatment with odanacatib maintains normal trabecular biomechanical properties in ovariectomized adult monkeys as demonstrated by micro-CT based finite element analysis of the vertebral cores

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The cathepsin K inhibitor odanacatib (ODN) is a bone formation-sparing inhibitor of osteoclastic resorption activity. This drug is currently under development for the treatment of postmenopausal osteoporosis. To support the bone safety profile of ODN, we evaluated the effects of ODN on trabecular bone hard tissue properties in the estrogen-deficient model of the ovariectomized (OVX) rhesus monkeys. Animals ($n=16$/group, age 11–22 years) were treated immediately after OVX'd with either ODN (2 mg/kg per day; equivalent to estimated daily clinical exposure), Alendronate (ALN; 30 μg/kg per weekly) or vehicle (VEH) for 20 months. Trabecular (Tb) cylindrical cores (~4.25 mm dia.) were extracted from lumbar spine (LV3), and scanned at an isotropic 12 μm resolution using a Scanco μCT 35 scanner. The Tb cores were endcapped and subjected to mechanical compression to record the load–displacement curve. Regression analysis of all groups showed a positive correlation between vBMD and the peak force (PF, $r^2=0.59$), and between peak stress (PS) and bone volume fraction (BV/TV, $r^2=0.51$). These results demonstrated that a normal apparent material property relationship was maintained in the lumbar spine of ODN ($r^2=0.65$ for vBMD vs PF; $r^2=0.51$ for BV/TV vs PS) and ALN ($r^2=0.54$ for vBMD vs PF; $r^2=0.53$ for BV/TV vs PS) treated monkeys. Finite element (FE) models were generated from the cores' μCT images and subsequently used to estimate the Tb bone hard tissue property (Young's Modulus) *in-silico*. The results showed only minor differences between the mean Young's Modulus of different treatment arms and were not statistically significant ($P=0.4$, Mean ± S.E.M. in GPa: VEH = 5.61 ± 0.22; ALN = 5.52 ± 0.21; ODN = 5.91 ± 0.21). Together with the experimental strength measurements, the FE result demonstrated that ODN treatment for 20 months maintained normal trabecular bone material hard tissue properties in the OVX-monkeys, and was comparable to ALN.

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## PP28

### Dietary creatine influence on bone metabolism in rats

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Objective
We investigated the effect of creatine administered in the diet for 10 weeks on bone turnover and bone quality in male Wistar rats.
Methods
Rats were divided (after approval of Ethical committee 9814/2008–30 and 30793/2010–30) into four groups, ten animals in each group. *G1 SLD-A* group were fed a standard laboratory diet (SLD). *G2 SLD-B* group were fed SLD and 12 h before rats were sacrificed diet was removed. *G3 CREA-A* group was fed with 10% creatine (CREA) enriched SLD. *G4 CREA-B* were fed CREA and 12 h before rats were sacrificed diet was removed. The bone mineral density (BMD, g/cm$^2$) was determined using dual-energy X-ray absorptiometry. The femurs were used for biomechanical testing. Bone marker concentrations were examined of N-terminal propeptide of procollagen (PINP; pg/ml), bone alkaline phosphatase (bALP, ng/ml), bone morphogenetic protein 2 (BMP-2; pg/m), the carboxy-terminal cross-links of collagen (CTX, pg/ml) and insulin-like growth factor (IGF; pg/ml) in bone homogenate using the ELISA method. Results are expressed as the mean or median.
Results
Differences results in biochemical tests in rats A and B suggest that standard preanalytical conditions are essential particularly for analytes PINP, BMP-2 and IGF1, more resistant is bALP and CTX. Decrease of PINP and increase of IGF1 was observed in CREA-A and CREA-B in comparison with SLD-A and SLD-B groups. BMD did not show differences between groups. Body fat was higher in in SLD-A 17.830 (S.E.M. 1.298) versus CREA-A 10.920 (S.E.M. 0.669). Biomechanical properties of bone tended to decrease in CREA-A and -B in comparison with controls.
Conclusion
The results suggest that the use of products with the addition of creatine, often used by bodybuilders affects bone metabolic rate and long-term use can be expected to change BMD.
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## PP29

**Systemic treatment with strontium ranelate does not influence the healing of femoral mid-shaft defects in rats**

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The objective of the study was to investigate whether the anti-resorptive and bone-forming dual effect of Strontium Ranelate (SrR) influences the healing of a non-critical sized femoral mid-shaft bone defect.
Sixty 16-weeks-old female Wistar rats were randomized into four groups. Cylindrical defects with a diameter of 2 mm was drilled through the anterior cortex of both right and left femoral mid-shafts. Two groups were treated with SrR (900 mg/kg/day) mixed into the food and the remaining two groups served as control. The animals were killed 3 and 8 weeks after the surgical procedure, respectively. The healing of the bone defects were analyzed using çCT and three-point bending test. All procedures were approved by The Danish Animal Inspectorate.
Treatment with SrR did not affect the BV/TV of the newly formed bone in the defect or the bone formed in the medullary cavity. The bone material density was significant ($P < 0.001$) higher for both the newly formed bone in the defect and the bone formed in the medullary cavity after 3 and 8 weeks of SrR treatment compared with the respective control groups. Mechanical fracture strength and stiffness were not altered by SrR.
The present study showed that SrR did not influence the healing of a non-critical sized femoral mid-shaft bone defect in rats. The higher bone material density values in the defects of the SrR treated animals are most likely due to strontium being radiological heavier than calcium.

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## PP30

**Strength training is capable of stimulating transcription factors Runx2 and osterix and ensure better bone quality in wistar rats during aging**

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Osteoporosis is a multifactorial disease that represents an increase public health problem, given the impact on functional independence and quality of life. Among the favoring factors to the imbalance in bone cell activity, the hypoestrogenism is primordial. Strength training (ST) proves to be effective because of its ability to stimulate estrogen receptor independent of ligand. In this study, we analyzed the action of ST on bone quality of rats during the aging. For this study, 40 females Wistar rats of 18 months were distributed into the groups: G1- Sham G2 – Sham/ST, G3 – (ovariectomized) OVX and G4- OVX/ST. During 120 days (3×/week), the animals Sham/ST and OVX/ST performed exercise with 80% of maxima force. Finite elements and immunohistochemical analysis for Runx2, osterix, osteocalcin and TRAP were realized. The results show that ST was able to stimulate osteoblast differentiation through the positive regulation of Runx2 and osterix, culminating action in favor of bone formation by increasing osteocalcin and decreasing TRAP. This action of ST on bone cells resulted in tougher tissue, which is evident in the finite element analysis. The table below shows the maximum force (N) and the minimum and maximum tension (MPa) of the femoral neck region and demonstrate that given the force applied in OVX/ST, maximum tension are higher than in the OVX group, comparable with the Sham animals which do not demonstrate the situation of hypoestrogenism. According these results it can be suggested that postmenopausal women, with contra-indication for hormone replacement, can benefit with the realization of ST, which stimulates the differentiation to bone cells and facilitates the bone formation and reversal of bone loss.

| | | **Tension** (MPa) | |
|---|---|---|---|
| **Groups** | **Maximum force** (N) | Minimum | Maximum |
| SHAM | 161.63 | 45.667 | 330.72 |
| SHAM/ST | 152.99 | 39.967 | 326.05 |
| OVX | 137.83 | 30.4 | 217.5 |
| OVX/ST | 156.96 | 41.025 | 327.53 |

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## PP31

**Excessive intake of soy milk affects bone development**

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The purpose of this study was to analyze the influence of diet supplemented with hydrosoluble soy extract – HSE in bone mass of male and healthy Wistar rats. Sixteen (16) 30 days old animals were kept in controlled conditions with light/dark cicle and fed with commercial food and filtered water. Randomized into two groups: control group received 500 ml of water and 300 g of food, daily; supplemented group S, in addition of water and food, they also received 500 ml of soy milk. After 11 weeks, the animals were euthanasied, its tibias were removed and subjected to bone densitometry (DXA) and mechanical test (three-point bending) for assessment of bone mineral density – BMD (g/cm$^2$), maximum force (N) and stiffness (kN/m). The results underwent statistical analysis and demonstrated that the consumption of soy milk caused a significant decrease in bone properties: BMD was $0.178 \pm 0.008$ g/cm$^2$ for the control group (C) and $0.146 \pm 0.014$ g/cm$^2$ for the supplemented group (S); maximum force was $88.27 \pm 5.07$ N for C and $73.58 \pm 5.25$ N for S; Stiffness was $173.45 \pm 10.33$ kN/m for C and $139.64 \pm 11.55$ kN/m for S. We concluded that hydrosoluble soy extract caused a significant decrease in mechanical bone properties of the rats, probably because it interferes in the absorption of minerals and amino acids. We suggest that soy milk should not be used as a substitute of cow milk once it has lower calcium content that can worsen bone density.

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## PP32

**Dietary arginine influence on bone metabolism in rats**

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Objective
We investigated the effect of arginine administered for 10 weeks in diet on bone metabolism in male Wistar rats.
Methods
Rats were divided (after approval of Ethical Committee 9814/2008-30 and 30793/2010-30) into four groups, ten animals in each group: *G1 SLD-S* group were fed a standard laboratory diet (SLD). *G2 SLD-H* group were fed SLD and 12 h before rats were sacrificed diet was removed. *G3 ARG-S* group was fed with 10% arginine (ARG) enriched SLD. *G4 ARG-H* were fed ARG and 12 h before rats were sacrificed was diet removed. The bone mineral density (BMD, g/cm$^2$) was determined using dual-energy X-ray absorptiometry. The femurs were used for biomechanical testing. Bone marker concentrations were examined: N-terminal propeptide of procollagen (PINP; pg/ml), bone morphogenetic protein 2 (BMP2; pg/ml) and factor (IGF; pg/ml) in bone homogenate using the ELISA method. Results are expressed as the mean or median.
Results
No significant differences of BMD were observed between groups, the decrease in adipose tissue mass was observed in both fasted groups (G1, 75; G2, 51; G3, 57; and G4, 49). The forces necessary to fracture left tibia were not significantly lower in ARG fed rats (G1, 131; G2, 126; G3, 109; and G4, 120). Higher PINP values in both groups (SLD and ARG) have been found after fasting (median G1, 151; G2, 155; G3, 139; and G4, 147). Moreover in other indicators were no differences between groups SLD and ARG, but differences were observed between fed and starving rats. There was increase in IGF (G1, 1387; G2, 1497; G3, 905; and G4, 5189).

Conclusion
Short-fasting affect bone metabolism and fat tissue mass in rats. Effects of diets enriched with arginine only marginally decreased resistance to the three-point tibial fracture.
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## PP33

**Relationships between morphometric, densitometric and mechanical properties of tarsometatarsal bone in ostriches (*Struthio camelus*)**

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The aim of the study was to evaluate relationships between morphometric, densitometric and mechanical properties of tarsometatarsus in ostriches (*Struthio camelus*). Males ($n=9$) and females ($n=15$) were kept to slaughter age of 14 months of life to obtain left tarsometatarsus. Using computed tomography technique (Somatom Emotion, Siemens), volumetric bone mineral density (vBMD) of the trabecular (Td) and cortical bone (Cd) were measured. Areal BMD and bone mineral content (BMC) were evaluated with the use of dual-energy X-ray absorptiometry (DEXA). Geometrical properties such as cross-sectional area (A), second moment of inertia (Ix), mean relative wall thickness (MRWT) and cortical index (CI) were derived on the basis of measurements of horizontal and vertical diameters of tarsometatarsus in the midshaft. Using an INSTRON 3367 apparatus (Instron, USA) and three-point bending test, mechanical parameters such as maximum elastic strength (Wy) and ultimate strength (Wf) of tibia were estimated. 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, BMC, and Ix with final body weight of ostriches were found ($P<0.05$). BMD was positively correlated with Td, BMC, A, MRWT, CI, and Wf ($P<0.05$). BMC was positively corretated with bone length, weight, A, Ix, Wy, and Wf ($P<0.05$). Bone weight was positvely correlated with bone length, A, Ix, and Wf, while bone lenght was negatively correlated with Td ($P<0.05$). Cross-sectional area was positively correlated with Ix, Wy, and Wf, while Ix was negatively correlated with MRWT, CI ($P<0.05$). Positive correlation between Ix and Wy was found ($P<0.05$). Positive correlations between MRWT and CI as well as between Wy and Wf were also found ($P<0.05$). In conclusion, this study showed numerous interrelationships between morphometric, densitometric and mechanical properties of tarsometatarsus. Thus, this bone may be used as an attractive model for further studies on metabolic response of skeleton to physiological, nutritional, toxicological and pharmacological factors influencing bone tissue metabolism.

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## PP34

**The surface and the volume trace element compound of the of biological bone apatite**

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The bioapatite crystals have a multilayer hydrate shell, which contains impurity ions such as magnesium, sodium, potassium and it is not clear the qualitative and quantitative characteristics of noapatite entourage. The major component noapatite environment in bone is water and we studied the surface of nanocrystals from trabecular bone in healthy rats and during the water deficiency.
In experiment we use adult laboratory rats (8 months age), remove the calcaneal bone, clean it from muscles and tendons and dry it to the constant weight. The specimen was burn in temperature from 560 to 760 °C and dissolved in distilled deionized water and treated with ultrasound. In this solution was determined content of calcium, magnesium, potassium and sodium by atomic absorption method.
Pyrolytic degradation of crystals surrounding at 680 °C causes a rise of soluble $Ca^{2+}$ concentration that was significant higher in case of dehydratation. This effect shows a high $Ca^{2+}$ concentration in the noapatite component and a defect structure of apatite crystals.
$Mg^{2+}$ ions at the temperature 700–750 °C moves from the bound to a labile state on the nanocrystals surface which may indicate an increase of the crystallites size, disappearance of lattice microdeformations, and decomposition of carbonate biomineral complexes. The concentration of the 'labile' $Ca^{2+}$ at the same temperatures significant decrease that may indicates their transition from the surface layer to the apatite grate. Counter migration of $Mg^{2+}$ and $Ca^{2+}$ in samples from the experimental pathology ('dehydration') begins at a lower temperature than in control samples. Active mobility of K and Na in the samples with dehydration confirms the fact of defective crystal structure.
Thus, our research have shown the ability of replacement the vacant places of water by the trace elements that may affect structural integrity of bone apatite.

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## PP35

**Strength features of humerus in rats with defects in tibiae after 60-day administration of sodium benzoate**

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Aim
Investigate strength features of humerus in rats with defects in tibiae after 60-day *per os* administration of sodium benzoate (SB).
Materials and methods
The experiment involved 210 rats with initial body weight of 200–210 g. The animals were separated into six groups as follows: Group 1 comprised animals that received *per os* 1 ml of 0.9% solution of sodium chloride daily (C), Group 2 (D) comprised animals with plain defects of 2.2 mm of diameter in proximal part of the shaft; Groups 3 and 4 received daily SB in dosage of 500 or 1000 mg/kg of body weight (B1 and B2) and Groups 5 and 6 comprised the animals with the same defects in tibiae as those of Group D that received SB in the same way as Groups B1 and B2 (B1D and B2D). After termination of observation terms the humeri were taken for bending strength test.
Results
Obtained show that SB and fracture (a defect in tibia) have negative effects on bone strength of humerus as far as breaking point values in the Groups B1D and B2D were lower than those of the Group D by 6.20 and 6.84% respectively and breaking moment values were lower than values of the same Group D by 7.59 and 7.14% respectively. In readaptation period strength restored markedly slower than in other groups: by the 24th day after SB discontinue breaking point values in the Groups B1D and B2D were lower than those of the Group D by 6.98 and 9.81% respectively. Also by the same time showed breaking moment and facture energy lower than those of the Group D by 7.21 and 6.32%. By the 45th day in both B1D and B2D Groups the same values were not significantly different than those of Group D.
Conclusions
Defect in tibia after 60-day administration of SB result in slower restore of bone strength. SB in dosage of 100 mg/kg of body weight had more pronounced effects on bone strength. Significant changes were found up to the 24th day of observation and group B2D exhibited more marked deviations than other groups.

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## PP36

**Comparing two types of bovine bone composition, structure and hardness based on their ecological effect and nutrition**

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Two kinds of apatite powder were prepared separately from bovine bones originated from different geographical and ecological areas with different climates, in order to characterize and compare their components, hardness and structures by burning them and heat treating the bone ashes at 850 °C in an air furnace (Table 1). The black ashes were converted to white and yellow–white powders. X-ray diffraction (XRD) analysis indicate that the white powder was carbonate–hydroxyapatite and the yellow white powder was fluorapatite. Neither of the samples contained any organic components of bone. X-ray analysis revealed that calcium and phosphorous were the main elements for both powders and sodium and fluoride were presented as minor impurities in fluorapatite powder.

Furthermore, the micro-hardness tests on both types of bone revealed that the bone which was composed of fluorapatite was about twice harder than the bone which was composed of carbonate-hydroxyapatite. Fluoride and sodium in the former cause more symmetric, dense and as a result harder structure.

**Table 1** Hardness.

| Sample composed of carbonate-hydroxyapatite | | Sample composed of fluorapatite | | |
|---|---|---|---|---|
| Force: 50 gf | Force: 100 gf | Force: 50 gf | Force:100 gf | Force: 200 gf |
| Hardness (Vickers): 16.6, 15.2, 16.8, 14.9, 15.3, 16.6, 15.2, 16.8 Average: 15.92 | Hardness (Vickers): 16.4, 16.2, 16.9, 17.4, 17.6, 16.4, 16.2, 16.9 Average: 16.75 | Hardness (Vickers): 27.5, 27.4, 30.2 Average: 28.3 | Hardness (Vickers): 33.3, 32.4, 36.4, 29.8, 29.1, 32.2, 31.4 Average: 32.08 | Hardness (Vickers): 26.7, 29.7, 31.7, 32.5 Average: 30.15 |
| Average: 16.33 | | Average: 30.71 | | |

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## PP37

### Bone matrix mineralization after sclerostin antibody treatment in a mouse model of osteogenesis imperfecta

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Children with osteogenesis imperfecta (OI) still suffer from frequent fractures, despite bisphosphonate treatment. Thus new therapeutic approaches are needed. Sclerostin is a protein that is thought to inhibit bone formation. Treatment with sclerostin antibodies (SclAB) increases bone mass in animal models and in clinical trials and may be a rational therapy for OI as well.

Transgenic (TgOI) *Col1a1*$^{Jrt}$/+ mice were generated being a model of OI type IV. 8 weeks old TgOI ($n=8$) and Wt ($n=8$) were analyzed after 4 weeks of SclAB treatment and compared to untreated animals ($n=7$, $n=8$). The bone mineralization density distribution (BMDD) of the cortical (Ct), metaphyseal spongiosa (MS) and epiphyseal spongiosa (ES) in the distal femur was measured by quantitative backscattered electron imaging. Additionally microCT parameters of the femurs were obtained.

TgOI mice exhibit increased bone matrix mineralization compared to Wt (most frequently occurring Ca concentration: Ct: +6.8% MS: +5.2% ES: +8.2%, $P<0.001$). The mineralization was also more homogenous in Ct. This fits previous findings in OI models. The percentage of lowly mineralized areas was increased in MS and ES most likely due to the decrease of bone volume (−86.3%, $P<0.001$).

Treatment with SclAB of Wt and TgOI mice overall led to shifts in the BMDD of the spongiosa towards higher and more homogenously mineralized bone. This is consistent with the observed decrease in percentage of lowly mineralized regions reflecting the increase in bone volume due to SclAB treatment. Two-way ANOVA tests revealed no interaction between genotype and treatment. After treatment, BV/TV of the Wt (+77.7%, $P<0.001$) and the TgOI (+65.9%, $P=0.02$) animals were elevated. Wt/SclAB and the TgOI/SclAB mice exhibit more bone volume and a similar increase in matrix mineralization compared to the corresponding untreated animals.

Therefore we conclude, that SclAB treatment has the similar effect on mineralization in TgOI and Wt mice.

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# Bone development/growth and fracture repair

## PP38

### Development and characterization of novel biodegradable scaffold materials

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The development of optimally performing biomaterials mimicking the natural physiological processes underlying bone repair is essential for non-healing large bone lesions. We sought to develop scaffolds that fill the lesions and improve bone healing. We tested three scaffolds comprised of varying percentages of lactide (LA), caprolactone (CL), and methacrylate produced by two-photon photopolymerization; LCM 3 (8:2; 85%), 4 (9:1; 90%), and 6 (9:1; 40%). We hypothesized that the quantity of specific polymer components may differentially impact bone regeneration. We first tested mouse calvarial-derived osteoblasts (OBs) in MTT and alkaline phosphatase assays as well as with alizarin red staining. We observed that LCM 3 and 4, but not LCM 6 induced mouse and human OBs to attach, proliferate, and produce mineralized matrix compared to negative controls. Secondly, we analyzed osteoclast (OC) development using co-culture-derived OCs and human CD14+OCs derived from blood. We observed that mouse and human OCs differentiated on LCM 3 and 4, but not on LCM 6, when compared to tissue culture plastic controls. Thirdly, we used a calvarial defect model in 3-month-old *BALB/c* mice to test scaffold integration. We found that implanted LCM 3 scaffolds induced a significant increase in bone regeneration in the defect compared to Vitoss and negative controls, as observed with quantum FX μCT. Remarkably, LCM 4 and 6 lacked osteoinductive ability. Taken together, these results indicate that a methacrylation degree of >50% seems to be a prerequisite for cellular attachment and that a higher content of CL promotes ossification and bone regeneration.

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## PP39

### Bone shaft revascularization after marrow ablation is dramatically accelerated in BSP−/− mice, along with faster haematopoietic recolonization

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The bone organ integrates the activity of bone tissue, bone marrow, and blood vessels and the factors ensuring this coordination remain ill defined. Bone sialoprotein (BSP) is with osteopontin (OPN) a member of the small integrin binding ligand *N*-linked glycoprotein (SIBLING) family, involved in bone formation, hematopoiesis, and angiogenesis. In rodents, bone marrow ablation induces a rapid formation of medullary bone which peaks by ~8 days (d8) and is blunted in BSP−/− mice. We investigated the coordinate hematopoietic and vascular recolonization of the bone shaft after marrow ablation of 2 months old BSP+/+ and BSP−/− mice.

Bone marrow was aspirated through the right femur epiphysis. Hematopoietic recolonization was analyzed by flow cytometry after labeling with specific antibodies. The bone vascular network was contrasted by barium sulfate infusion. RT-PCR was performed on tri-reagent flushed bone marrow.

At d3, the ablated area in BSP−/− femurs showed higher vessel density (99.2±4.9 vs 23.2±8.5 mm$^2$) and vascular volume than BSP+/+, along with higher VEGF (×1.95) and OPN (×1.8) expression. Interestingly, unablated BSP−/− femur marrow also contains more blood vessels than BSP+/+ (74.2±8.4 vs 58.0±7.6 mm$^2$). Vessel numbers in the shaft of ablated BSP+/+ mice reached BSP−/− values only by d8, but with a vascular volume which was twice the value in BSP−/−, reflecting smaller vessel size in mutants. At d6, a much higher number of Lin–as well as LSK (Lin- IL-7Rα- Sca-1hi c-Kithi,) and hematopoietic stem cells (HSC: Flt3- LSK) were counted in BSP−/− marrow, indicating a faster recolonization.

In conclusion, bone marrow ablation in BSP−/− mice is followed by a faster vascular and hematopoietic recolonization, along with lower medullary bone formation. Thus, lack of BSP affects the interplay between hematopoiesis, angiogenesis and osteogenesis. The higher expression OPN an angiogenic SIBLING, concomitant with massive revascularization in BSP−/− mice suggests a functional role, presently investigated with siRNA targeting OPN.

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## PP40

### NFI-C regulates osteoblast differentiation via control of osterix expression

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In bone marrow, bone marrow stromal cells (BMSCs) have the capacity to differentiate into osteoblasts and adipocytes. Onset of osteoporosis with age stems in part from the enhanced bone marrow adipogenesis that represses osteogenesis. In this study, we demonstrate that disruption of nuclear factor I-C (NFI-C) impairs osteoblast differentiation and bone formation, and increases bone marrow adipocytes. Interestingly, NFI-C controls postnatal bone formation but does not influence prenatal bone development. Moreover, Nfic-deficiency remarkably increases bone marrow fat in mice similar to osteoporotic patients. We also found decreased NFI-C expression in osteogenic cells from human osteoporotic patients. Notably, transplantation of Nfic-overexpressing BMSCs stimulates osteoblast differentiation and new bone formation, but inhibits adipocyte differentiation by suppressing PPARγ expression in $Nfic^{-/-}$ mice showing an age-related osteoporosis-like phenotype. Finally, NFI-C directly regulates Osterix expression but acts downstream of the BMP 2–Runx2 pathway. These results suggest that NFI-C acts as a transcriptional switch in cell fate determination between osteoblast and adipocyte differentiation in BMSCs. Therefore, regulation of NFI-C expression in BMSCs could be a novel therapeutic approach for treating age-related osteoporosis.

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## PP41

### Exosomes derived from human platelet lysate affect MSC functions *in vitro*

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Introduction
Despite the popularity of platet lysate (PL) treatments in orthopaedics, the mechanism of action and the effectiveness of this therapeutic tool is still controversial. So far, the activity of PL has been associated with different growth factors (GFs) released upon platelet degranulation. However, PL activity might also be due to the efficient cell to cell transport system of GF and other bioactive molecules by their encapsulation into exosomes. In this study, we characterized exosomes from human PL, and investigated their effect on MSC proliferation, migration and osteogenic differentiation *in vitro*.
Methods
Exosomes were isolated from human PL by differential ultracentrifugation. Their purity was assessed by electron microscopy and evaluating CD63 expression by western blot analysis. To test the effect of exosomes on MSC functions, bone marrow-derived MSC were cultured in presence of two different exosome concentrations or with PL. At specific time points, cell proliferation, migration, and osteogenic differentiation were evaluated by Alamar blue assay, Boyden chamber assay, and Alizarin red staining respectively.
Results
Electron microscopy revealed the presence of vesicles within the expected size range of exosomes (30–100 nm) which expressed the specific exosomal marker CD63. MSC treated with PL-derived exosomes showed a significant and dose-dependent increase of cell proliferation and migration. Furthermore, osteogenic differentiation assay demonstrated that exosome concentration differently affected the ability of MSC to deposit mineralized matrix.
Conclusions
In this study, we demonstrated that exosomes can be successfully isolated from human PL. PL-derived exosomes increase cell proliferation and migration at an higher extent than PL. In addition, exosome concentration affects osteogenic differentiation of MSC. Our results provide evidence of exosomes as putative effectors of PL, and highlight the importance of these vesicles as a potential nanodelivery system for cell-free regeneration therapies.

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## PP42

### Bone age in hemiplegic cerebral palsy: is there a correlation with hand function and limb length?

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Limb length discrepancy has been observed in children with hemiplegic cerebral palsy (HCP). However, the difference of bone age and limb lengths have been a controversial issue. The purpose of this study was to analyze the bone age and the upper extremity segmental lengths between affected and naffected in HCP patients.
Seventy-eight patients (39 boys and 39 girls; mean age $71.3 \pm 28.1$ months). Fourty-five children were right and 33 were left hemiplegia. The bone ages of affected and unaffected hand-wrists were determined by the Greulich and Pyle atlas. Each of the upper extremity segmental lengths composed of humerus, ulna, radius, and 3rd metacarpal bones were measured by radiograph. Hand function was classified by manual ability classification system (MACS).
The mean bone age was $66.7 \pm 33.5$ months in the affected side and $68.9 \pm 33.7$ months in the unaffected side ($P < 0.001$). Fifty-one of the patients performed X-rays of the upper extremities and all segmental lengths of the upper extremities, and showed significant differences between affected and unaffected side in the upper extremities ($P < 0.001$). However, the difference of side-to-side bone age revealed no statistical significant correlation with segmental upper limb length discrepancies. Hand function of fifty-six patients was evaluated by MACS and the MACS level of the affected hand showed correlation with difference of side-to-side bone age ($r = 0.29$, $P = 0.03$) and all segmental upper limb length discrepancies ($P < 0.05$). The hand function of affected side was delayed compared to unaffected side in the bone-age-delayed group ($P < 0.01$).
The bone age of the affected side compared to the unaffected side is delayed in the HCP and the hand function of the affected side is correlated with the difference of side-to-side bone age and the upper limb length discrepancy in the HCP. Through this study, hand function might be helpful for predicting potential limb shortness and delayed bone age in HCP.

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## PP43

### Subchronic co-administration to cadmium, diazinon and selenium causes apparent symptoms of osteoporosis in adult male rats

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Osteoporosis, as a global public health concern, may be induced by various hazardous substances such as cadmium (Cd) and pesticide diazinon (DZN). Selenium (Se), an essential trace element, can adversely modify bone structure in higher concentrations. The current study was designed to investigate structural changes in femoral bone of adult male rats after simultaneous co-administration to Cd, DZN, and Se. A total of 20 male Wistar rats were randomly divided into two experimental groups. In the first group, young males (1-month-old) were exposed to a combination of 30 mg $CdCl_2$/L, 40 mg DZN/L, and 5 mg $Na_2SeO_3$/L in their drinking water, for 90 days. Ten 1-month-old males without toxicant application served as a control group. At the end of experiment, macroscopic and microscopic analyses of femora were performed. Our results showed no significant differences in body weight, femoral weight and femoral length between the two groups. On the other hand, cortical bone thickness was considerable decreased in rats simultaneously administered to Cd, DZN, and Se ($P < 0.05$). Additionally, a smaller number of primary and secondary osteons was identified in these rats. Apparent symptoms of osteoporosis such as resorption lacunae and osteoporotic fractures were observed in Cd-DZN-Se-intoxicated rats. Histomorphometric evaluation revealed that area, perimeter, maximum, and minimum diameters of primary osteons' vascular canals, Haversian canals, and secondary osteons were significantly decreased ($P < 0.05$) in rats exposed to Cd, DZN, and Se. Our study demonstrates that subchronic co-administration to Cd, DZN, and Se harmfully affects cortical bone thickness, vascularization, and induces evident clinical manifestations of osteoporosis in male rats.

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## PP44

### Sex-related differences of morphometric and densitometric properties of lumbar vertebrae in silver foxes (*Vulpes vulpes*)

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The aim of this study was to determine morphological and densitometric properties of lumbar vertebrae obtained from male and female silver foxes. Five lumbar vertebrae (L2–L6) were isolated from 1-year-old males ($n=5$) and females ($n=6$) and cleaned from remaining soft tissues to determine bone weight and vertebral body length. Quantitative computed tomography (QCT) and SOMATOM EMOTION SIEMENS apparatus (Siemens, Erlangen, Germany) were used to determine the volumetric bone mineral density (vBMD) of trabecular bone (Td) and cross-sectional area ($A_{VB}$) of the vertebral body. Td was measured using 2 mm thick, cross-sectional QCT scans, placed at 60% of each vertebral body length, and $A_{VB}$ the at this place was measured automatically. Total bone volume (Bvol) of each lumbar vertebra and mean volumetric bone mineral density (MvBMD) were determined and the volume-of-interest (VOI) was defined by limiting the minimum and maximum density for the investigated bone at 0 and 3000 Hounsfield units (HU) respectively. The quantitative determination of calcium hydroxyapatite (Ca-HA) in middle part of vertebral body was performed. Bone mineral density (BMD) and bone mineral content (BMC) of whole femur were determined using dual-energy X-ray absorptiometry (DEXA) and Norland Excell Plus Densitometer (Fort Atkinson, WI, USA) equipped with Research Scan Software. Mean values obtained from all measurements were averaged for each single vertabrae (L2–L6) and were compared using non-paired Student's *t*-test. Differences showing $P>0.05$ were not considered statistically significant. Mean values of bone weight, length, $A_{VB}$, Bvol and BMC were found to be significantly higher by 22.7, 14.1, 13.1, 23.8, and 14.8% in males when compared to the females respectively ($P\leq0.05$). However, MvBMD of the whole vertebra was significantly lower in males by 2.5% when compared to the females ($P=0.0006$).
This study has shown sex-related differences of morphometric and densitometric parameters of lumbar spine in silver foxes. The obtained results indicate that silver foxes may serve as an attractive experimental model for further studies on axial skeleton properties and bone metabolism regulation in mammals in response to physiological, pharmacological, nutritional, and toxicological factors, being an alternative model for other species of monogastric animals such as dogs.

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## PP45

### 1,25-Dihydroxyvitamin $D_3$ modulates the cross-talk between mesenchymal stem cells and macrophages

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The regeneration of traumatized, damaged or lost bone is still a major clinical and socio-economical problem. Bone-tissue engineering approaches involve culturing mesenchymal stem cells (MSCs) on scaffolds, in order to create a 3D microenvironment in which MSCs are able to generate functional tissues and regulate foreign body reaction to implanted construct. Excessive or non-resolving inflammation, characterized by the presence of monocytes, macrophages and giant cells, makes a major contribution to osteolysis and implant failure. In recent years, it has been postulated that one of the mechanisms of MSC action in tissue repair is the modulation of the macrophage-mediated inflammatory response through the secretion of soluble factors. Despite its long-standing association with calcium homeostasis and bone metabolism, accumulating evidence suggests that $1,25(OH)_2D_3$ has immunomodulatory effects on cells of the monocyte/macrophage lineage. In this context, we investigated the effect of $1,25(OH)_2D_3$ on the production of soluble factors involved in fracture healing in a co-culture model of MSCs and macrophages. To this aim, human MSCs and TPA differentiated THP1 cells were either cultured separately or co-cultured in the presence or absence of 10 nM $1,25(OH)_2D_3$. Treatment with $1,25(OH)_2D_3$ reduced TNFα–IL-6, MCP1, $PGE_2$, and VEGF release from macrophages. IL6 and VEGF secretion from MSCs increased in response to $1,25(OH)_2D_3$ while MCP1 levels decreased. Compared to isolated macrophages, lower levels of TNFα and RANTES were detected in co-cultures with MSCs while IL6, MCP1, $PGE_2$, and VEGF secretion increased. Among these factors, TNFα, IL6, and $PGE_2$ were regulated by $1,25(OH)_2D_3$ in MSC/macrophage co-cultures. Taking together, these data suggest that $1,25(OH)_2D_3$ is involved in the modulation of the local complex interplay of soluble factors during tissue regeneration.

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## PP46

### Midkine deficiency significantly delayed chondrogenesis during fracture healing in mice

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One growth factor that potentially plays a role in fracture healing is midkine (Mdk). Mdk is expressed in chondrocytes during bone repair (Ohta *et al.* 1999) and has been shown to influence bone mass and mechanotransduction (Neunaber *et al.* 2010, Liedert *et al.* 2011). The aim of the present study was to evaluate the effects of Mdk-deficiency on bone repair in a standardized mouse femur osteotomy model.
Mdk-deficient and wildtype mice (C57BL/6) were used for the study. After induction of a femur osteotomy stabilized with an external fixator, mice were sacrificed at day 10 or 21 and fracture healing was assessed by three-point-bending test, microCT based evaluation and histomorphometry. Furthermore, immunohistochemical staining for β-catenin was performed.
Mdk−/− mice displayed a significantly decreased flexural rigidity of the fractured femur 21 days post-surgery. MicroCT data indicated that bone mineral density in the periosteal callus in Mdk-deficient mice did not differ significantly from WT littermates. The moment of inertia in the bending axis was significantly reduced in animals lacking Mdk indicating an altered callus geometry during fracture healing. We observed that Mdk-deficient mice showed a significantly decreased amount of cartilage in the callus after 10 days, whereas the cartilage content was significantly increased after 21 days. The expression level of β-catenin was lower in Mdk−/− mice 10 days after fracture.
Our data indicate an impaired fracture healing due to a delayed chondrogenesis in Mdk-deficient mice. Together with the finding that a chondrogenic cell line overexpressing Mdk displays enhanced chondrogenesis (Ohta *et al.* 1999), we hypthothesize that Mdk plays a pivotal role in the development of cartilage tissue in the early fracture callus.

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## PP47

### Mechanisms of action and osteogenic activity of bone marrow mesenchymal stromal cells are donor dependent

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Human mesenchymal stromal cells (hMSC) have been investigated as a clinical therapy to promote tissue repair. However, the disappearance of grafted cells soon after engraftment suggests that hMSC could principally act as initiators of repair through paracrine mechanisms.
The aim of this study was to evaluate the relative contribution of grafted hMSC and host cells in promoting bone tissue repair. We isolated hMSC from three bone marrow (BM) donors, then directly loaded into scaffolds and subcutaneously implanted in mice in order to induce ectopic bone formation (no. 94–612).
This work revealed a cell heterogeneity between hMSC from different BM donors regarding their mechanisms of action and their osteogenic activity. We demonstrated by species-specific qPCR that grafted hMSC were able to survive up to 6 weeks post-implantation but with a different manner depends of the donor. This was not dependent on vascularisation as hMSC induced vascularisation with the same efficacy in less than a week as evaluated by the expression of VE-cad

and PECAM. However, it seems to depend on MSC apoptosis as hMSC with a poor cell survival show either an higher and/or prolong entry in apoptosis as mesured by Bax/Bcl2 expression. Then, by species-specific RT-qPCR we highlighted the importance of direct and indirect role of the hMSC for optimal bone tissue regeneration. Whereas in our mouse model, hMSC does not modulate macrophage (MP) polarization, they seem to affect MP differentiation into osteoclasts as bone formation is associated with increased osteoclast activity. However, this was not dependent of the osteoclastic canonical pathway as hMSC express RANKL and OPG with the same ratio level.
In conclusion, we validated that grafted hMSC could survive and we demonstrated that mechanisms of action and bone-forming capacities of BM-derived hMSC were donor dependent, which is of critical importance for MSC studies and clinical applications of tissue regeneration.

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## PP48

**Spatial arrangement of mesenchymal stem cells regulates their immunomodulatory properties on macrophages**

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Bone-tissue engineering approaches involve culturing mesenchymal stem cells (MSCs) on 3D scaffolds designed to mimic the composition, structure, and biomechanics of the native bone matrix. One of the key challenges in bone–tissue engineering is to understand the host response to implantable engineered constructs. Macrophages play an important role in fracture healing and tissue repair and are mainly involved in the inflammatory response at the early stage upon scaffold implantation and during its degradation. Growing evidence suggests that MSCs regulate inflammatory response mediated by macrophages through the secretion of soluble factors. 3D tissue scaffold structures drive MSCs into spatial dispositions that may regulate their immunomodulatory properties. The objective of this study was to determine the role of 3D scaffold geometry in regulating paracrine factors secreted by MSC in a co-culture model with macrophages. To this aim, MSCs were seeded on highly porous polystyrene scaffolds, which provide a 3D spatial environment, and co-cultured with TPA-differentiated THP1 cells using a transwell insert system. As controls, MSC were co-cultured with macrophages in 2D conditions. 3D growth of MSCs modulated the secretion patterns of $PGE_2$, TSG6, MCP1, and RANKL. Compared to isolated MSCs, the presence of macrophages induced an increase in $PGE_2$, TSG6, and MCP1 levels. Scaffolding structure of co-cultured MSCs further increased $PGE_2$ and TSG6 secretion but led to a decrease in MCP1 levels. Secretion of RANTES, MIP1α, RANKL, MCSF, and GM–CSF to the co-culture medium was attenuated when MSCs were cultured on 3D scaffolds. Monocyte migration rate was lower when assayed using conditioned medium from 3D than 2D co-cultures. Taking together, these data suggest that MSCs sense and respond to 3D environment modulating secretion profiles of chemokines and anti-inflammatory molecules that regulate their cross-talk with macrophages. Spatial arrangement of MSCs on 3D scaffolds may be responsible of their inhibitory action on monocyte migration.

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## PP49

**Whole-body vibration with extremely low-amplitude accelerates early-stage bone defect healing with reducing angiogenesis**

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This study was undertaken to evaluate the effect of whole-body vibration (WBV) with extremely low-amplitude on early-stage bone healing. Experiments were conducted with an approval of the Animal Research Committee of Osaka University Graduate School of Engineering Science. Male BALB/cByJJcl mice were subjected to a 0.5 mm drill-hole surgery on a tibial diaphysis at 14 weeks of age and divided into three groups ($n=8$ each) from the day after the surgery, which received 0.03 g WBV at either 30 Hz (W30), 90 Hz (W90), or 0 Hz, i.e. sham WBV (SW) for 20 min/day respectively. After 12-day WBV, each animal was perfused with agarose solution containing zirconia nanoparticles for vascular casting. Following euthanasia, the defect portion was harvested, fixed with 4% paraformaldehyde solution, and scanned by synchrotron light at 17.9 and 18.1 keV, below and above the zirconia k-edge respectively. The two scan data sets were reconstructed, and vascular and bone images (2.7 μm voxel resolution) were obtained through image subtraction. $K_2HPO_4$ phantom solutions were also scanned for bone mineral quantification. Between-group differences in volume fractions of newly formed bone (B.Vf) and angiogenic vessels (V.Vf) as well as the mean degree of mineralization (DM) in the defect were tested for significance ($P<0.05$) with the Kruskal–Wallis test followed by Dunn's multiple comparison test. B.Vf was higher in W30 ($52\pm1\%$) than in SW ($41\pm4\%$) while V.Vf was lower in W30 ($4\pm2\%$) than in SW ($10\pm4\%$). DM was higher in W30 ($0.79\pm0.02$ g/cm$^3$) than in SW ($0.76\pm0.03$ g/cm$^3$). B.Vf ($45\pm2\%$) and DM ($0.78\pm0.02$ g/cm$^3$) in W90 tended to be higher than those in SW. These results suggest that WBV with extremely low-amplitude facilitates early-stage bone defect repair. Reduced angiogenesis, which could still suffice to promote osteogenesis, implies that the present WBV drives the differentiation of mesenchymal stem cells preferentially toward bone-forming cells rather than endothelial cells.

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## PP50

**Forearm fracture in premenopausal women, a disorder of trabecular corticalization**

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Postmenopausal women with forearm fracture have higher cortical porosity and lower trabecular density perhaps due to excessive age-related bone loss[1]. Remodelling becomes unbalanced and rapid only after ~45 years of age. We therefore proposed that bone fragility in premenopausal women with a forearm fracture originates during growth. At metaphyses, trabeculae emerging from the periphery of the growth plate form cortex by 'corticalization')[2]. We speculated that fewer and/or thinner trabeculae from the growth plate may impair corticalization so the spacing between more separated trabeculae leave a higher porosity (forming wider transitional zone), reduced cortical area and a larger medullary area (by lack of adsorption of trabeculae upon the endocortical surface).
In a previously reported cohort of 40 premenopausal women (mean age $30\pm8$ years) with forearm fractures[3], and 80 controls, we assessed trabecular architecture and cortical porosity of the compact-appearing cortex (CC), outer- and inner transitional zones (OTZ and ITZ) at the ultradistal radius acquired using HR-pQCT and quantified using StrAx1.0[4]. Cortical cross-sectional area (CSA)/total CSA was used as a measure of the cortical area corrected for bone size.
Cases had 4% lower cortical CSA/total CSA, a reciprocally higher medullary CSA/total CSA (both $P=0.067$), 2–3% higher porosity of the OTZ and ITZ (both $P<0.04$), and 19% lower trabecular vBMD due to 8% fewer and 3% thinner trabeculae than controls ($P<0.04$). A SD increment in ITZ porosity and trabecular vBMD conferred odds ratios for fracture of 1.33 (1.12–1.60) and 1.03 (1.01–1.04) respectively.
Within the constraints of this cross-sectional study, we infer that reduced trabecular number and thickness and impaired corticalization contribute to thinner and more porous cortices, reduced trabecular density and so bone fragility.

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## PP51

**Alkaline phosphatase interacts with collagen during mineralization**

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Background
Alkaline phosphatase (ALP) has an essential role in bone mineralization. ALP is attached to the surface of matrix vesicles (MVs) in which hydroxyapatite crystals are initially formed. Formation of a tentative collagen–ALP complex may be an early step in the calcification process. The present study was designed to investigate the binding properties of different ALPs to collagen present in bone and cartilage, i.e. types 1 and 2 collagen.

Methods
The binding properties between ALP and collagen were studied by surface plasmon resonance using a BIAcore system. Human type 1 collagen was coupled to a sensor chip and human bone ALP was introduced in solution. The quantity of ALP bound to collagen was monitored in real time and in relation to the response from a reference surface where no collagen was present. Collagen (human type 1 and bovine types 1 and 2) was separated on a gel, blotted to a membrane and incubated with different human ALPs (bone, liver, kidney, and intestinal) and *E. coli* ALP. The binding of ALP to a collagen type 1 coupled column was studied with an ÄKTA liquid chromatography system.
Results
Surface plasmon resonance studies indicated significant binding between bone ALP and collagen. The various ALPs bound with different affinities to collagen blotted to the membrane. ALP eluted as two fractions from the collagen type 1 coupled column indicating some electrostatic binding.
Conclusion
Our results, from applying various methods, suggest that ALP binds to collagen, which could be part of the process that occurs at the surface of and between collagen fibrils during mineralization. Some types of ALP show higher affinity for collagen, possibly due to posttranslational glycosylation differences, or differences in molecular structures among various ALPs.

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## PP52

### Cadmium induced embryopathy: nitric oxide rescues teratogenic effects of cadmium

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Although the heavy metal pollutant cadmium (Cd) is a well known teratogen, the mechanism behind Cd mediated teratogenicity is still unclear and it mainly targets the bone development. Previously we and other groups reported on the protective role of nitric oxide (NO), a key signaling molecule in the embryonic developmental process, against thalidomide and other teratogenic assaults also NO is considered to be a vital signaling molecule in the regulation of bone formation. The objective of this study was to investigate the effects of Cd on the mechanistic interplay between reactive oxygen species (ROS) and NO signaling in the developing embryo. Chick embryonic model was used to determine the time and dose dependent effects of Cd and NO recovery against Cd assault. The effects of Cd and NO recovery on the developing embryos were assessed by morphometric analysis using alcian blue and benzidine staining and various angiogenic assays. ROS and NO, levels were measured using ultrasensitive oxygen and NO electrodes respectively. Results showed that Cd treatment during early development caused multiple birth defects, with more frequency of bone related defects such as, micromelia and exencephaly in chick embryos. Exposure to Cd suppressed endogenous NO level and cGMP signaling, inhibiting angioblast activation and subsequently impairing yolk sac vascular development. Furthermore Cd induced superoxide and lipid peroxidation mediated activation of pro apoptotic markers p21 and p53 on developing embryo. Cd also caused down-regulation of FOXO1 and activation of FOXO3 and caspase 3 resulting in apoptosis. Addition of exogenous NO via a NO donor was able to blunt Cd mediated effects and restore normal embryo development. In conclusion, Cd mediated embryopathy occurs via impairing NO-cGMP signaling and inducing oxidative stress and activation of apoptotic pathways. Supplementation of exogenous NO via NO donor negates Cd mediated effects and protects the developing embryo.

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## PP53

### Caspase-7 participates in osteogenic molecular networks

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Caspases are the basic enzymatic components in the apoptotic process. Recently, these molecules seem to participate not only in apoptotic but also in other cellular events, particularly in differentiation. This work aimed to further investigate novel roles of executive caspases such as caspase-7. The investigation is based on our previously published findings showing non-apoptotic roles of caspase-7 in odontogenesis.
In this study, we followed the localization of the activated form of caspase-7 during alveolar and mandibular bone formation and ossification in mice. As cleaved caspase-7 was present in number of cells that did not show signs of apoptosis, we approached the PCR array technology to examine osteogenic molecular networks in the WT vs caspase-7 deficient mice. Samples of the mandibular bone at the embryonic stage 15.5 were used. Mandibular bones fixed in RNALater were accurately cut off tissue slices. Examination of 84 genes was performed in the assay. Total mRNA was transcribed into cDNA and the Mouse Osteogenenic PCR array (SA Biosciences, PAM-026A-24) performed.
PCR array analysis of the mandibular bone of caspase-7 deficient vs WT mice showed a significant decrease in mRNA levels of several genes. One of them was Smad1, the key player in BMP pathway and induction of bone formation. Moreover, a decreasing trend but not a statistically significant drop was observed in other genes important in osteogenesis such as ameloblastin, BMPR1A, DMP1, FGF2, FGF2R or MMP8.
Caspase-7 apparently interacts with osteogenic signals during early mandibular bone development. Further investigations are under process to specify the participation of caspase-7 in intramembranous osteogenesis.
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## PP54

### MicroCT analysis of caspase-7 deficient mice revealed impaired osteogenesis

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Caspase-7 belongs to executive apoptotic and pro-inflammatory caspases. Recent reports point to novel roles of these caspases, particularly in cell differentiation. As we revealed such function of caspase-7 in odontogenesis, we turned our attention to participation of this molecule in formation of hard tissue in general. In this work, both type of bones, intramembranous (mandibular/alveolar bone), and endochondral (long bones) were investigated. Mouse heads and front limbs at ED13.5, 14.5, 15.5, 17.5, and P1 and 5 were used for immunohistochemical localisation of activated form of caspase-7 in bone formation.
In both bone types, the activated form of caspase-7 was detected from the beginning of ossification during embryonic development and persisted up to postnatal stages. The activation of caspase-7 positively correlated with osteocalcin, a marker of osteogenesis. The adult bone status was investigated by microCT in the WT vs caspase-7-deficient adult mice. MicroCT revealed differences in both models of ossification. Intramembranous bone in the knock-out mice showed a statistically significant decrease in volume, while the mineral density was not altered. Conversely, endochondral bone showed constant volume but a significant decrease in mineral density in caspase-7 knock-out mice. The trabecular separation and thickness were not altered, however, changes in spatial organization of trabeculae in caspase-7-deficient mice were evident as well as endosteal and periosteal surfaces.
The microCT results confirmed a specific role of caspase-7 in osteogenesis. The different consequence of caspase-7 deficiency in the intramembranous vs endochondral bones corresponds to previously reported specificities in both types of ossification related to the expression patterns of periostin, BMPs or variations in the vascularisation.
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## PP55

**Roles of Bone morphogenetic proteins signalling in the bony repair of injured growth plate cartilage in young rats**

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Growth plate cartilage is solely responsible for bone lengthening in children. Injury upon growth plate is often repaired by bony tissue which leads to bone growth defects such as limb length discrepancies or bone angulation deformity at later life. Earlier studies using an established rat growth plate injury model have identified a series of repair phases; inflammatory, fibrogenic, osteogenesis and remodelling repair phases. Currently, the cellular and molecular mechanisms involving the bony repair are unknown. A recent microarray study during the growth plate injury has shown BMP signalling activity during the growth plate injury repair. BMP signalling plays a vital role in regulating bone fracture repair. However, its role in the growth plate injury repair is unknown. This *in vivo* study investigated the potential roles of BMP signalling during the growth plate injury bony repair in rats using BMP inhibitor, human noggin recombinant protein, rhNoggin (delivered systemically using osmotic pump at 6 ug/ml for 14 days post growth plate injury) showed lower percentage of bone volume formation within the growth plate injury site for noggin-treated group on days 14 and 35 time-points through Micro-CT scan and analysis ($n=4$, $P<0.05$). Histological analysis revealed significant lower percentage of bone trabeculae formation when treated with rhNoggin protein for both D14 and D35 time-points ($n=5$, $P<0.05$). Immunohistochemical analysis on both treatment groups showed P-Smad1/5/8 immunopositive cells to be present however, the intensity and quantity were lesser when compared to the vehicle treated. Consistent with RT-qPCR, level of Runx2 and osteocalcin gene expression are lower when compared to the vehicle treated group at days 14 and 35 time-points although non-significant. These results suggest that BMP signalling is involved in regulating the bony repair during the growth plate injury repair by promoting osteoblast differentiation and osteogenesis.

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## PP56

**The influence of dexamethasone administered prenatally on cartilage in newborns of spiny mouse (*Acomys cahirinus*)**

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Glucocorticoids are the most commonly used drugs in medical therapy, released in stress and necessary for normal development in both humans and animals.
The aim of this study was to investigate the effect of prenatally administered dexamethasone (Dex), a synthetic glucocorticoid, on the histomorphometry of the femur in the offsprings of spiny mice, as a precocial species, characterized by high fertility, and the rapid development of the fetal, allowing the offsprings to the large autonomy shortly after birth.
The study was performed on four pregnant spiny mice. Time of the experiment included the prenatal period between the 20th day of gestation until birth (pregnancy lasts an average of 36–38 days). The mice from the experimental group received dexamethasone *per os* in a dose of 125 mg/kg BW daily. At the end newborns were weighted and euthanized. The femora were isolated, weighed and measured. Histology and histomorphometry of articular and growth plate were performed.
Maternal Dex treatment resulted in lower birth weight by 17%. Dex administration significantly reduced the thickness of the hypertrophy zone of growth plate by 34% and total thickness by 8.7%. In addition Dex decreased number of cells in the articular cartilage by 27% and decreased their diameter by 5%, significantly though. Dex also affected the structure and spatial distribution of thick and thin collagen fibers, lowering the proportion of thin fibers compared with control group. Moreover, Dex treatment considerably lowered amount of proteoglycans in articular and growth cartilages.
Glucocorticoids exposure to pregnant spiny mice affects cartilage development by accelerating maturity of collagen fibers and growth plate, along with presumably disrupts further longitudinal growth of long bones.
Declaration of interest
There is no conflict of interest.

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## PP57

**Anabolic and anti-catabolic effect of Strontium ranelate in an *in vivo* bone healing model**

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We assessed whether systemic Strontium ranelate (SrRan) administration accelerates the healing of a bone defect and could modulate local bone cellular activities. Proximal tibia bone defects were created in 6-month-old female rats, which received then orally SrRan (625 mg/kg per day, 5/7 days) or vehicle (controls) for 4, 8, or 12 weeks. Bone samples were analysed by micro-computed tomography and histomorphometry in various compartments, ie metaphyseal second spongiosa (MC), a region close to the defect (CDC), within the healing defect (DC) and in cortical defect bridging region. All mentioned results are statistically significant.
From 8 weeks of treatment and independently of the site, SrRan decreased bone resorption as indicated by reduced active osteoclast surfaces. In contrast, bone formation was stimulated within DC and CDC at early stage of healing as shown by increased mineral apposition rate (MAR) at endosteal compartment by week 4 and increased bone formation rate in CDC and DC by weeks 8 and 12. Osteoid surface and thickness were not altered in DC, suggesting that osteoblast function was modulated toward mineral apposition rather than differentiation. This is supported by increased adjusted apposition rate and a trend to lower mineralization lag time in early healing phase. This led to an increase in trabecular bone mass by weeks 8 and 12 at each trabecular site investigated in SrRan vs time-matched controls. Cortical defect bridging was detected earlier compared to changes in trabecular compartment and was accelerated in SrRan compared to controls. Increased endosteal MAR in early stage of healing led to higher cortical thickness by week 4 in SrRan vs controls, with a trend to lower periosteal osteoclast surfaces by week 8.
In healing of both trabecular and cortical compartments bone defect, SrRan early stimulated formation and later decreased resorption, suggesting potential advantages in orthopaedic surgery.

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## PP58

**Single cell analysis of caspase-3 in apoptotic and non-apoptotic cells during mouse limb development**

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Caspases belong to cysteine proteases participating in apoptosis and inflammation. However, there is recent evidence about their functions also in other events such as cell differentiation. This is supported also by our latest research of odontogenesis and osteogenesis. With the increasing interest in caspases due to their apoptosis-related therapies but also emerging non-apoptotic roles, exact evaluation of their impact at single cell level becomes challenging.
We have recently reported about caspase-3 detection at femtogram level ($10^{-15}$ g) in cell populations of embryonic micromasses of chondrogenic mesenchymal cells. To evaluate active caspase-3 in individual cells, a miniaturized device was developed.
Such miniaturized device enables quantitation of caspase-3 just in single apoptotic and non-apoptotic cell. The detection is based on the specific cleavage of modified luciferin by caspase-3, bioluminescent emissions of photons and their detection by photomultiplier tube working in the photon counting regime. Mouse front limbs at the digitalization stage were examined. Digital vs intedigital cells were used to verify the novel method. Caspase-3 in these cells was simultaneously evaluated by immunohistochemistry and flow cytometry.
Mass of caspase-3 per cell in non-apoptotic cells was under detection limit, whereas the mass in interdigital apoptotic cells was around 5–6 fg corresponding to $10^5$ of molecules. These masses of caspase-3 in mouse apoptotic cells are in a good agreement with the data published so far. Recently, further precision of instrumentation is under process using a low volume technology to quantify the natural content of active caspase-3 in nonapoptotic mouse cells.

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## PP59

**Comparison of a novel bone device OSTEOGROW with commercial bone devices in regard of a local inflammatory reaction**

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BMP6 is a member of the TGFβ superfamily with a high potential to induce new bone formation. Recently, we discovered that blood coagulum from the patient's own blood (WBCD) modified with calcium salts serves as an appropriate autologous carrier for BMPs (OSTEOGROW device). Preclinical experiments indicate that BMP6 is efficacious when used in a significantly lower amounts than BMP2 and BMP7. Besides testing its osteogenic activity, we also tested the influence of the novel OSTEOGROW device on inflammatory reaction in the surrounding tissue, as compared to commercially available bone devices. Male CD1 mice and female Sprague–Dawley rats were allocated into five experimental groups as follows: negative control (vehicle); treatment groups (0.6, 3 and 6 μg rhBMP6 Genera Research); and rat groups were: negative control (vehicle); treatment groups (2, 11, and 22 μg rhBMP6 Genera Research). WBCD was prepared from animals' autologous blood according to the standard protocol and the device was implanted deep into the thoracic sc pockets. Implants were removed on day 14 for μCT and histological analysis. The implantation of the WBCD device prepared with rhBMP6 protein (Genera Research) resulted in the equivalent formation of ectopic bone as assessed by μCT. High and medium doses induced noticeable ectopic bone formation. New bone in the ectopic bone fragment was dominantly located at the periphery of the implants with the signs of new trabecular bone formation in the middle of the implant. Observation of the inflammatory reaction in the surrounding tissue in rats and mice with implanted WBCD devices revealed no inflammation at the implantation site. The Helistat collagen sponge implant used with BMP2 and BMP7 commercial bone devices triggers a pronounced inflammatory response. The OSTEOGROW device is non-immunogenic and non-inflammatory, and therefore safe device to use in future applications.

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## PP60

**Effects of treatment with different bone-resorption inhibitors on alveolar wound healing process of old acyclic female rats**

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To evaluate the regeneration of alveolar bone after treatment with bone-resorption inhibitors in old acyclic rats that had been through a long period of low estrogen. Thirty-two female Wistar rats with 20 months old intact and ovariectomized (OVX at 4 months of age), were randomized into four groups ($n = 8$/group): i) intact; ii) OVX/O (corn oil); iii) OVX/$E_2$ (17β-estradiol, 400 μg) and iv) OVX/RLX (Raloxifene, 1 mg/kg per day). All treatments began on the 420th day after OVX and lasted for 60 days. At 20 months old, all groups had their right-upper incisors extracted. At 28 days after the tooth extraction, the right maxilla was prepared to histological analyses and immunohistochemistry reaction for osteocalcin. Plasma samples were submitted to RIA for estradiol and ELISA assay to determine osteocalcin concentration. In OVX/$E_2$ group higher plasmatic concentration of estradiol was verified. The histomorphometric analysis showed 45.26% of bone formation in the intact groups with a higher number of osteocytes and presented bone lining cells more positive for osteocalcin immunolabeling than groups OVX/O and OVX/RLX. The OVX/O groups showed 30.99% of bone formation and discrete presence of osteoblastic lineage cells with positive immunolabel for osteocalcin. The group OVX/$E_2$ showed 50.28% of bone formation and the number of osteocytes lower than the intact animals, showed yet a more expressive positive label for osteocalcin than other OVXs groups. The OVX/RLX group showed 38.60% of bone formation, greater positive osteocalcin immunolabeling with the presence of osteoblastic cells immunopositive for osteocalcin and a lower number of osteocytes, similar to the animals of group OVX/O. The plasmatic osteocalcin concentration were significantly elevated in OVX animals in comparison with the intact group. Furthermore, plasmatic osteocalcin values were significantly reduced by $E_2$ and RLX. Our results show that administration of $E_2$ or RLX to oestrogen-deficient rats leads to a better recovered of alveolar bone healing.

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## PP61

**Fas ligand in formation of hard tissues**

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Among activation of apoptotic machinery, Fas (CD95)/FasL (CD178) were suggested to act in cell proliferation and differentiation. Expression of Fas and FasL was reported during tooth and bone formation. The examination of gld mice showed increased total body bone mass and number of osteoblasts in long bones. However, Fas and FasL functions in osteogenesis remain controversial. As most of studies dealing with Fas/FasL system in bone formation were performed in endochondral models, we turned to intramembranous bones.
The aim of our study was to investigate functions of FasL in development of the mandibular and alveolar bones using immunohistochemistry and impact of FasL deficiency by microCT examination of gld mice. This project continues our earlier published investigations of Fas/FasL in prenatal molar tooth development. Heads at embryonic (E) 12.5, 15.5, 17.5, and postnatal (P) 0, 1, 4, 11 days were used for immunohistochemical analysis. FasL was localized in the dental epithelium, dental follicle and a few cells of the dental papilla. In general, as the tooth development progressed, FasL expression decreased. Notably, similar pattern was observed in mandibular/alveolar bone; FasL was detected in osteoblasts at the E15.5 and the number of positive cells decreased at later stages. Importantly, FasL expression did not copy the apoptosis pattern suggesting non-apoptotic engagement in formation of hard tissues. Additionally, preliminary results of microCT analysis of the adult gld mice showed increased enamel volume in molars but no impact on crown formation. Detailed analysis of bone parameters is in progress to support the data pointing to dual function of Fas/FasL in odontogenesis and osteogenesis.
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## PP62

**Effects of maternal administration of β-hydroxy-β-methylbutyrate on humerus properties in newborn piglets**

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Alterations in fetal nutrition result in developmental adaptations that permanently alter the structure, physiology and metabolism of the offspring.

β-hydroxy-β-methylbutyrate (HMB) is the bioactive metabolite of leucine responsible for inhibiting proteolysis and for modulating protein turnover *in vitro* and *in vivo*.
The aim of the study was to determine the effect of maternal administration with HMB on mineralization, geometry and mechanical properties of long bones in male piglets.
β-hydroxy-β-methylbutyrate was given *per os* to two sows in the daily dose of (0.2 g/kg) BW from the 70th upto 90th day of pregnancy. After birth, six randomly chosen male piglets from HMB group and six from the control (without HMB) were euthanized and both humeri were analyzed to assess the bone morphometry, bone mineral density (BMD), geometry (A, cross section area; IC, cortical index; and MRWT, mean relative wall thickness), maximum elastic strength (Wy) and ultimate strength (Wf).
Maternal treatment with HMB significantly increased bone mass comparing to the control ($8.8 \pm 0.82$ and $5.0 \pm 0.29$ g respectively, $P < 0.05$). Similarly, the length increased from $43.8 \pm 2.32$ to $56.3 \pm 1.79$ mm in the HMB group. Moreover, Wy, Wf, A and MRWT were increased by 45, 119, 56, and 33% respectively due to HMB treatment. Whereas BMD of distal ($2.2 \pm 0.03$ g/cm$^2$) and proximal ($2.2 \pm 0.07$ g/cm$^2$) part of humerus decreased in HMB group compared to control ($5.6 \pm 0.36$ and $2.6 \pm 0.25$ g/cm$^2$ respectively). Furthermore, BMD of articular cartilage was lower in HMB group when compared with the control group.
Maternal HMB treatment stimulated ossein formation hence BMD was decreased in larger and heavier bones. It can be assumed that β-hydroxy-β-methylbutyrate exerted a great positive effect on morphology, geometry, and mechanical properties of humeri resulted in development of more mature bone in male offsprings.
Declaration of Interest
There is no conflict of interest.

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## PP63

**Adiponectin induce elasticity of bones**

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It is known that body weight is positively correlated with increased bone mineral density and decreased fracture risk. Adiponectin is expressed in bone forming cells, and administration of adiponectin has been demonstrated to stimulated osteoblast proliferation. The overall role of adiponectin in bone metabolism remains unrevealed as both stimulated and inhibiting effects on osteoclastogenesis have been reported. We aimed at describing the relationship between adiponectin and mechanical properties of bone, and characterize possible autocrine and paracrine effects of adiponectin on osteoblast and osteoclast.
There were no significant relationship between plasma adiponectin levels and bone biomechanical data in rats. In contrast, the relative expression of adiponectin mRNA in femur correlated positively to ultimate bending moment, ultimate energy absorption and deflection, however, negative to bending stiffness. Recombinant adiponectin (80 ng/ml) induced spongier and more fragile spheres of three-dimensional bone formation models produced in rotating co-cultures of human osteoblasts and osteoclasts.
Adiponectin mRNA are expressed in human monocytes and the expression decreased upon stimulation with RANKL and MC-CSF and differentiation to osteoclasts. We found recombinant adiponectin (0.08–10 μg/ml) to stimulate human osteoclast differentiation and activity, whereas no effect was observed on osteoblast differentiation and the expression of markers involved in osseoid mineralization (alkaline phosphatase, collagen type 2, osteocalcin and CD44) in mesenchymal stem cells. Beside acutely up-regulating adiponectin mRNA expression, adiponectin also stimulated leptin expression and secretion.
Adiponectin has effects on the elasticity and flexibility of the bones, by regulating recruitment and differentiated endpoint of cells involved in bone remodelling.

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## PP64

**Efficacy of a combination of simvastatin and poly(DL-lactic-co-glycolic acid) in stimulating the regeneration of bone defects**

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The proper healing of bone defects requires a bone graft or bone substitute and synthetic materials have been developed as alternatives to autografts and allografts. Poly(DL-lactic-co-glycolic acid) (PLGA) is a synthetic polymer widely used for bone healing because of its biocompatibility and biodegradability. PLGA scaffolds have also been used in drug delivery devices, such as in combination with simvastatin, to stimulate bone growth. In this work, we examined the usefulness of a combination of PLGA with simvastatin for treating bone defects. For this, two defects were created in rat calvaria and in half of the animals the right sides were filled with PLGA scaffolds while the other half received PLGA plus simvastatin; the left sides remained empty. The rats were killed for histological analysis after 4 and 8 weeks. There was a significant increase in the amount of bone formation in the treated lesions, particularly those that received PLGA plus simvastatin. The animal use described here was approved by an institutional Committee for Ethics in Animal Use of University of Paraiba Valley (UNIVAP) (protocol n° A051/CEP/2009) and the experiments were done within the general guidelines of the Brazilian Society for Laboratory Animal Science (SBCAL) and the principles of laboratory animal care (NIH publication 42–75, 2011).

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## PP65

**Influence of BMI on pubertal development and bone mass accrual in apparently healthy school children aged 6-17 years**

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Aim
To evaluate progression of bone mineral density (BMD) and bone mineral content (BMC) during pubertal development and influence of body composition and vitamin D on BMD and BMC in children and adolescents.
Materials and methods
This cross-sectional study was part of an ongoing health survey of Delhi school children. Total 1905 apparently healthy school children (835 boys; and 1070 girls) in the age group of 6–17 years were studied. After brief history, tailored clinical examination and anthropometry, blood samples were collected for measurement of serum 25-hydroxy vitamin D (S.25VitD) and parathyroid hormone (iPTH). Whole body DXA scans were performed (GE Lunar Prodigy scanner). Fat mass index (FMI) was calculated.
Results
The mean age of subjects was $13.27 \pm 2.48$ years while mean FMI was $5.59 \pm 3.1$ kg/sqM. Vitamin D deficiency (VDD; S.25Vit D $< 20$ ng/ml) was present in 96.8% subjects. BMD and BMC increased progressively with progression of puberty in both boys and girls but maximum gain was observed from pubertal stages 2 to 4 while no significant gain in BMD or BMC was seen between pubertal stages 4 and 5. Boys showed higher percentage rise in BMD from stages 1 to 5 in comparison to girls. FMI did not show any significant correlation with S.25VitD or iPTH but showed significant positive correlation with total body bone mineral content (TBMC; $r = 0.40$, $P \leq 0.001$), lumber spine BMD (LSBMD; $r = 0.11$, $P \leq 0.001$) and with femoral neck BMD (FNBMD; $r = 0.13$, $P \leq 0.001$). Similarly, total lean mass also showed positive correlation with TBMC, LSBMD and FNBMD in all subjects as well in all pubertal groups. S.25VitD was also positively correlated with TBMC ($r = 0.13$, $P \leq 0.001$), LSBMD ($r = 0.15$, $P \leq 0.001$) and FNBMD ($r = 0.06$, $P = 0.006$).
Conclusion
BMD progressively increases during pubertal development with maximum gain occurring between pubertal stages 2 and 4 with non-significant change between stages 4–5.

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## PP66

### Interrelationships between body weight, bone-specific alkaline phosphatase BAP, IGF1 and morphometric, densitometric and mechanical properties of deciduous mandibular teeth properties in Polish Merino Sheep

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The aim of this study was to determine interrelationships between final body weight, bone-specific alkaline phosphatase (BAP), IGF1 and morphological, densitometric and mechanical properties of deciduous mandibular teeth isolated from seven healthy Polish Merino rams. Four incisors ($I_1$–$I_4$) and second premolar ($P_2$) were obtained from 5-month-old sheep. Teeth were cleaned, weighed, and their length was measured. Mean volumetric tooth mineral density (MvTMD) and total tooth volume (Tvol) were determined using quantitative computed tomography (QCT) and SOMATOM EMOTION SIEMENS apparatus (Siemens, Erlangen, Germany) equipped with Somaris/5 VB10B software and Volume Evaluation application package. For Tvol and MvTMD determinations, volume-of-interest was defined between minimum and maximum density of the investigated samples at 0 and 3071 Hounsfield units (HU), respectively. Micro computed tomography (micro CT) and SkyScan 1174 apparatus (SkyScan n.v., Kontich, Belgium) were used to measure total enamel volume (Evol), volumetric enamel mineral density (vEMD), total dentine volume (Dvol) and volumetric dentine mineral density (vDMD). The volume-of-interest was defined between minimum and maximum density of the investigated samples at –1000 and 3071 HU. To determine mechanical properties, all incisors were subjected to a three-point bending test and maximum elastic strength (Wy) and ultimate force (Wf) were determined. In compression test Wf of $P_2$ was determined (Zwick/Roell Z010 apparatus; Zwick, Ulm, Germany). BAP and IGF1 were determined in serum of 28- and 150-day-old sheep using immunoenzymometric assays (Ostease BAP and IGF1 ELISA, Immunodiagnostic Systems Ltd, Boldon, Tyne & Wear, UK). Pearson's correlation coefficient ($r$) between final body weight, BAP, IGF1 and morphological, densitometric and mechanical properties of teeth was determined. Final body weight was positively correlated with Tvol and Dvol of $I_1$, $I_4$ and $P_2$ ($r>0.88$; $P<0.05$). IGF1 in 28-day-old sheep was negatively correlated with MvBMD of $I_1$, $I_2$, $I_3$, $I_4$ and $P_2$ ($r<-0.82$; $P<0.05$). IGF1 in 28-day-old sheep was positively correlated with Wf of $P_2$ ($r=0.84$; $P<0.05$). IGF1 in 150-day-old sheep was negatively correlated with vDMD of $I_2$, and Tvol and Dvol of $I_4$ ($r<-0.86$; $P<0.05$). BAP in 150-day-old sheep was negatively correlated with Wy and Wf of $I_2$ and MvBMD of $P_2$ ($r<-0.76$; $P<0.05$). In conclusion, this study revealed that body weight of sheep is associated with morphological properties of teeth. Interrelationships of IGF1 and BAP concentration in serum with morphological, densitometric and mechanical properties were proven.

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## PP67

### Prevalence of vertebral fractures, after 50 years old

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Introduction
Fragility fractures are number growing. They reduced quality life and increased mortality. After 50 years, one woman and two men will have an osteoporotic fracture in their lifetime. The presence of a vertebral fracture (VF) multiplied the risk of new vertebral fracture and the risk of hip fracture. They are pauci or asymptomatic.
Study purpose
Evaluate the prevalence of fragility vertebral fractures after 50 years, the number of asymptomatic fractures, and other forms of spinal disease which may constitute a differential diagnosis.
Materials and methods
Prospective study conducted between December 2012 and March 2013. We included 51 patients $\geq$ 50 years old. Analysis of thoraco-lumar radiographs was based on the semi-quantitative method of Genant (grade,seat and VF number). The prevalence of FV was calculate, and the percentage of asymptomatic fractures.
Results
Our population was predominantly female (86.2%). The mean age was 61 years. 66.6% of patients had back pain. 19 cases had at least one VF. Among them, six were asymptomatic. In the FV group, six patients had a personal history of bone fragility factors. Two had a history of non-traumatic fracture and one case among parents. The majorities of patients were in vitamin D or already supplemented. Five cases treated for osteoporosis, ten patients were under anti-osteoporotic, four unknown osteoporotic. 11 cases followed for osteoarthritis. Three cases had the notion of size loss between 2 and 5 cm. 13 had a fracture grade I, four grade II and two grade III. Ten patients had a single fracture, 8 two fractures, and only one patient, three fractures. All fractures were lumbar seat and attributed to osteoporosis.
Discussion and conclusion
The clinical signs of vertebral fractures are not specific. Their severity is variable. They pose problem of differential diagnosis of vertebral deformities or neoplastic. View prognosis, research vertebral fractures after 50 years is essential.

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## PP68

### Increased expression of PTX3 in non-hematopoietic periosteal cells during fracture healing

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Pentraxin 3 (PTX3) is a highly conserved member of the long pentraxins subfamily produced in response to proinflammatory stimuli. Its biological roles have been associated to infection, female fertility and angiogenesis. We aimed to elucidate the role of PTX3 in the process of fracture healing.
WT mice and mice deficient for the PTX3 gene (PTX3-KO) were used, after obtaining the approval from the Ethical committee. Cells from bone marrow and endosteal/periosteal compartments were cultured to stimulate differentiation into osteoblast or osteoclast lineage. Same populations were analyzed by flow-cytometry to assess the proportion of putative osteoclast and osteoblast progenitor cells. Bone metabolism *in vivo* was determined by histomorphometry and micro-computerized tomography (μCT). The method of Bonnarens and Einhorn was modified to produce standardized closed tibial fracture. Bones were analyzed at early (2 and 6 days) and late stage (3 weeks) of fracture healing.
PTX3-KO mice had lower bone mass (BV/TV $2.72 \pm 1.23$ for females and $5.39 \pm 1.73$ for males) than their WT littermates (BV/TV $5.03 \pm 0.87$ for females and $7.04 \pm 0.87$ for males, $P<0.05$). Although, we found increased PTX3 expression with the maturation of osteoblast and osteoclast *in vitro*, no differences were observed in the osteoblastogenic and osteoclastogenic *ex vivo* potential between WT and PTX3-KO mice. Nevertheless, we found lower bone formation rate in PTX3-KO mice. Non-hematopoietic periosteal cells highly up-regulate PTX3 expression during initial phase of fracture healing. Moreover, PTX3-KO mice formed significantly less mineralized callus following bone fracture (BV/TV $15.33 \pm 2.32$ vs $19.66 \pm 4.32$, $P<0.05$).
Our results confirmed that PTX3 has a positive impact on bone mass. Particularly, we showed that bone regeneration requires PTX3 for the induction of periosteal reaction, supporting previous studies that indicate important role of various inflammatory molecules in the initial phase of fracture repair.

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## PP69

### Enhancement of fracture repair by upregulation of the innate immune response

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Osteoporotic fractures are very common and represent an enormous unmet medical need. Our group has previously reported that addition of rTNF to the fracture site promotes fracture healing in C57/BL6 mice (Glass *et al. PNAS* 2011). Using a murine fracture model of endochondral healing, we observed that local addition of rTNF only accelerates fracture repair if administered within the first 24 h following injury. The optimal therapeutic dose is 1 ng. TNF is first expressed by neutrophils in the first 72 h followed by F4/80+ monocytes/macrophages. Furthermore, downregulation of early inflammation using anti-TNF or rIL10 impaired fracture healing. To quantify the recruitment of innate immune cells, we used a murine air-pouch model whereby murine fracture supernatants +/− rTNF was injected. Fracture supernatants are generated by incubating fracture fragments in media overnight to capture the local cytokine environment of the fracture site. We found that addition of rTNF promoted neutrophil recruitment, which in turn promoted recruitment of monocytes/macrophages by CCL2 production. Macrophages have previously been reported to be critical in bone repair (Alexander *et al*. *JBMR* 2011). Furthermore, neutrophil depletion using anti-Ly6G antibody and inhibition of the chemokine receptor for CCL2, CCR2, led to significantly impaired fracture healing. Finally, local treatment with rTNF enhanced fracture repair in ovariectomy-induced osteoporotic mice by 40%. Thus, we have shown that TNF is a key upstream inflammatory mediator in fracture repair. Mechanistically, addition of rTNF upregulates CCL2 production and monocyte recruitment. Our findings provide evidence that the innate immune response represents a viable therapeutic target in the enhancement of fracture healing.

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## PP70

### The roles of CDC42 in bone development

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Members of the Rho family including small GTPase Cdc42 have been shown to play multiple roles in cell regulation, including regulation of cytoskeletal organization, cell migration, proliferation, and apoptosis. However, their tissue-specific roles *in vivo*, especially in limb bud mesenchyme, remain largely unknown. Herein, we report that conditional deletion of Cdc42 in AER dose not change the limb development, however, knockout of Cdc42 in mesenchymal stem cells of limb bud (PrxCre;$Cdc42^{f/f}$) results in a severe phenotype of bone formation. Knockout of Cdc42 demonstrated short limbs and body, abnormal calcification of the cranium. Severe defects were also found in long bone growth plate cartilage, characterized by loss of columnar organization of chondrocytes, and thickening and massive accumulation of hypertrophic chondrocytes, resulting in delayed endochondral bone formation. Analysis of micromass cultures suggest that deletion of Cdc42 in C3H10T1/2 leads to defects in cartilage condensation and reducing the expression of N-cadherin, p-Smad1/5 and p-p38, suggesting that Cdc42 regulates the mesenchymal stem cells condensation via BMP and p38 signaling. In situ hybridization analysis revealed that expression patterning of Col10, Ihh, PTHRPr and Mmp13 were changed. Our results demonstrated that knock-down of Cdc42 in the chondrocyte cell line ATDC5 resulted in earlier induction of hypertrophic markers, such as Col10 expression and matrix mineralization. The results point out that Cdc42 regulates prehypertrophy via PI3K-AKT signaling and undergo vigorous apoptosis. These results demonstrate that Cdc42 is essential for bone development, especially the cartilage formation.

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# Calciotropic and phosphotropic hormones and mineral metabolism

## PP71

### Effect of recombinant human parathyroid hormone, rhPTH(1–84), on bone turnover markers and bone mineral density in patients with hypoparathyroidism: 24-week, open-label REPEAT study

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Patients with hypoparathyroidism lack sufficient parathyroid hormone (PTH) and exhibit reduced bone turnover, abnormally increased bone mineral density (BMD), and abnormal bone microarchitecture. Current treatment regimens fail to address underlying PTH deficiency. In the REPLACE phase III trial, treatment with rhPTH(1–84) restored mineral homeostasis, increased bone turnover markers (BTMs), and decreased BMD in patients with hypoparathyroidism.
REPEAT was a 24-week, open-label, flexible-dose extension study at three sites in Hungary. Patients received 50 μg/day (escalated to 75 and then 100 μg/day, if needed) rhPTH(1–84). Serum BTMs analyzed were bone-specific alkaline phosphatase (BSAP), cross-linked C-telopeptide of type 1 collagen (CTX), aminoterminal propeptide of type 1 collagen (P1NP), and osteocalcin (OCN). BMD was determined by dual-energy X-ray absorptiometry (DXA).
At the initiation of REPEAT, enrolled patients ($n=16$, previously treated with rhPTH(1–84) in the REPLACE study; $n=8$, rhPTH(1–84)-naive) had mean BSAP, CTX, and P1NP levels within normal limits, but OCN levels ($3.82\pm1.89$ μg/l) were lower than normal. At week 24, mean BSAP, CTX, P1NP, and OCN levels increased by $21.2\pm14.0$ μg/l (229%), $651.7\pm390.8$ ng/l (398%), $213.4\pm118.5$ μg/l (728%), and $27.6\pm24.2$ μg/l (748%), respectively. Compared with patients who received rhPTH(1–84) in REPLACE, rhPTH(1–84)-naive patients had greater increases in BTMs, with the exception of OCN, for which both groups had similar changes from baseline. Changes in mean absolute DXA values for BMD direct measurements ranged from $0.0349\pm0.0786$ to $-0.0138\pm0.0658$ g/cm$^2$ for the seven locations evaluated. Trends toward decreased BMD measurements were greater among rhPTH(1–84)-naive patients. *Z*-scores showed minimal differences from baseline among all patients, but a trend toward decreased *Z*-scores was observed among treatment-naive patients, with the exception of the distal one-third radius. rhPTH(1–84) was generally well tolerated.
Treatment with rhPTH(1–84) was associated with increases in BTMs for all patients, even when retreated with rhPTH, and decreases in most BMD measurements and *Z*-scores for rhPTH(1–84)-naive patients.

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## PP72

### Candidate reference methods for the harmonisation of parathyroid hormone (PTH) assays: consideration of variant forms including phosphorylated PTH

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The analysis of intact parathyroid hormone (PTH) (PTH1–84) is useful in the diagnosis of hyper- and hypocalcaemia, hyperparathyroidism, and in the prevention of bone mineral disorders in renal patients. However, the analysis is complicated by the presence of PTH fragments. These are especially prone to accumulation in renal failure, and cross-react differently with different immunoassays, including the most recent, third-generation immunoassays. As such, large variability exists between different commercially available assays, as well as reference ranges for guiding treatment. This variability may be considered a critical governance issue in renal patients, and harmonisation of testing is urgently required.
Following the 50th anniversary of the introduction of the first PTH R/A last year, there has been little standardisation of PTH testing, despite our increased understanding of PTH biology. Recently, mass spectrometric methods have been developed as 'reference methods' for harmonising the quantitation of PTH1–84, but even these methods are subject to significant interferences from variants including oxidised PTH. In this work, we report the use of mass spectrometric immunoassay for the identification of phosphorylated PTH in clinical samples. The phosphorylated tryptic peptide, $PTH_{14–20}$ ($HLNS_{(P)}MER$), was detected in 58 (16%), and quantified in 15 (4%) of samples (LOD and LLoQ 10 and 30 pg/ml

respectively). The median (range) concentration in samples with quantifiable phosphorylated $PTH_{14–20}$ was 52 (32–767) pg/ml, compared to a median (range) concentration of 287 (43–1883) pg/ml for the equivalent non-phosphorylated $PTH_{14–20}$ peptide (LLoQ 30 pg/ml). There was no correlation between concentrations of HLNSMER and $HLNS_{(P)}MER$ ($R^2=0.01$).
Further clinical investigation into the biological activity of these variants is necessary, plus further analytical work including i) the use of high-resolution mass spectrometry, and ii) the analysis of PTH without prior protease digestion, is required before mass spectrometry-based approaches can be considered as reference methods against which other methods should be harmonised.

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## PP73

**S.C. injection of recombinant human parathyroid hormone rhPTH(1–84) in thigh provides a more prolonged pharmacokinetic profile and a greater calcemic response when compared with injection in abdomen**

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Recombinant human parathyroid hormone (rhPTH) (1–84) is currently being developed as PTH replacement therapy for patients with hypoparathyroidism. Because rhPTH(1–84) is administered subcutaneously, we compared the pharmacokinetic profile of PTH(1–84) and the calcemic response following S.C. rhPTH(1–84) injection in thigh and abdomen.
In this phase 1, open-label, three-way crossover study, healthy postmenopausal women received three randomized doses of 100 µg rhPTH(1–84) S.C. administered with an injection pen cartridge in the thigh (testosterone) or abdomen (A1 and A2 as two separate injections). At least 5 days separated the three injections. Blood samples were collected immediately before and up to 24 h after each injection for measurement of plasma PTH(1–84) and serum total calcium levels.
Eighteen women (mean age, $60.5\pm7.6$ years; baseline PTH(1–84) 14.8–38.7 pg/ml) received each of the three injections; 16 women had PTH(1–84) levels above baseline for all three injections and were included in this analysis. The PTH(1–84) concentration vs 24 h time profile following injection in testosterone was more prolonged compared with A. Maximum levels occurred at a mean 1.04 (testosterone), 0.99 (A1), and 0.93 (A2) h after injection. The mean baseline-corrected PTH(1–84) maximum concentration achieved ($C_{max}$) was 303 (testosterone), 546 (A1), and 473 (A2) pg/ml, and exposure ($AUC_{0–t}$) was 1349 (testosterone), 1565 (A1), and 1524 (A2) pg•h/ml. Despite the lower $C_{max}$ and $AUC_{0–t,}$ in testosterone, the mean time to return to predose levels was 12–24 (testosterone) vs 10 h (A1/A2). The serum total calcium profile with injection in testosterone showed a more sustained increase (return to mean baseline levels in $>24$ h) compared with A (return to baseline in 14–24 h).
The longer duration of exposure to elevated PTH(1–84) levels following rhPTH(1–84) S.C. in thigh and the resulting extended calcemic response suggests that thigh is the preferred route of rhPTH(1–84) administration for the treatment of hypoparathyroidism.

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## PP74

**Bone turnover and FGF-23 levels in vitamin D-deficient critically ill patients with and without acute kidney injury**

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Introduction
Elevated FGF-23 serum levels are induced by hyperphosphatemia and are linked to poor skeletal mineralization and adverse outcomes including vascular calcification and mortality. Recently, it was shown that FGF-23 levels are substantially elevated in acute kidney injury (AKI), and that higher levels in AKI are associated with a greater risk of adverse outcomes.
Methods
In 25 vitamin D deficient (25(OH)D $<20$ ng/ml) critically ill adults with and without AKI, markers of bone and mineral metabolism (25-hydroxyvitamin D, 1,25-dihydroxyvitamin D, parathyroid hormone, ionized and total serum calcium, phosphorus, bone specific alkaline phosphatase, osteocalcin, tartrate resistant acid phosphatase, β-crosslaps) were measured. FGF-23 was measured using a C-terminal FGF-23 ELISA (Immutopics, San Clemente).
Results
The mean age was $63\pm16$ years and 76% were men. FGF-23 levels were significantly higher in patients with AKI (median 2720 RU/ml, range 351–8708) compared to patients without AKI (150 RU/ml, 55–14 000; $P<0.001$) and nonsignificantly higher in nonsurvivors ($n=12$, 624 RU/ml, 61–8709) compared to survivors (312 RU/ml, 55–14 000, $P=0.453$). There was no significant correlation between FGF-23 and any of the measured biochemical markers.
Discussion
In our small cohort of medical critically ill patients, FGF-23 levels were substantially higher than in other reported populations. In patients with AKI these were significantly higher than in patients without AKI. There was no significant correlation of FGF-23 and any of the determined markers of bone and mineral metabolism, although this may primarily be a matter of small sample size, as the correlation coefficient was strongest for serum phosphorus, ionized calcium and osteocalcin and reached values of almost 0.3. In conclusion, the role of FGF-23 in critical illness remains unclear and further studies on this topic are warranted.

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## PP75

**Low calcium-phosphate diet attenuates dietary protein deficiency-mediated impairment of bone growth by blunting the decrease in serum IGF1 and in hepatic GH receptor gene expression**

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Low protein diet (LPD) impairs body growth, decreases serum IGF1 and increases serum FGF21, two hepatokines influencing bone growth. Low calcium-phosphate diet (LCaPiD) increases serum calcitriol. We hypothesized that LPD effects on bone growth may differ according to calcium and phosphate intakes through the hormonal modulation of calcitriol, IGF1 and FGF21. One-month old rats were fed isocaloric diets containing 10 or 5% casein (10Prot, 5Prot), with 1.0% calcium and 0.8% phosphorus (normal; NCaPi) or 0.2% calcium and 0.16% phosphorus (low; LCaPi) for 8 weeks. Tibia bone microarchitecture was analyzed by microCT, BMC by DXA, tibia midshaft and proximal strength by flexion and compression tests, respectively, and cortical tissue hardness by nanoindentation. Independently of CaPi intakes, LPD led to body growth retardation. In NCaPi, LPD decreased bone strength and altered microstructure in association with higher serum FGF21 ($+466\%$, $P<0.01$), lower serum IGF1 ($-24\%$ $P<0.05$) and reduced hepatic GH receptor (GHR) gene expression ($-48\%$ $P<0.01$). In LCaPi, LPD-related effects were attenuated in association with a lower decrease in serum IGF1 ($-11\%$, vs 10ProtLCaPi, $P=0.14$) and hepatic GHR mRNA levels ($-30\%$ vs 10ProtLCaPi, $P<0.01$). The latter was however higher than 5ProtNCaPi ($+48\%$ vs 5ProtNCaPi, $P<0.05$). In LCaPi, LPD still maintained high serum FGF21. Cortical tissue hardness was not affected in 5ProtNCaPiD, while it was lower in 5ProtLCaPiD ($-15\%$ vs 10ProtLCaPi; $P<0.01$). Positive correlations were observed between serum IGF1 and midshaft Ct.BV ($r=0.73$, $P<0.01$) and proximal BV/TV ($r=0.52$, $P<0.01$). In LCaPi groups, normocalcemia and normophosphatemia were maintained together with increased serum calcitriol. In conclusion, LCaPiD attenuated the LPD–related alteration of bone growth by reducing hepatic GH resistance, however at the expense of cortical tissue hardness. Our results suggest that a factor induced by LCaPiD could act at the liver level to attenuate the LPD effects.

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## PP76

**Relationship between vitamin D levels and carotid arterial stiffness in postmenopausal women with hypertension**

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Arterial stiffness or pulse wave velocity (PWV) is increasingly recognized as a strong predictor of future cardiovascular events and all cause mortality. Literature date indicate a relationship between vitamin D (25-OHD) deficiency and arterial

stiffness in isolated systolic hypertension (ISH), but it is still unclear how 25-OHD deficiency may contribute to functional changes of the arterial wall in hypertensive patients. This study aimed to evaluate the relationships between PWV at carotid artery, 25-OHD and parathyroid hormone (PTH) in postmenopausal women with ISH. Seventy five consecutive ambulatory postmenopausal women with ISH (mean $66.9 \pm 7.8$) were recruited for the study. In the morning, systolic and diastolic blood pressure (BP) were recorded in seated position by a semi-automated oscillometric method, and a venous sample was taken to measure 25-OHD and PTH levels. Finally, in all patients, an echocolor-doppler of the carotid vessels was performed (MyLab 60 with a 7.5 MHz probe, Esaote, GE, Italy), to assess, by automatic RF echo-tracking software, intima-media thickening (IMT) and RF quality arterial stiffness (QAS). After adjusting for age, PWV showed a positive correlation with systolic BP ($r=0.33$; $P<0.01$) and with PTH ($r=0.35$; $P<0.01$) and a negative correlation with 25-OHD serum levels ($r=-0.40$; $P<0.01$). Moreover, in a multivariate regression model 25-OHD levels were independently associated with PWV at carotid artery. In conclusion, in postmenopausal women with ISH the serum levels of 25-OHD are independent predictor of PWV and the negative effect of reduced levels of 25-OHD might mediated by PTH. Further longitudinal studies are warranted to define the role of reduced levels of vitamin D in the pathogenesis of hypertension.

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## PP77

### Elevated blood serotonin decreases bone volume via a mechanism involving insulin and calcitriol

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To further characterize the role of serotonin (5HT) in bone metabolism, Wistar-Zagreb rat model consisting of the high-5HT and low-5HT subline with different platelet 5HT content and transporter (5HTT) activity has been used.
Primary osteoclasts from the high-5HT subline had an increased expression of 5HTT, 5HT–2A receptor, Trap and cathepsin K, while osteoblasts showed no difference in the expression of 5HT elements or osteoblastic markers. However, upon addition of 5HT and insulin combination, expression of Alph and osteocalcin in osteoblasts was significantly upregulated.
In 8 weeks old rats the bone volume of distal femur and lumbar spine was significantly decreased in high-5HT rats. Histomorphometric analysis revealed increased osteoblast activity in these animals, while no change in the number of bone cells was observed. Serum CTX value was significantly increased in high-5HT animals while no change in the osteocalcin level was detected. 5HT level had no effect on the PTH value, but calcitriol and FGF23 were decreased in high-5HT rats. Treatment with calcitriol reduced the platelet 5HT content by 20% in high-5HT rats while PTH had no effect on platelet 5HT content.
High-5HT rats had a higher level of blood glucose and insulin, resulting in a higher body mass as compared to low-5HT rats. A single streptozotocin injection lowered the insulin plasma value with a consequent decrease in the platelet 5HT by 20%. Treatment with fluvoxamine, a 5HTT inhibitor, reduced the 5HT level in both sublines by 50% that was accompanied by decreased insulin in plasma of low-5HT rats.
These results demonstrate that 5HT affects the bone volume via a complex autocrine/paracrine and endocrine mechanism involving insulin and calcitriol. Thus, 5HT is not a single mediator of bone metabolism, but influences the bone volume via a complex biochemical network of hormones and growth factors associated with 5HT homeostasis.

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## PP78

### Effects of TSH and calcitriol on bone metabolism: *in vivo* and *in vitro* study

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TSH exerts both antiresorptive and anabolic effects on bone remodeling in aged ovariectomized rats and $TSH^{-/-}$ mice, supported by clinical results demonstrating that low TSH level is associated with increased bone loss. To further explore the effect of TSH on bone metabolism we here introduced a rat model with removed thyroid and parathyroid glands to obtain low serum concentrations of thyroid and parathyroid hormone, calcitonin and $1,25(OH)_2D_3$ (approval for the experiment was obtained from ethical committee of University of Zagreb). Surgery resulted in hypocalcemia, low parathyroid and thyroid hormone, $1,25(OH)_2D_3$, C-telopeptide and osteocalcin serum level. Intermittent administration of TSH resulted in a further decrease of serum calcium and decreased level of serum C-telopeptide due to the suppression of bone resorption, while serum osteocalcin level was higher indicating an increased bone formation rate. MicroCT analyses of the distal femur and proximal tibia showed that rats treated with $1,25(OH)_2D_3$ alone or in a combination with TSH had an increased trabecular bone volume, and enhanced trabecular bone quality. Biomechanical testing of the trabecular bone showed an increased maximal load for 105 and 235%, respectively, in rats treated with $1,25(OH)_2D_3$ alone, or in a combination with TSH. Rats treated with TSH had a significantly decreased number of osteoclasts in comparison to TPTx control animals. The decline of the osteoclast number was even greater in rats treated with a combination of TSH and $1,25(OH)_2D_3$, despite the fact that $1,25(OH)_2D_3$ increased the number of osteoclasts. Addition of TSH to osteoblasts increased the production of the bone specific alkaline phosphatase and had a synergistic effect when combined with $1,25(OH)_2D_3$. We suggest that TSH independently of calciotropic hormones suppressed bone resorption and stimulated bone formation, while in combination with $1,25(OH)_2D_3$ acted synergistically on bone formation resulting in an increased bone volume.

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## PP79

### Associations among vitamin D binding protein polymorphisms and concentrations and total, free, and bioavailable serum 25-hydroxyvitamin D concentrations in finnish adults

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Vitamin D and its metabolites are bound to vitamin D binding protein (DBP) in the circulation. Only a small proportion is in unbound, free form. According to the 'free-hormone-hypothesis' only the free form is biologically active. DBP is a polymorphic protein and different variants of the protein differ in their ability to bind vitamin D. This may have an impact on the amount of free and bioavailable (free + albumin-bound) 25-hydroxyvitamin D (25(OH)D) in the circulation. We investigated the associations among DBP polymorphisms, total-, free- and bioavailable serum 25(OH)D in 617 healthy 37–47 year old Caucasian women and men in a cross-sectional study. Fasting blood samples were collected and concentrations of S-25(OH)D and DBP were determined from the blood. Dietary and supplement intake of vitamin D and calcium (Ca) as well as background data about lifestyle were collected. SNP's 4588 and 7041 were genotyped based on polymorphisms in the DBP coding gene. They combine to form six common diplotypes and three haplotypes. The concentrations of free and bioavailable S-25(OH)D were calculated for the diplotypes and haplotypes according to published binding coefficients. The calculated amount of free and bioavailable S-25(OH)D was 0.02 and 8.8%, respectively, of the total measured S-25(OHD). We found a significant difference in the DBP concentrations among the haplotypes and almost significant between the diplotypes. S-25(OH)D concentrations did not differ between the haplotypes but there was a significant difference among the diplotypes. When the haplotype and diplotype free and bioavailable S-25(OH) D concentrations were SNP adjusted, both the free and bioavailable S-25OHD among the genotypes differed significantly. In conclusion, DBP polymorphism affects the total, free and bioavailable concentrations. Free

and bioavailable S-25(OH)D may bring new aspects to associations between vitamin D status and health outcomes.

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## PP80

**Histological and analytical studies in the role of melatonin in the formation and composition of incremental lines in dentin**

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The purpose of the present study is to examine the relationship between the formation and composition of incremental lines in tooth dentin and the role of melatonin through histological and analytical studies. In this experiment, 5, 6 and 7 day old SD rats were used. These rats were divided into three groups: i) a control group (0.5% alcohol content drinking water); ii) a low concentration group (0.5% alcohol + 20 μg/ml melatonin content drinking water); and iii) a high concentration group (0.5% alcohol + 100 μg/ml melatonin content drinking water). The animal protocol was approved by the Animal Care and Use Committee of Mekai University. The specimens were observed and analyzed using light microscopy, scanning electron microscopy, transmission electron microscopy, laser Raman microprobe spectrometry, and an electron-probe microanalyzer (EPMA). In the control group, two dark-staining incremental lines of hematoxylin and one light staining layer were observed in incisor dentin. In the low-melatonin concentration group, the light staining layer became narrow. In the high-melatonin concentration group, this layer disappeared. The number and size of calcospherites in predentin increased in proportion to the concentration of melatonin administered. A new incremental line was confirmed in both the incisor and molar dentin of the melatonin treated groups. In the high-concentration group, a strong expression of ALP activity was observed. In EPMA analysis, Ca and P content were increased in the melatonin treated group. By laser Raman microprobe spectrometry, the peak of $PO_4^{3-}$ was higher in the high-concentration group. It is considered that melatonin participates in the formation of incremental lines and the calcification mechanism of dentin. The work was supported by KAKENHI (23592727).

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## PP81

**1,25-Dihydroxy vitamin D assay with on-board sample purification on the IDS-iSYS automated system**

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1,25-Dihydroxyvitamin D (1,25D) is one of the major regulators of calcium metabolism. Due to its lipophilic nature and low circulating concentration, the measurement of 1,25D concentration levels has been labour intensive and technique dependent in addition to multiple equipments required for the sample purification procedure. We reported the results of soon to be commercialised fully on-board IDS-iSYS 1,25 VitD$^{Xp}$ assay.

IDS-iSYS 1,25 VitD$^{Xp}$ assay purifies the human sera utilising the anti-1,25D antibody coated magnetic particles to capture 1,25D in cuvette 1. After incubation, the magnetic particles are washed and 1,25D is eluted. The eluate is transferred to cuvette 2 where the immunoassay procedure will take place, using the currently available IDS-iSYS 1,25-dihydroxy vitamin D test. Below are the preliminary analytical performance of the IDS-iSYS 1,25 VitD$^{Xp}$ (iSYS XP125) assay:

Combining the innovative on-board sample purification procedure with the already proven IDS-iSYS 1,25-dihydroxy vitamin D test, the IDS-iSYS 1,25 VitD$^{Xp}$ not only enhances the clinical laboratory 1,25D testing efficiency, it also delivers accurate results for patient care.

| Performance | Result |
|---|---|
| Inter-assay precision | 9.8% (18.7 pg/ml)<br>8.3% (59.3 pg/ml)<br>5.8% (95.5 pg/ml)<br>6.1% (163.9 pg/ml) |
| Linearity | 90–113% |
| Method comparison against current IDS-iSYS 125D ($n$=121) | ISYS XP125 = 0.99 × (iSYS 125D) − 0.75 pg/ml $r$=0.97 |

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## PP82

**Comparison of proportions of T lymphocyte subsets according to serum 25(OH) vitamin D levels in postmenopausal women**

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Many reports have proposed significant role of vitamin D on immune mediated disease. This study analyzed lymphocyte subsets according to serum 25(OH) vitamin D levels in postmenopausal women to determine the potential effect of vitamin D on immune-mediated disease.

On a prospective observational basis, we enrolled 31 postmenopausal women who underwent health checkup in our hospital. Peripheral blood drawn for the estimation of total T, B cell, NK cell count, proportion of CD3+, CD4+,CD8+, Th1, Th2, Th17 and Treg subsets of T lymphocyte after fasting on the morning. We also measured BMI, estrogen, fasting glucose, hs-CRP, lipid profile, serum 25(OH)D, calcium, phosphate.

The subjects were divided into three groups according to serum vitamin D levels. Proportion of CD4$^+$ ($P$=0.024) T cells were significantly decreased in three tertile group compared with one tertile group. Proportion of CD8+ ($P$=0.004) T cells in three tertile group were significantly increased than that of the one tertile group and three tertile group. Also, CD4$^+$/CD8$^+$T cell ratio in one tertile group was significantly increased than that of the two tertile group and three tertile group. However there was no differences in total T, B, NK cell count, Th1, Th2 cytokine producing T cell population, Th17 and Treg cells according to vitamin D levels.

In this study, serum vitamin D levels in postmenopausal women is associated with changes in the peripheral CD4+, CD8+T cell compartment.

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## PP83

**Serum serotonin: useful for the assessment of the bone metabolism status?**

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Introduction

Atypical markers will provide interesting information in order to assess the bone metabolism. Serotonin has a dual action related to the bone and its serum level is the easiest to use in daily clinical practice also the exact value is not very well described up to this moment.

Aim

We correlate to serum levels of serotonin with different results of clinical bone health evaluation in menopausal women.

Materials and methods

A transversal study enrolled menopausal women with no previous specific medication for osteoporosis. The study was conducted in Parhon National Institute of Endocrinology, Bucharest, Romania. No patient with previous neuroendocrine tumor or carcinoid syndrome was enrolled. The serum serotonin

(ELISA) had normal levels between 40 and 400 ng/ml. The bone evaluation was performed by central DXA (GE Lunar Prodigy) and Heel Quantitative Ultrasound (Achilles Insight). The WHO groups based on central DXA were formed. The SPSS21 was used for statistic. Statistical significance was at $P$ value $<0.05$.
Results
132 women had mean age of 56.3 years, and mean left Achilles stiffness index (ASI) of 80; mean lumbar bone mineral density (BMD) was 1.04 g/cm$^2$. 52 subjects had normal DXA, 62 patients had osteopenia, and 18 women had osteoporosis. The mean serotonin (of 159 ng/ml) was in osteoporosis groups of 147.8 ng/ml, in osteopenia of 164.2 ng/ml, and in women with normal DXA of 156.5 ng/ml. The linear correlation between serotonin levels and lumbar BMD, as well as ASI was not statistical significant.
Discussion
The serotonin has not yet been placed in current clinical practice, but this represents an intro into two different domains: the non-metastatic carcinoid bone loss, and the anti-depression medication (including serotonin mediators) and fracture risk.
Conclusion
No trend line was detected in serotonin levels in patients with different levels of BMD.

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## PP84

**Relation between 25-hydroxyvitamin D level and blood pressure in healthy young Saudi women: effect of adiposity**

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Objectives
The aim of this study was to determine the relationship between 25-hydroxyvitamin D (25(OH) D) level and blood pressure (BP) in Saudi women and to assess the effect of BMI, as a measure of adiposity, on this relationship.
Methods
Three-hundred and three apparently healthy Saudi women (20–40 years old) were randomly selected for this cross-sectional study. All women signed an informed consent and the study was approved by the Ethical Committee. BP was measured using an automated BP monitor (BPTru) following a standardized protocol. Anthropometric measures were taken and fasting blood samples were obtained for the determination of serum 25(OH) D and parathyroid hormone (PTH). Both hormones were determined by chemiluminescence immunoassay method (DiaSorin, Italy). Linear regression models were used to determine the independent correlation between 25(OH) D level and BP.
Results
Vitamin D deficiency was highly prevalent among the studied women with 96.7% having 25(OH) D levels $<$ 50 nmol/l and 70% having levels $<$25 nmol/l. Linear regression analysis showed that 25(OH) D concentrations were negatively correlated to both systolic ($\beta = -$ 5.962, $P=0.001$) and diastolic BP ($\beta=-3.483$, $P=0.029$) in models adjusted for age and PTH. When adding BMI to the model, this correlation was attenuated for the systolic BP ($\beta=-4.848$, $P=0.005$), but became stronger for the diastolic BP ($\beta=-4.291$, $P=0.004$).
Conclusion
In young Saudi women, the correlation between 25(OH) D and BP is independent of BMI. Since both vitamin D deficiency and obesity are prevalent among Saudi women, proper measures for the management of these health problems have to be taken in order to prevent future hypertension and other cardiovascular events. Longitudinal studies to confirm these findings, and intervention studies to assess the BP lowering effects of 25(OH) D on hypertensive and obese subjects are needed.

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## PP85

**The impact on calcium metabolism when replacing intact PTH with teriparatide treatment in patients with hypoparathyroidism**

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PTH replacement therapy in hypoparathyroidism (hypoPT) has become more accepted after proving successful in several clinical studies. Intact PTH ($PTH_{1–84}$) was in 2012 withdrawn, leaving teriparatide ($PTH_{1–34}$) the only therapeutic option available.
All patients with postoperative HypoPT who changed medication from $PTH_{1–84}$ (100 μg) to $PTH_{1–34}$ (20 μg), after at least 12 months of conventional therapy and a minimum of 6 months of $PTH_{1–84}$ were included. The following treatment with $PTH_{1–34}$ was for at least 3 months. Plasma ionized calcium, daily dose of 1α-hydroxylated-vitamin D metabolites (Etalpha), calcium and PTH was collected.
Eight patients (women = 88%) with a mean age of 54 ± 12 years and a duration of hypoPT of 13 ± 6 years were included. Before initiation of $PTH_{1-84}$ the mean daily dose of Etalpha was 1.9 ± 1.1 μg and calcium supplements were 1550 ± 705 mg. Etalpha dose was reduced with 86 ± 35% ($P=0.01$) after 6 months of $PTH_{1–84}$ treatment and terminated in seven patients. Calcium were reduced with 78 ± 36% ($P=0.02$) to 273 ± 353 mg and stopped in four patients. Six patients received 100 μg $PTH_{1–84}$ a day, the seventh received PTH 2 out of 3 days and the last one received $PTH_{1–84}$ every other day.
When changing from $PTH_{1–84}$ to $PTH_{1–34,}$ plasma ionized calcium initially dropped and the demand for supplements increased. Etalpha was resumed in four patients; mean daily dose increased to 0.99 ± 1.26 μg ($P=0.04$) and calcium increased to 329 ± 368 mg ($P=0.72$). Five patients received 20 μg $PTH_{1-34}$ a day; two patients twice a day and one 20/40 μg alternately.
Compared with $PTH_{1–34}$, $PTH_{1–84}$ has a longer plasma half-life and a higher calcemic response. We have shown a need for higher doses of Etalpha and calcium supplements to maintain normal serum calcium when treated with $PTH_{1–34}$ compared to $PTH_{1–84}$ and in some a need for more than one daily dose.

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## PP86

**Circulating myostatin in type 2 diabetes subjects: relationship with bone metabolism and fractures**

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Introduction
Myostatin (growth differentiation factor 8, GDF-8) has an important role in the regulation of muscle mass, and mice lacking the myostatin gene show a generalized increase in bone density and strength. Type 2 diabetes subjects have an increased risk of fragility fractures despite of higher bone mass. Taking into account the myostatin influence in bone strength a better understanding of myostatin actions in type 2 diabetes is of interest.
Objectives
Our aims were to evaluate serum myostatin concentrations in type 2 diabetes patients, and to explore its relationship with bone mineral density (BMD), bone turnover markers and fractures.
Methods
Our study was a cross-sectional one including 73 type 2 diabetes patients. Concentrations of myostatin were measured by ELISA (R&D systems). BMD was evaluated by DXA (Hologic QDR 4500).
Results
Mean age was 56 ± 6 years and duration of diabetes was 13 ± 7 years. Serum myostatin showed no correlation with BMD at lumbar spine ($r=0.074$), femoral neck ($r=0.130$), or total hip ($r=0.174$), $P>0.05$ for all. Moreover, there was no relationship with bone turnover markers: OC: $r=0.080$; BSAP: $r=0.150$; TRAP: $r=0.027$; CTX: $r=0.001$, $P>0.05$ for all. Finally, myostatin showed no differences according to the presence of prevalent fractures: fractures 2800 ± 1309 pg/ml vs no fractures 2542 ± 957 pg/ml, $P=0.372$.
Conclusion
Our data does not support an association between serum myostatin and bone parameters in type 2 diabetes. A true lack of relationship in humans may be an explanation, although a disrupted regulation of this pathway in type 2 diabetes may also take place.

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## PP87

**Serum concentration of bone tissue metabolism markers in 28 and 180-day-old Polish Large White pigs**

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The aim of the study was to evaluate time-related changes in serum concentration of bone tissue metabolism markers in Polish Large White male pigs. At birth, the piglets were divided into four groups. The first control group ($n=7$) received physiological saline i.m. (placebo). The second group (NanoCa group; $n=7$) were administered p.o. with nanopartical calcium (Ace Nano Calcium, NanoTechWorld, Pohang, Korea). The third group (Dex group; $n=7$) received dexamethasone (dexamethasone 0.2% solution; Rapidexon, Novartis, The Netherlands) in i.m. injections at a dose of 1 mg/kg per 48 h. The fourth group (NanoCa/Dex group; $n=6$) received simultaneously p.o. nanopartical calcium and dexamethasone in the same dosage as the second and third group. Nanopartical calcium was administered p.o. in the second and fourth groups at two different dosages – namely 250 mg/pig per day (since birth up to 4 months of life) and 500 mg/pig per day (up to 6 months). Administration with dexamethasone and nanopartical calcium was applied in this study to accelerate bone tissue metabolism of the experimental animals. Blood samples for serum were collected from piglets at the age of 28 and 180 days of life. Bone-specific alkaline phosphatase (BAP) concentration in serum of pigs was determined using an immunoenzymometric assay (Ostease BAP, Immunodiagnostic Systems Ltd, Boldon, Tyne & Wear, UK). Osteocalcin (OC) concentration of was assessed using MicroVue Human Osteocalcin EIA Kit (QUIDEL, San Diego, CA, U.S.A). C-terminal telopeptide of type-I collagen (CTX-I) was evaluated using Serum CrossLaps ELISA (IDS Ltd, UK). IGF1 was determined immunoenzymometric assay (OCTEIA IGF1, IDS Ltd, UK). Concentration of parathormone (PTH) was evaluated using Porcine Intact PTH Elisa Kit (Immunotopics, Inc., San Clemente, CA, U.S.A). Statistical comparison of the differences between age-differentiated groups was performed using student *t*-test for dependent variables and $P<0.05$ was considered as statistically significant. Serum concentrations of BAP, OC and PTH were significantly lowered by 54, 20 and 16% in 180-day-old pigs when compared to 28-day-old group ($P\leq0.001$). Serum concentrations of CTX-I and IGF1 were significantly increased by 181 and 60% in 180-day-old pigs when compared to 28-day-old group of animals ($P<0.001$). In conclusion, this study has shown higher leveles of bone formation markers such as BAP and OC in younger pigs confirming intensive skeletal formation in rapidly growing pigs. Bone resorption marker (CTX-I) level in serum was nearly threefold higher in the older group of pigs when compared to the group at the age of 28 days of life, confirming higher resorption rate of bone tissue in animals with significantly higher bone mass of the skeleton. IGF1 concentration was significantly elevated in the older group of pigs while an opposite results were stated in case of PTH evaluation.

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## PP88

**Ectopic parathyroid glands: imaging methods and strategy of surgical treatment**

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There is the question, if routine screening of hypercalcemia and hypovitaminosis D in the whole Czech population may be considered meaningful and economical. Primary hyperparathyroidism is a frequent cause of hypercalcemia. At our clinic we surgically treat 180 patients diagnosed for primary hyperparathyroidism on average per year. In the sample we detect 24% rate of ectopic gland localization. Such ectopic localization complicates effective surgery, stretches out the operating and anaesthesia time and increases the rate of surgery failure if not diagnosed properly before the operation. Besides the obvious US visualisation we routinely perform MIBI-SPECT/CT, which we consider the most effective method. SPECT /CT has 87–93% sensitivity and 90% specificity in detection of hyperparathyroid tissue. Selected case studies will be presented to depict the typical ectopic localizations, the benefits and downsides of the respective localization methods will be demonstrated and the resulting surgical strategies will be discussed. The 3D SPECT/CT image allows the decision between mini-invasive approach, uni-lateral neck exploration or the necessity of sternotomy, which happens in 5% of the time. Overall surgery success rate of 98.5% (significant drop in intact serum PTH and Ca) is achieved thanks to careful pre-operational localization. Such rate should be considered excellent enough to justify routine screening of hypercalcemia and hypovitaminosis D in the whole Czech population.

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## PP89

**Zoledronate prevents lactation induced loss of bone strength and micro-architecture**

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Objective
In rodents, lactation is associated with a considerable and very rapid bone loss, which almost completely recovers after weaning. The aim of the present study was to investigate whether the bisphosphonate zoledronate (Zln) inhibits lactation induced bone loss during lactation. In addition, to study if Zln interferes with recovery of bone mass after lactation has ceased.
Materials and methods
70 NMRI mice were divided into the following groups: baseline, pregnant, lactation, lactation + Zln, recovery, recovery + Zln, and recovery control (virgin). The lactation period was 12 days, then the pups were removed, and recovery took place for 28 days. Zln, 100 µg/kg s.c., was given at the day of delivery, and 4 and 8 days after delivery. The experiment was approved by the Danish Animal Experiments Inspectorate.
Results
In L4, lactation resulted in substantial loss of trabecular BV/TV (−40% vs pregnant, $P<0.001$), trabecular thickness (Tb.Th*) (−29% vs pregnant, $P<0.001$), bone material density (−4% vs pregnant, $P<0.001$) and bone strength (−55% vs pregnant, $P<0.001$). Zln completely prevented lactation induced changes in L4: BV/TV (+10% vs pregnant, NS), Tb.Th* (+0.03% vs pregnant, NS), bone material density (1.2% vs pregnant, NS), bone strength (+11% vs pregnant, NS). Similar results were found in the proximal tibia. Full recovery was found 4 weeks after weaning. Interestingly, L4 of the recovery group treated with Zln during the lactation period had higher BV/TV (+45%, $P<0.05$), Tb.Th* (+16%, $P<0.05$), and bone strength (+38%, $P<0.05$) compared with recovery controls. This indicates that Zln did not interfere with the substantial anabolic response, which takes place during recovery.
Conclusion
Zln fully prevented lactation induced loss of bone strength and architecture, and did not inhibit the anabolic response taking place after weaning.

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## PP90

**A randomized, double-blind, placebo-controlled, ascending, single-dose study of a human monoclonal anti-FGF23 antibody (KRN23) in X-linked hypophosphatemia**

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Purpose
In X-linked hypophosphatemia (XLH), elevated serum FGF23 causes low serum phosphorus (Pi) and inappropriately normal 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) levels. We report safety, tolerability and biochemistry markers following single ascending dose administration of KRN23 in adults with XLH.

Methods
38 XLH subjects with baseline FGF23 ≥ 30 pg/ml were randomized to receive single doses of KRN23 (K) or placebo (P) either (0.003–0.3 mg/kg) or (0.1–1.0 mg/kg). Biochemistry and immunogenicity were assessed at baseline and at various time points up to day 50.
Results
22 subjects were in the i.v. cohort (17K, 5P), and 16 in the s.c. cohort (12K, 4P). At baseline, demographic characteristics were comparable in all groups Serum Pi and renal threshold maximum for phosphate reabsorption (TmP/GFR) were lower than reference ranges in all subjects. At the higher doses, both i.v. and s.c. KRN23 increased serum Pi, TmP/GFR and $1,25(OH)_2D$; peak serum Pi occurred later with s.c. (8–15 days) than with i.v. dosing (4–5 days). Duration of effect on Pi was dose-related and longer with s.c. than i.v., persisting beyond 29 days with s.c. Adverse events (AEs) occurred with higher frequency in patients receiving KRN23 (82% i.v., 83% s.c.) compared to those receiving placebo (40% i.v., 50% s.c.). AEs related to the study drug occurred in six patients. Single-dose administration of KRN23 was well-tolerated as assessed by AEs, laboratory parameters, vital signs, calcium homeostasis, renal ultrasonography, and ECGs. No patient had an increase in nephrocalcinosis or developed hypercalciuria, hypercalcemia or a rise in serum parathyroid hormone. No anti-KRN23 antibody was detected in any patients.
Conclusions
Blocking FGF23 activity with KRN23 is a promising treatment for XLH. Single i.v. or s.c. doses increased serum TmP/GFR, serum Pi and $1,25(OH)_2D$ with effects lasting for more than 4 weeks with s.c. dosing.

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## PP91

**Pharmacokinetics and pharmacodynamics of a human monoclonal anti-FGF23 antibody (KRN23) after ascending single-dose administration in patients with X-linked hypophosphatemia**

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Purpose
In X-linked hypophosphatemia (XLH), elevated serum FGF23 causes low serum phosphorus (Pi) and inappropriately normal 125-dihydroxyvitamin D ($125(OH)_2D$) levels. We report PK and PD of KRN23 following single ascending dose administration in adults with XLH.
Methods
38 XLH patients with baseline FGF23 ≥ 30 pg/ml were randomized to receive a single dose of KRN23 (K) or placebo (P) either i.v. (0.003–0.3 mg/kg) or s.c. (0.1–1.0 mg/kg). PK and PD samples were obtained from baseline through day 50. A validated ELISA method was used for PK and commercial assay kits were used for PD assays.
Results
22 subjects participated in the i.v. cohort (17K, 5P), and 16 in the s.c. cohort (12K, 4P). Baseline age, sex, weight, and height of all groups were comparable in all groups. Mean KRN23 terminal half-life was shorter for i.v. (11–12 days) than for s.c. (13–19 days) administration. Absolute bioavailability was ~100% with s.c. dosing. There was a dose-proportional increase in serum KRN23 levels in both i.v. and s.c. cohorts. Following s.c. administration, peak serum KRN23 and Pi occurred at the same time. Area under the curve ($AUC_{last}$) for changes from baseline in serum Pi, $125(OH)_2D$, and TmP/GFR were linearly correlated with $AUC_{inf}$ for serum KRN23. s.c. dosing exhibited a slower absorption profile (peak time: 8–11 days) and a more sustained effect than i.v. dosing as indicated by the delayed time to reach maximum mean serum Pi level (peak time: 8–15 vs 0.5–4 days) and longer time to return to baseline (50 vs 29 days).
Conclusions
KRN23 is a promising treatment for XLH patients. Complete absorption, sustained effect on serum Pi beyond 4 weeks, and a direct linear relationship between PK and PD effects supports a s.c. treatment regimen of once every 4 weeks for KRN23 in adults with XLH.

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## PP92

**Effect of subcutaneous recombinant human parathyroid hormone, rhPTH(1–84), on skeletal dynamics in hypoparathyroidism: findings from the 24-week replace and 8-week relay phase III clinical trials**

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Hypoparathyroidism results in low bone turnover and increased bone mineral density (BMD). Replacing deficient PTH with rhPTH(1–84) has the potential to correct these skeletal abnormalities. To investigate the effect of rhPTH(1–84) on BMD and bone turnover markers (BTMs), data from two studies were assessed. REPLACE, a double-blind, multicenter, placebo-controlled study, randomized 134 patients with hypoparathyroidism to receive once-daily rhPTH(1–84) (50 μg initially, increased to 75, then 100 μg if needed) or placebo. In RELAY, a dose-blind, multicenter study, 47 patients with hypoparathyroidism were randomized to receive 25 or 50 μg/day rhPTH(1–84). Twenty-one patients in RELAY (enrolled after 4-week washout) were previously treated in REPLACE. In both studies, BTMs (bone-specific alkaline phosphatase (BSAP), carboxy-terminal telopeptide of type 1 collagen (CTX), osteocalcin (OCN), and aminoterminal propeptide of type 1 collagen (P1NP)) were assessed at baseline and at weeks 8 (RELAY) and 24 (REPLACE). BMD was assessed in REPLACE.
In REPLACE, treatment with rhPTH(1–84) significantly increased all BTMs from low-normal baseline values to significantly higher levels at week 24 (Table 1) compared with placebo ($P \leq 0.001$). In RELAY, all BTMs increased with both rhPTH(1–84) doses at week 8 (Table), with no significant differences between doses for any marker. In REPLACE, BMD decreased toward normal in patients receiving rhPTH(1–84) at lumbar spine, total hip, femoral neck, and distal one-third radius. rhPTH(1–84) was generally well tolerated in both studies. Patients with hypoparathyroidism with low bone turnover and high BMD respond to rhPTH(1–84) in 8 weeks. BTMs reflect restoration of turnover toward normal, and BMD also returns toward normal.

**Table 1** BTMs: change from baseline with rhPTH(1–84) (mean ± s.d.).

| | **BSAP** (μg/l) | **CTX** (pg/ml) | **P1NP** (μg/l) | **OCN** (μg/l) |
|---|---|---|---|---|
| REPLACE | 20.5 (18.1) | 798.4 (648.0) | 299.5 (234.6) | 25.9 (27.7) |
| RELAY 25 μg | 0.66 (3.8) | 79.5 (239.6) | 13.7 (38.5) | 0.27 (2.7) |
| RELAY 50 μg | 0.08 (4.3) | 92.7 (141.6) | 22.0 (39.6) | 1.4 (2.9) |

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# Cancer and bone: basic, translational and clinical

## PP93

**Inhibition of TGF-β signaling pathway blocks the development of osteosarcoma lung metastases**

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Osteosarcoma is the main malignant primary bone tumor in children and adolescents for whom the prognosis remains poor, especially when metastases are present at diagnosis (survival rate drops to 20% when lung metastases were detected). Because TGF-β has been shown to promote metastases in many solid tumors, we investigated the effects of inhibition of the TGF-β/Smad cascade on osteosarcoma behavior. To this end, two independent procedures, a pharmacological approach with TGF-β receptor I inhibitor (SD-208) and a molecular

approach using the natural Smad inhibitor (Smad7), was used. The impact of these procedures was assessed on tumor growth, tumor microenvironment, bone remodeling and lung metastases development by using a mouse model of osteosarcoma induced by paratibial injection of osteosarcoma cells, in accordance with the institutional guidelines of the French Ethical Committee.
We also showed that Smad7 slows the growth of the primary tumor and increases mice survival. In this context, we demonstrated that Smad7 expression does not affect osteosarcoma cell proliferation but affects the microarchitectural parameters of bone. In addition, Smad7-osteosarcoma bone tumors expressed lower levels of osteolytic factor RANKL, suggesting that Smad7 overexpression affects the 'vicious cycle' established between tumor cells and bone cells by its ability to decrease osteoclast activity. Interestingly, we finally showed that Smad7 overexpression in osteosarcoma cells and SD-208 inhibits the development of lung metastases. These effects are correlated to the fact that Smad7 and SD-208 reduced two key functions in tumor progression, cell migration and invasion, in part by inhibiting the ability of TGF-β to stimulate the expression and activity of MMP2.These results suggest that the inhibition of TGF-β/Smad signaling pathway could be a promising therapeutic strategy against the tumor progression of osteosarcoma.

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## PP94

### Preclinical efficacy of PF-04942847, a novel HSP90 inhibitor, in osteosarcoma

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Introduction and objective
Despite recent improvements in therapeutic management of osteosarcoma, ongoing challenges in improving the response to chemotherapy warrants the development of new strategies to improve overall patient survival. Among new therapeutic approaches, heat shock protein (HSP) 90 is a molecular chaperone involved in the maturation and stability of various oncogenic proteins leading to tumor cells survival. In this study, we assessed the *in vitro* and *in vivo* antitumor properties of a novel synthetic HSP90 inhibitor, PF-04942847 both *in vitro* and *in vivo* in osteosarcoma.
Methods
The effects of the HSP90 inhibitor were evaluated *in vitro* on U2OS, SaOS2, MNNG/HOS, KHOS, MG63, G292, CAL72, SJSA1 and 143B cell growth and apoptosis. The signaling pathways were analyzed by western blotting. The consequence of HSP90 therapy *in vivo* was evaluated in athymic mice bearing MNNG/HOS xenografts. The effect of PF-04942847 on osteoclastogenesis was assessed *in vitro* on purified human CD14+ monocytes.
Results
In our panel of osteosarcoma cell lines, PF-04942847 inhibited cell growth in a dose-dependent manner (IC50 ± 50 nM) and induced apoptosis with an increase of sub-G1 fraction and PARP cleavage. These biologic events were accompanied by decreased expression of Akt, Erk, MYC, c-MET, STAT-3, and modulation of HSP expression. When administered orally 3 times/week (25 mg/kg) to mice bearing osteosarcoma tumors, PF-04942847 significantly inhibited tumor growth by 40% and prolonged survival compared to controls. Moreover, in contrast to 17-AAG, the novel PF-04942847 compound inhibits osteoclast differentiation *in vitro*.
Conclusions
Targeting HSP90 using PF-04942847 inhibited osteosarcoma tumor growth *in vitro* and *in vivo* and appears to inhibit osteoclastogenesis. All these data provides a strong rationale for clinical evaluation of PF-04942847 in osteosarcoma.

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## PP95

### Interleukin 34 promotes angiogenesis and increases blood cells adherence to endothelial cells

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Tumour growth and metastatic dissemination are significantly reduced in mice bearing an inactivation of the macrophage-colony stimulating factor (M-CSF) gene, due to angiogenesis impairment. In fact, M-CSF directly induces angiogenesis by increasing vascular endothelial growth factor (VEGF) production. Interleukin 34 (IL34), the M-CSF's 'twin' cytokine, was characterized as a new cytokine promoting the growth, survival and differentiation of the myeloid lineage. Consistent with these findings, this work studied the involvement of IL34 in *in vitro* and *in vivo* angiogenesis.
IL34 activities on endothelial cells were first analyzed *in vitro* using endothelial progenitors or differentiated endothelial cells and by studying cell proliferation and the differentiation into vascular cord on Matrigel. Furthermore, the adherence of hematopoietic stem cells to endothelial cells under physiological conditions of blood flow and the intracellular signaling pathways by western blot were investigated. The effects of IL34 on angiogenesis were also studied *in vivo,* using two murine models: subcutaneous implants of Matrigel plugs containing fibroblast growth factor (FGF2) ± IL34 (authorization n°C75.06.02) and paratibial inoculation of human osteosarcoma cells overexpressing IL34 in nude mice (authorization no 1280.01).
In association with the FGF2, IL34 enhanced the vascular tube formation *in vitro* as demonstrated by their significant higher size and number. Western blot analyses revealed that IL34 effects were partially mediated by the MAPK pathway. In addition, IL34 increased adherence of both $CD34^+$ hematopoietic stem cells and $CD14^+$ monocytes on activated endothelium. The *in vitro* pro-angiogenic effect of IL34 was confirmed *in vivo*. Results showed that IL34 induced a neovascularisation of Matrigel plugs in mice and IL34 promoted tumour angiogenesis in osteosarcoma.
This work demonstrates that similar to M-CSF, IL34 promotes angiogenesis, inducing vascularisation both *in vitro* and *in vivo*. By promoting new vessel formation and extravasation of cells from the immune system, IL34 could play a key role in tumour developmet.

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## PP96

### Osteoblast-secreted extracellular vesicles stimulate the expansion of $CD34^+$ human umbilical cord blood cells

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Introduction
Umbilical cord blood (UCB) is increasingly used in hematopoietic stem cell (HSC) transplantations; however, the low cell numbers are still remaining as a limiting factor for proper engraftment. Osteoblasts are major constituents of HSC niche and play important roles in regulating HSC self-renewal and differentiation. Recently, extracellular vesicles (EVs) have been implicated in stem cell fate regulation via horizontal transfer of proteins and nucleic acids between cells. Therefore, in this study we focused on the characterization of human osteoblast EVs and investigated their potential in *ex vivo* expansion of human UCB–HSCs for clinical use.
Methods
We used human pre-osteoblasts (SV-HFO cells) to isolate EVs by a series of ultracentrifugation steps. We characterized osteoblast EVs by electron microscopy, proteomics, and miRNA sequencing, and investigated their effect on $CD34^+$ UCB cells by single-platform counting and subset immunophenotyping using flow cytometry and qPCR.
Results
Treatment of $CD34^+$ UCB cells with osteoblast EVs led to twofold expansion of the phenotypic HSCs three- and fivefold expansion of the $CD34^+$ expressing progenitors. Microscopic analyses demonstrated that osteoblast EVs are very heterogenic in size and morphology. Mass spectrometry-based proteomic

analyses identified an interesting range of novel osteoblast EV proteins primarily linked to ribosomal activity and RNA processing in addition to the well-known vesicle proteins. Moreover, EVs were enriched with small RNAs, and contained miRNAs known be involved in the regulation of early hematopoiesis. Interestingly, we discovered that EV treatment downregulated the expression of HMG-box transcription factor 1 (HBP1), which is one of the miR-29a targets, in $CD34^+$ UCB cells.

Conclusion

In this study we demonstrated that osteoblasts secrete EVs that hold the potential to expand UCB–HSCs *ex vivo*. Elucidating the molecular mechanism of EV function is likely to provide us the means to increase the expansion efficiency and develop improved grafts for stem cell transplantations.

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## PP97

**TRAF2, but not TRAF6, regulates breast cancer induced osteoclastogenesis and osteolysis.**

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Tumour necrosis factor receptor associated factors (TRAFs) play a key role in signal transduction in mammalian cells. Several members of the TRAF family have been identified but only TRAF2 and TRAF6 are implicated in the regulation of osteoclastogenesis. Here we studied the role of TRAF2 and TRAF6 in breast cancer induced bone cell activity and osteolysis. We observed that TRAF2, but not TRAF6, is highly expressed in the human MDA-MB-231 (MDA-231) bone-seeking breast cancer cells when compared to parental cells (TRAF2; 43% increase, $P<0.01$). Targeted knockdown of TRAF2, but not TRAF6, in MDA-231 cells by small interfering RNAs markedly reduced cell migration (38% reduction, $P<0.05$), significantly reduced the ability of MDA-231 cells (41% reduction, $P<0.05$) and their conditioned medium (69% reduction, $P<0.05$) to induce osteoclast formation in bone marrow cultures. Next, we successfully generated stable parental and bone seeking MDA-231 cell lines over-expressing TRAF2 using a retroviral approach. Over-expression of TRAF2 in the parental MDA cell lines (eightfold increase) significantly enhanced cell migration (30% increase, $P<0.001$) and invasion (50% increase, $P<0.05$). Over-expression of TRAF2 in bone-seeking MDA-231 cells significantly enhanced the stimulatory effects of these cells (187% increase, $P< 0.001$) and their conditioned medium (24% increase, $P<0.005$) on osteoclast formation in RANKL stimulated bone marrow cultures. Moreover, studies in human MDA-231–mouse calvaria organ co-cultures showed that conditioned medium obtained from MDA-231 cells over-expressing TRAF2 caused profound increase in osteolysis (20% increase, $P<0.05$) when compared to conditioned medium from control cells. In conclusion, our studies showed that TRAF2, but not TRAF6, activity in breast cancer cells regulates breast cancer cell motility *in vitro* and osteolysis *ex vivo*. However, the role of TRAF2 in bone metastasis and osteolytic bone loss associated with breast cancer *in vivo* will require further investigation.

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## PP98

**Biological markers of aggressive giant cell tumour of bone: an immunohistochemical study**

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Giant cell tumour of bone (GCTb) is an osteolytic neoplasia with tendency to local recurrence (10–25%), while metastases or malignant transformation are described in 1–4% of cases.

It is composed by three distinct populations that cross-talk each other generating unbalance in bone remodeling and activation of NF-kB signaling pathway.

To identify new candidate biological markers useful for improving clinical management of GCTb we investigate the expression of key proteins involved in cell tumour-bone microenvironment interaction and related their immunostaining intensity and frequency with clinical features of the patients.

The protein expression was evaluated by tissue microarray technique on a series of primary GCTb from 83 disease-free, 72 locally relapsed, and 33 metastatic patients.

The most important proteins promoting osteolysis such as RANKL, RANK, MMP2, interleukins were more strongly and frequently expressed in relapsed group when compared with disease free group, also showing a significant association with pro-survival signaling proteins including NFIB and c-Fos. The metastatic rate based on protein immunoreactivity indicated a significantly higher probability of metastasis in patients with moderate to high than low or absent positive expression.

These data demonstrated that the linkage between matrix remodelling and tumour cell activity could recognize pathway interconnection endpoints useful for new therapeutic strategies.

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## PP99

**Changes in bone mineral density and biochemical markers of bone turnover in postmenopausal women with breast cancer initiating aromatase inhibitors therapy**

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Postmenopausal women with early stage hormone-receptor-positive breast cancer (EBC) are standardly treated with aromatase inhibitors (AIs). However, one side-effect of AIs treatment is a decrease in bone mineral density (BMD) and an increased risk of fracture. The objectives of this study were to examine: i) changes in bone formation (N-terminal propeptide of type I procollagen; PINP) and bone resorption (cross-linked C-telopeptides of bone type I collagen; CTX) markers, as well as changes in serum intact parathyroid hormone, 25 hydroxyvitamin D (25OHD) and plasma sclerostin levels over the first 6 months of AIs therapy in a cohort of breast cancer patients initiating aromatase inhibitors therapy and ii) the association between early changes in bone markers and subsequent BMD changes after 12 months of AIs treatment. Eligible breast cancer patients ($n=50$) were recruited and followed for 12 months. The results showed that low BMD and vitamin D insufficiency is highly prevalent among women with EBC treated with adjuvant AIs. AIs treatment was associated with significant increases in serum CTX ($P<0.01$) and plasma sclerostin ($P<0.001$) within the first 6 months and significant decreases in BMD at all measured sites ($P<0.001$) after 12 months. The changes in CTX were significantly ($P<0.05$) and negatively related with lumbar spine ($r=0.309$) and femoral neck ($r=0.351$) BMD changes. The changes in sclerostin were significantly ($P< 0.05$) and positively associated with femoral neck BMD changes ($r=0.396$). Conclusion: Findings from this study suggest the importance of early assessment of BMD and 25OHD levels when starting AIs treatment. Early identification of women with poor bone health and/or rapid increase of bone markers offer us possible intervention to prevent bone loss and fracture risk.

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## PP100

**Bone quality of metaplastic woven bone of mixed metastases: a FTIRI analysis**

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Objectives

Most osteolytic tumors are in fact mixed and contain an osteoblastic component associated with the predominant osteolytic areas. This metaplastic woven bone can be evidenced on X-rays as blurred white areas but is always evidenced by histological analysis even in the absence of radiological expression. Metaplastic bone formation reflects the activation of new osteoblasts, the stimulation of the dormant lining cells due to the release of growth factors buried in the bone matrix during the osteoclastic resorption. The production of bone matrix by malignant cells has also been advocated.

Materials and methods
Twelve patients with secondary metastases of the iliac crest evidenced by hot spots on a $^{99}$Tc-MBP san were diagnosed by histomorphometry on bone biopsies. Fourier transformed infrared analysis and imaging (FTIRI) was used on 4 μm thick sections of undecalcified bone. The mineral, the collagen and the cross links ratio (1660:1690/cm bands) were determined. The matrix characteristics were analyzed and imaged in the 'old' residual bone and in the newly formed woven bone in the vicinity of the tumor cells. Imaging was done after peak selection and transfer of the selected files into the MalLab software.
Results
FTIRI provided images of the phosphate, amide and combination of peak ratio after having selected the peaks of interest. In addition, the matrix properties can be measured and compare between the old and newly formed bones. Woven bone appeared poorly calcified with a low phosphate:amide ratio ($P=0.03$) crystallinity ($P<0.0001$) and carbonate substitution ($P=0.003$). Collagen was less mature as evidenced by lower cross-links ($P=0.01$).
Conclusions
Woven bone observed in normal conditions (fetal bone and callus) is known to be highly mineralized by the process of matrix vesicles. Woven bone associated with bone metastasis appears on the contrary poorly mineralized and rapidly elaborated by osteoblasts. The collagen matrix has a low level of reticulation. FTIRI is a powerful tool to measure and visualize the various components of the bone matrix in human diseases.

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## PP101

**The effect of granulocyte colony-stimulating factor in adults undergoing autologous peripheral blood stem cell collection**

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Introduction
Granulocyte colony-stimulating factor (G-CSF) is widely used to mobilize peripheral blood stem cells (PBSC) and enable PBSC collection by apheresis. Although bone pain is a common adverse event following G-CSF treatment, little is known on its effect on bone metabolism.
Methods
Markers of bone turnover (OC, osteocalcin, β-CTx, bALP, C-terminal telopeptide of type I collagen, bone specific alkaline phosphatase, TRAP, tartrate resistant acid phosphatase) and mineral metabolism were assessed in adult patients with haematological malignancy who received G-CSF for autologous PBSC collection. Analyses were repeated after G-CSF stimulation. Patients with glomerular filtration rate $<30$ ml/min were excluded from analysis.
Results
Eighteen subjects were included (ten men, eight women, mean age $48\pm11$ years, BMI $24.5\pm2.9$ kg/m$^2$). ß-CTX and bALP were elevated already at baseline. OC, bALP and TRAP were significantly altered by G-CSF, while ß-CTX and calcium levels remained unchanged (Table 1).

**Table 1** Markers of bone turnover in adult patients before and after G-CSF

| Biochemical marker, reference range | Before G-CSF | After G-CSF | *P* |
|---|---|---|---|
| β-CTx, 0.06–0.35 ng/ml | $0.40\pm0.34$ | $0.42\pm0.32$ | 0.57 |
| Osteocalcin, 1.0–35.0 ng/ml | $25.3\pm18.7$ | $16.5\pm10.0$ | 0.007 |
| bALP, 7.5–20.6 U/l | $22.8\pm17.7$ | $33.7\pm17.9$ | 0.002 |
| TRAP, 2.59–4.03 μg/l | $3.41\pm1.57$ | $2.78\pm1.15$ | 0.002 |
| Total calcium, 2.25–2.65 mmol/l | $2.30\pm0.14$ | $2.31\pm0.19$ | 0.88 |

Conclusion
Our results demonstrate that high-dose G-CSF acutely affects bone metabolism. The clinical relevance of these finding remains unclear, but further research is warranted to confirm our findings and ascertain long-term skeletal health in this vulnerable population.

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## PP102

**Paget's sarcoma – A case report in Korea**

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Paget's disease of bone is a condition where the process of bone remodeling is disrupted; Its primary event is an increased bone resorption followed by a subsequent reactive bone formation. The disease is most common in central Europe, the United Kingdom, Australia, and New Zealand. It is also found, though with lower incidence, in southern Europe, Scandinavia, and the United States. It is extremely rare in East Asian countries, especially in Korea, Japan, China, the Middle East, India and Africa. Through the English literature, none of the Paget's sarcoma were reported from these East Asian countries. We recently experienced a case of Paget's sarcoma from Korean male subject. The patient is 74-year-old male suffering several years with Paget's disease affecting both ilium, pubis and ischium. Recently he was noticed huge right hip mass and the mass were excised. Histologically it was high grade sarcoma and the ischium showed high grade osteoblastic osteosarcoma. We did gene analysis for *Q16STM1* and *TNFRSF11A* from the sarcoma area. No *Q16STM1* gene mutation was found however, in *TNFRSF11A* gene, there was exon 6 mutation 192 codon cytosine to thymine, which means alanine to valine mutation.

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## PP103

**Selective inhibition of BET bromodomains epigenetic signaling interferes with the bone-associated tumor vicious cycle**

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The vicious cycle established between bone associated tumors and bone resorption is the central problem with therapeutic strategies against primary bone tumors and bone metastasis. The bromodomain and extra-terminal domain (BET) protein family is an important class of 'histone reading protein' capable to recognize the N-acetylation of lysine residues on histone tails. BET bromain proteins have recently been described as regulators of MYC expression in various tumors. In this study, we report data to support inhibition of BET bromodomain as a novel and promising therapeutic strategy that target simultaneously the three partners of the vicious cycle. Treatment with JQ1, a unique BET bromodomain inhibitor, reduced cell viability and induced apoptosis in osteosarcoma cells and, inhibited osteoblastic differentiation *in vitro*. *In vivo*, BET inhibitor (IP; 50mg/kg) significantly inhibits tumor growth by 70% and prolongs survival in both POS-1 syngenic and MMNG/HOS xenograft models compared to control. Additionally, these results were accompanied by a decrease of associated bone lesions.
These effects were associated with transcriptional silencing of *MYC* and *RUNX2*, resulting from the depletion of *BRD4* from their respective loci. Moreover, JQ1 also inhibited osteoclast differentiation by interfering with BRD4-dependent RANKL activation of *NFATC1* transcription. Collectively, our data indicates that JQ1 is a potent inhibitor of osteoblast and osteoclast differentiation as well as bone tumor development.

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## Cell biology: osteoblasts and bone formation

### PP104

**Overexpression of Sp7 in osteoblasts inhibits osteoblast maturation but enhances angiogenesis in bone**

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Sp7 and Runx2 are essential transcription factors for osteoblast differentiation at an early stage, and Runx2 inhibits osteoblast maturation at a late stage. However, the functions of Sp7 in bone development are not fully elucidated. Thus, we pursued the functions of Sp7 in bone development by generating osteoblast-specific *Sp7* transgenic (tg) mice using 2.3 kb *Col1a1* promoter. *Sp7* tg mice showed osteopenia and woven-bone like structure in the cortical bone, which was thin and less mineralized. The frequency of BrdU incorporation was increased in the osteoblastic cells, while the expression of *Col1a1*, *Spp1*, *Ibsp*, and *Bglap2* was reduced. Further, the osteopenia in *Sp7* or *Runx2* tg mice was worsened in *Sp7/Runx2* double tg mice and the expression of *Col1a1* and *Bglap2* was reduced. Histological analysis showed that blood vessels were increased in the cortical bone, the numbers of canaliculi and osteocyte processes were reduced, and the osteocytes were accumulated around the blood vessels in *Sp7* tg mice. Immunohistochemical analysis showed an increase in CD34-positive vascular endothelial cells, and micro-CT analysis showed an increase of vascular volume in the cortical bone. In contrast, the introduction of sh-*Sp7* into WT metatarsal bones suppressed the angiogenesis in the cortical bones in organ cultures. Immunohistochemical and western blot analyses showed that vascular endothelial growth factor (VEGF) A expression was increased in the osteoblasts in *Sp7* tg mice. Immunohistochemical analysis using WT bone sections showed that osteoblasts, which were positive for endogenous Sp7, were also detected by VEGF antibody. These findings indicated that Sp7 inhibits osteoblast maturation at a late stage, and suggested that Sp7 regulates angiogenesis in bone through the regulation of VEGF.

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### PP105

**Reduced bone formation and increased adiposity with insulin resistance in interleukin-11 deficient mice**

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We previously reported that interleukin (IL)-11 plays an important role in the mechanical stress and PTH-induced stimulation of osteoblast differentiation and bone formation. Mechanical stress and PTH enhance IL-11 gene promoter activity and increase IL-11 expression. Increased IL-11 down-regulates dikkopf 1/2 (Dkk1/2) expression and stimulates canonical Wnt signaling, which enhances osteoblast differentiation. The present study was undertaken to clarify the physiological role of IL-11 in osteogenesis and adipogenesis by creating IL-11 deficient (IL-11KO) mice.

The growth curves of IL-11KO and WT mice were similar. Bone mineral density (BMD) measured by μCT revealed that lumbar and total femoral BMD was lower in IL-11KO mice compared with those in WT mice after 8 to 12 weeks of age. The reduction in BMD was observed at both cortical and cancellous bones in both male and female IL-11KO mice. Serum osteocalcin was lower in IL-11KO than in WT mice, while serum TRAP5b was similar in both groups. Bone histomorphometry revealed that Tb.Th, Ob.S/BS and N.Ob/BS were reduced in IL-11KO mice, but there was no significant difference in Oc.S/BS or N.Oc/BS between IL-11KO and WT mice. Immunohistochemical staining of sclerostin and SOST mRNA expression in the femur increased in IL-11KO mice compared with WT mice. Furthermore, adipose tissue mass, adipocyte number and size were increased with impaired glucose tolerance, higher fasting serum insulin level and higher HOMA-IR in IL-11KO mice compared with WT mice fed with high fat diet.

These observations are consistent with the notion that IL-11 plays an important physiological role in maintaining bone formation and bone mass without affecting bone resorption. In addition, the increased adiposity and insulin resistance observed in IL-11KO mice suggest the role of IL-11 in regulating energy metabolism, which may be mediated *via* its effect on canonical Wnt signaling.

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### PP106

**Downregulation of Smurf1 is involved in odontoblast differentiation induced by histone deacetylase inhibitors**

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Smurf1 is an E3 ubiquitin ligase for Smad1/5, Runx2 and MEKK2 and plays a role as a negative regulator for osteoblast differentiation and bone formation. However, the role of Smurf1 in odontoblast differentiation has not been elucidated. Previously, we reported that histone deacetylase inhibitors (HDACi) enhance odontoblast differentiation through the upregulation of Nfic expression. In this study, we investigated the regulatory effect of HDACi on Smurf1 expression and the role of Smurf1 in odontoblast differentiation. The effects of HDACi (*SAHA*, Trichostatin A) on the Smurf1 expression and odontoblast differentiation were observed in MDPC23 cells, a murine pre-odontoblast cell line. HDACi increased Nfic expression while decreasing Smurf1 expression. Overexpression of Nfic decreased Smurf1 expression whereas Nfic knockdown increased Smurf1 expression. Treatment with HDACi or overexpression of Nfic reduced the levels of RNA polymerase II binding, acetyl-H3 and methyl-H3K4 in the DNA region spanning the Smurf1 promoter and transcription start site, suggesting that HDACi/Nfic downregulate Smurf1 transcription. Smurf1 overexpression decreased the expression levels of odontoblast differentiation marker genes such as DMP1, Nestin and DSPP and matrix mineralization. Similar to HDACi treatment, Smurf1 knockdown enhanced the expression levels of odontoblast differentiation marker genes. These results suggest that HDACi downregulate Smurf1 expression through the induction of Nfic expression and that reduction in Smurf1 level contribute to HDACi-induced odontoblast differentiation.

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### PP107

**Osteoblast-specific overexpression of amphiregulin leads to transient increase in cancellous bone mass in mice**

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It is well known that the epidermal growth factor receptor (EGFR), its ligands, and the structurally related receptor ERBB2/neu are expressed in skeletal cells. However, the functions of EGFR ligands in bone cells remain poorly defined. In this study, we employed a transgenic mouse line overexpressing the EGFR ligand amphiregulin (AREG) specifically in osteoblasts under the α1(I)-collagen promoter. AREG-tg mice did not show changes in body weight or gross phenotype. Compared to WT littermates, expression of AREG mRNA was 94-fold higher in femurs of 4-week-old AREG-tg mice. pQCT analysis of the femoral metaphysis revealed increased trabecular BMD in AREG-tg mice at 4, 8, and 10 weeks of age. However, the high bone mass phenotype was transient and disappeared in 20- and 72-week-old animals. Micro-CT analysis of the secondary spongiosa confirmed increased trabecular BMD, trabecular bone volume and trabecular number in the distal femur of 4-week-old AREG-tg mice as compared to WT controls. Furthermore, μ-CT analysis of the primary spongiosa did not show evidence of alterations in the production of new bone trabeculae in distal femora of AREG-tg mice. Histomorphometric analysis revealed a reduced number of osteoclasts in 4-week-old AREG-tg mice, but not at later time points. Cancellous bone formation rate remained unchanged in AREG-tg mice at all time points. In addition, bone mass and bone turnover in lumbar vertebral bodies were similar in AREG-tg and WT mice at all ages examined. Proliferation and differentiation of osteoblasts isolated from neonatal calvariae did not differ between AREG-tg and WT mice. Taken together, these data suggest that AREG overexpression in osteoblasts leads to a transient high bone mass phenotype in the trabecular compartment of the appendicular skeleton by a growth-related, non-cell autonomous mechanism, leading to a positive bone balance with unchanged bone formation and lowered bone resorption.

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## PP108

### Collagen XIII in bone homeostasis

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Collagen XIII is a conserved transmembrane collagen with wide distribution in various tissues. It can be enzymatically cleaved to form a soluble bioactive molecule with relevance to cell proliferation, migration and adhesion. Collagen XIII overexpression in mice causes a massive bone overgrowth with no defects in early skeletal development. The bone phenotype of Col13a1$^{OE}$ mice is most apparent in long tubular bones but also present in calvariae. Our findings show that type XIII collagen is a significant regulator in the early osteoblast differentiation.

We have found major differences in proliferation rate, morphology and activity of primary osteoblasts derived from Col13a1$^{OE}$ mice compared to controls. We have also discovered qualitative changes in bones of Col13a1$^{OE}$ mice. Micro-computed tomography (μCT) and X-ray photoelectron spectroscopy (XPS) analyses of 25 week old Col13a1$^{OE}$ mouse femurs reveal tenfold increase in cortical bone volume whereas the calcium content is decreased by 51% compared to WT mice.

In summary, while collagen I is the main protein component of bones, our mouse models show that altered collagen XIII expression has striking effects on bone. Collagen XIII enhances the early steps of osteogenic differentiation and is an important regulator of bone remodeling and homeostasis.

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## PP109

### Advanced glycation end products inhibit the mineralization of marrow stromal cells by binding the receptor for AGEs and increasing TGF-β expression and secretion

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We and others have recently showed that patients with type 2 diabetes mellitus (T2DM) have a higher risk for fracture, although they have normal or slightly higher bone mineral density (BMD). These findings suggest that bone fragility in T2DM, which is not defined by BMD, may contribute to fracture risk. Hyperglycemia accelerates the formation of advanced glycation end products (AGEs) and causes diabetic complications. AGEs including pentosidine are also known to cause bone fragility due to quality deterioration in diabetic patients. We found that AGEs suppressed mineralization of mouse stromal ST2 cells by inhibiting their differentiation with reduced expressions of osteocalcin (OCN) and osterix (OSX) mRNA in the cells. It is known that TGF-β is especially abundant in bone, and that enhancement of its signals deteriorates bone quality in animal experiments. However, it is still unclear whether or not TGF-β signal is involved in the AGEs-induced suppression of osteoblastic bone formation. In this study, we made AGE3 by incubating BSA with glycolaldehyde, and examined the roles of TGF-β in the suppression of mineralization of ST2 cells induced by AGE3. Treatments of the cells with AGE3 (200 μg/ml) significantly inhibited mineralization by 71.2% on experimental day 21 ($P<0.001$). Simultaneously, AGE3 significantly increased the mRNA expression and protein level of TGF-β by RT-PCR and ELISA of whole cell lysates, respectively, on days 3, 5, and 7 ($P<0.001$). Transfection of siRNA of the receptor for AGEs (RAGE) significantly inhibited the AGE3-induced increase in TGF-β protein level ($P<0.001$), and recovered mineralization in the cells ($P<0.05$). Moreover, treatments of TGF-β type I receptor kinase inhibitors (2.5 uM SD208 and 3.0 uM SB431542) significantly antagonized the AGE3-induced suppression in OCN and OSX mRNA expressions ($P<0.05$), and recovered mineralization completely. Similar results were also obtained in human primary mesenchymal stem cells differentiated into osteoblasts. These findings indicate that the AGEs-RAGE pathway inhibits the mineralization of marrow stromal cells by increasing TGF-β expression and secretion, suggesting that TGF-β action stimulated by AGEs adversely affects bone in the process of diabetic complications.

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## PP110

### Age-dependent loss of microvesicular galectin-3 and its consequences on bone formation *in vitro* and *in vivo*

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Introduction

Mesenchymal stem cells (MSCs) counteract the decline of physiologic functions but their regenerative power decreases with age. In particular osteogenic differentiation capacity of MSCs has been shown to decrease with age thereby contributing to slowed down bone formation and osteoporosis. While much is known about cellular aging of MSCs, little is known about extrinsic factors influencing their functionality. Here we set out to identify circulating factors of the aged systemic environment that influence osteogenesis

Results

While searching for influential factors extracellular vesicles (EVs) were found. Exposition of MSCs to EVs isolated from plasma of human elderly donors failed to induce osteogenesis compared to EVs of young donors raising the question which age-dependent vesicularly secreted components impact on the differentiation capacity. We identified vesicular galectin-3 as an influential component. Overexpression of galectin-3 in MSCs was shown to boost osteogenic differentiation capacity while reducing its protein expression by siRNA inhibited osteogenesis *in vitro*. Moreover intracellular galectin-3 levels of MSCs correlated with their osteogenic differentiation potential. Next we could demonstrate that plasma as well as vesicular galectin-3 levels were reduced in elderly human donors compared to young donors and that vesicular galectin-3 levels indeed impact on osteogenic differentiation capacity of MSCs. Finally nano-CT scan on galectin-3 knock out mice revealed a previously unknown reduction of femoral cortical as well as trabecular thickness compared to WT littermates.

Conclusion

We could demonstrate that the composition of circulating EVs changes with age and that they deliver factors impacting on the osteogenic differentiation capacity of MSCs. Among other factors vesicular galectin-3 was shown to be enriched within EVs isolated from young human donors and to enhance osteogenesis. Reduction in vesicular galectin-3 plasma levels with age might lead to a reduced uptake of galectin-3 by MSC and therefore contribute to impaired osteogenesis with age.

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## PP111

### Osteopontin as a novel substrate for proprotein convertase 5/6 (PCSK5) in bone

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Proprotein convertase PC5/6 (*Pcsk5*) is expressed in mouse bone and *Pcsk5* epiblast-specific conditional knockout mice have a bone phenotype displaying small size, delayed ossification and additional thoracic segments and ribs. Some features are attributed to growth and developmental factor 11 (GDF11) – a known substrate for PC5/6 – while the delayed mineralization has yet to be explained. Osteopontin (OPN) is a bone matrix protein with roles in mineralization, cell adhesion and migration, and contains the consensus sequence, K/R-$X_n$-K/R↓ ($n=0,2,4$ or 6 variable amino acids) for cleavage by PC5/6. This study investigated OPN as a substrate for PC5/6. *In situ* hybridization of normal mouse bone using *Pcsk5*, *Opn/Spp1* and *Dmp1* anti-sense and control sense cRNA probes, and quantitative RT-PCR on primary murine bone cells and bone cell lines showed expression of Pcsk5 in bone-forming cells (similar to *Opn* and *Dmp1*). Long-bone extracts from *Pcsk5* conditional-knockout E18.5 embryos revealed an OPN fragment at ~15 kDa in normal bone that was not present in PC5/6-deficient bone, as determined by OPN immunoblotting. *Pcsk5*

conditional-knockout E18.5 embryos were also analyzed by micro-computed tomography, confirming the delayed skeletal mineralization. In addition, enzyme-substrate assays using recombinant human PC5/6 and OPN, as well as co-transfections of V5-tagged *OPN/Opn* and *Pcsk5* into Cos-1 cells, showed that PC5/6 efficiently cleaved human OPN (~65 kDa) into ~55 kDa and 17 kDa fragments, consistent with the co-transfection results. Co-transfections with mouse *Opn* cDNA did not show any direct cleavage by PC5/6. In conclusion, we demonstrate that *Pcsk5* is expressed in bone-forming cells, and that OPN is a novel substrate for PC5/6. Cleavage of OPN by PC5/6 could modify the function of OPN in bone, and/or modify downstream enzymatic processing of OPN leading to the bone phenotype. Animal studies were approved by the institutional animal care committee. Funded by CIHR.

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## PP112

### Levels of circulating vesicular microRNA-31 increase with age as well as in the case of osteoporosis and inhibit osteogenic differentiation capacity of mesenchymal stem cells

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Aging is a complex process that results in the decline of physiologic functions due to accumulation of damage in cells and tissues. Mesenchymal stem cells (MSCs) counteract this decline but their regeneration capacity decreases with age. In particular osteogenic differentiation potential of MSCs has been shown to decrease with age thereby contributing to slowed down bone formation and osteoporosis. While much is known about cellular aging of MSCs, little is known about factors of the aged systemic environment influencing their functionality.
While searching for extrinsic factors that influence osteogenesis of MSCs extracellular vesicles (EVs) were found.
Exposition of MSCs to EVs secreted by senescent endothelial cells (senECs), which were shown to accumulate with age *in vivo*, or isolated from plasma of human elderly donors failed to induce osteogenesis compared to MSCs incubated with secreted EVs of young endothelial cells or plasma derived EVs of young donors. We attributed the age-dependent impairment of osteogenesis by EVs to vesicular miR-31 which was shown to be enriched within EVs of senECs and within plasma derived EVs of elderly donors but also in EVs of patients suffering from osteoporosis.
Overexpression of miR-31 in MSCs reduced osteogenic differentiation capacity while inhibiting miR-31 enhanced osteogenesis *in vitro*. MiR-31s underlying molecular inhibitory effect was illuminated by demonstrating that miRNA-31 targets FZD3, a factor which was previously unknown to be necessary for osteogenesis. Finally we were able to rescue MSCs from the inhibitory effect of EVs isolated from senECs or from plasma of elderly donors by transfecting them with a miR-31 inhibitor.
Summarizing our data suggest that vesicular miR-31 is enriched within EVs of elderly donors as well as in the case of osteoporosis and that it is able to inhibit osteogenesis. Thus it might serve as a diagnostic and therapeutic target whenever osteogenesis is a limiting factor

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## PP113

### Bone-anabolic effects of sulforaphane, a naturally occurring isothiocyanate on bone metabolism

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Few drugs generate bone-stimulatory effects *via* epigenetic mechanisms. Modulation of CpG-residues hydroxymethylation in gene-promoters of key osteoblast-related factors (e.g., DLX5) induces their expression and increases osteoblast differentiation *in vitro*. The chemical properties of sulforaphane (SFN), a natural compound abundantly present in cruciferous vegetables like broccoli, suggest that it may have similar molecular and biological effects. Previous studies suggest that SFN blocks nuclear factor-kappaB and thus osteoclastogenesis *in vitro*.
Here, we evaluated the effect of SFN on biological properties of osteoblasts and osteoclasts in cell culture models. Mouse MC3T3-E1 osteoblasts, RAW-264.7 osteoclasts, MLO-Y4 osteocytes, murine bone marrow-derived mesenchymal stem cells (mBMSCs), human adipose-derived mesenchymal stem cells (hAMSCs), mouse pre-osteoclasts (mOC) and mouse calvaria were treated with increasing concentrations of SFN for up to 6 weeks. The *in vivo* relevance of SFN in promoting bone mass was examined in C57Bl6/J mice (at 8 weeks) treated intraperitoneally with SFN for 5 weeks.
After 24 h, 3 μM SFN inhibits cell proliferation and activates caspases 3/7 and 8 significantly stronger in pre-osteoclastic RAW-264.7 cells than in MLO-Y4 and MC3T3-E1 cells. Furthermore, mOC-resorption on ivory is significantly inhibited (~40%) after 12 days. Consistently, SFN significantly decreases expression of RANKL/TNFSF11 in MLO-Y4 cells and mice calvaria. Direct anabolic effects of SNF are reflected by significantly increased expression of bone markers (e.g., *Bglap2*, *Runx2*, *Col1a1*) in MC3T3-E1 cells (after 14 days) and mBMSC (after 20 days). In hAMSCs, SFN transiently stimulates expression of selected markers (e.g., *BGLAP2, OPG/TNFRSF11B, SATB2*) with increased alkaline phosphatase deposition after 13 days. Furthermore, all cell-types and mice calvaria exhibited robust stimulation of extra cellular matrix mineralization after SFN exposure. As measured by μCT, SFN significantly increases trabecular number in bone and an increasing trend ($P=0.07$) was observed in trabecular bone volume in C57Bl6/J mice.
Our findings suggest SFN as potential bone-anabolic mediator suppressing osteoclast function and promoting osteogenic differentiation.

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## PP114

### N-linked glycosylation as a critical mechanism of PTH-resistance in osteoblasts in high glucose conditions

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Type 2 diabetes mellitus impairs bone quality and increases fracture risk. We showed that diabetic ZDF rats have low bone mass due to impaired osteoblastogenesis, which can be partially reversed with an intermittent parathyroid hormone 1–84 (PTH) therapy. It remains unclear, why PTH treatment does not fully restore osteoblast (OB) function in diabetic conditions. Here, we tested if high glucose (HG) conditions lead to a partial PTH resistance in osteoblasts. Pre-osteoblastic MC3T3-E1 cells were cultured in osteogenic medium in HG concentrations for 21d. HG decreased the production of mineralized matrix by −69% as compared to control cells. Intermittent PTH treatment reversed this effect (+115%), but did not reach the level of control cells. PTH dose-dependently increased cAMP levels up to 40-fold, whereas this increase was blunted by HG. Similarly, PTH-induced activation of protein kinase

A, the downstream target of cAMP, was decreased in HG conditions. Gene expression of IGF1, alkaline phosphatase, osteopontin, connexin43 and RANKL significantly increased after intermittent treatment of PTH in control and HG conditions. To determine whether the blunted cAMP activation was due to a decreased expression of the PTH receptor 1 (PTHR1), we determined protein levels of PTHR1 using western blot. However, PTHR1 expression was not affected by HG or PTH. Instead N-linked glycosylation was increased in HG. Combined treatment of PTH with tunicamycin, blocking N-linked glycoprotein synthesis, elevated the amount of mineralization in HG treated cells similarly to PTH treatment (fourfold). Interestingly, tunicamycin alone was able to reverse the inhibition of mineralization of HG treated OB. These results suggest that increased N-linked glycosylation is a critical mechanism of OB function in HG conditions in MC3T3-E1 cells. Thus, preventing these posttranslational modifications may improve OB function and augment the response to PTH treatment in type 2 diabetes mellitus.

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## PP115

**Epigenetic modifications and canonical WNT signaling enable direct programming of non-osteogenic cells into osteoblasts**

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Mesenchymal cells alter and retain their phenotype during skeletal development through activation or suppression of signaling pathways. For example, we have shown that Wnt3a only stimulates osteoblast differentiation in cells with intrinsic osteogenic potential (e.g., MC3T3-E1 pre-osteoblasts) and not in fat cell precursors or fibroblasts (respectively, 3T3-L1 pre-adipocytes or NIH3T3 fibroblasts). Wnt3a promotes osteogenesis in part by stimulating autocrine production of the osteoinductive ligand Bmp2. Here, we show that the promoter regions of the genes for Bmp2 and the osteoblast marker Alp are epigenetically locked to prevent their expression in non-osteogenic cells. Both genes have conserved CpG islands that exhibit increased CpG methylation, as well as decreased acetylation and increased methylation of histone H3 lysine 9 (H3-K9) specifically in non-osteogenic cells. Treatment of pre-adipocytes or fibroblasts with the CpG demethylating agent 5′-aza-2′-deoxycytidine (5′-aza-dC) or the histone deacetylase inhbitor trichostatin-A (TSA) renders Bmp2 and Alp responsive to Wnt3a. Hence, drug-induced epigenetic activation of Bmp2 gene expression contributes to Wnt3a mediated direct programming ('trans-differentiation') of pre-adipocytes or fibroblasts into osteoblasts. We propose that direct conversion of non-osteogenic cells into osteoblastic cell types without inducing pluripotency may improve prospects for novel epigenetic therapies to treat skeletal afflictions.

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## PP116

**Inhibitory effect of GH on the adipogenic commitment of mesenchymal stromal cells derived from human trabecular bone**

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Besides its well known effect on longitudinal bone growth, GH plays a role in the maintenance of adult bone mass. As aging progresses GH levels decline, bone mass decreases and mesenchymal precursors show a reduced osteogenic differentiation capacity resulting in an increase in bone marrow adipocytes. We investigated the effect of GH on MSC differentiation and the possible involvement of microRNA in this process. Human MSC derived from trabecular specimens, waste material of orthopedic surgery (protocol approved by the Institutional Ethical Committee), were characterized by FACS analysis and cultured for 14 days in both normal growth and adipogenic medium in the presence or absence of GH (5 ng/ml). RT-PCR showed that in hMSCs cultivated in normal growth medium, GH enhances the expression of the osteogenic-related genes Runx2, although not significantly, osteoprotegerin (OPG, $P<0.05$) and osterix (OSX, $P<0.05$) and reduces the expression of the Wnt inhibitor DKK1, without any modulation of the adipogenesis related-genes adiponectin and C/EBP$\alpha$. Since miR22 targets a Runx2 inhibitor and miR29c regulates DKK1 expression we evaluated the levels of miR22 and miR29c. Results showed that GH exposure induces an increase in both miRNAs.

In hMSCs cultivated in adipogenic medium GH does not increase the expression of the osteogenic genes OPG and OSX, but decreases adiponectin ($P<0.01$) and C/EBP$\alpha$ ($P<0.01$) expression. GH also downregulates miR-204 ($P<0.05$), which is known to be involved in adipogenesis. OilRedO staining of hMSCs confirmed these data showing that GH treatment significantly reduces lipid droplets formation ($P<0.001$).

Taken together these data suggest that GH plays a role in MSCs differentiation by increasing osteoblastogenesis in normal conditions. Moreover it might reverse MSCs commitment towards fat tissue in an adipogenic environment, such as the bone marrow milieu during the aging process.

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## PP117

**EGF inhibits Wnt/β-catenin-induced osteoblast differentiation in a Smurf1-dependent manner**

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BMPs and canonical Wnts are the representative developmental signals that enhance osteoblast differentiation and bone formation. Previously, we demonstrated that EGF inhibits BMP2-induced osteoblast differentiation through the induction of Smurf1 expression. However, the regulatory role of EGF in Wnt/β-catenin-induced osteoblast differentiation has not been elucidated. In this study, we investigated the effect of EGF on Wnt/β-catenin signaling-induced osteoblast differentiation using C2C12 cell line. EGF significantly suppressed the expression of osteoblast marker genes, which was induced by Wnt3a and GSK-3β inhibitor. EGF increased the expression levels of Smurf1 mRNA and protein. Smurf1 knockdown rescued Wnt/β-catenin-induced osteogenic marker gene expression in the presence of EGF. EGF treatment or Smurf1 overexpression did not affect the expression level of β-catenin mRNA but reduced β-catenin protein level and TOPFLASH activity. EGF and Smurf1 promoted β-catenin ubiquitination. Co-immunoprecipitation and GST pull-down assays showed that Smurf1 associates with β-catenin. These results suggest that EGF/Smurf1 inhibit Wnt/β-catenin-induced osteogenic differentiation and that Smurf1 downregulate Wnt/β-catenin signalling *via* enhancing proteasomal degradation of β-catenin.

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## PP118

**HBO increased osteogenic differentiation of MSCs *via* regulating Wnt processing, secretion, and signaling**

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Background
Autocrine and paracrine Wnt signaling operates in stem cell populations and regulates mesenchymal lineage specification. In Wnt producing cells, Wntless

(GPR177) supports the transport of Wnt from the trans-Golgi network (TGN) to the cell surface in vesicles, from which Wnt is then released. The retromer complex (VPS35) interacts with Wntless and retrieves Wntless from endosomes back to TGN, thereby maintaining the normal levels of Wntless protein. V-ATPases-driven proton pumping and organellar acidification are essential for Wnt secretion. However, little is known about the effects of HBO on the Wnt processing, secretion, and signaling in MSCs.

Materials and methods

Cells were cultured in osteogenic induction medium. Control cells were maintained in 5% $CO_2$/95% air and the hyperoxic cells were exposed to 100% $O_2$ at 2.5 ATA (atmospheres absolute) in a hyperbaric chamber. To investigate the effects of HBO on Wnt processing and secretion, the protein levels of Wnt3a, GPR177, VPS35, ATP6V0, ATP6V1, and β-actin were detected by western blot. The secreted Wnt3a in the collected medium was quantified by ELISA. To investigate the effects of HBO on Wnt signaling, the mRNA and protein levels of Wnt3a, β-catenin, GSK-3β, Runx 2, as well as alkaline phosphatase activity and calcium deposition were analyzed after HBO treatment. The von Kossa staining was tested in osteogenetic differentiated MSCs after HBO treatment.

Results

Our data showed that HBO treatment increased the expression of Wnt3a, GPR17, VPS35, ATP6V0, and ATP6V1 to stimulate Wnt processing and secretion, and the effect was confirmed by siRNA treatment. The mRNA and protein levels β-catenin and Runx 2 were up-regulated, while GSK-3β was down-regulated after HBO treatment. HBO significantly increased alkaline phosphatase activity, calcium deposition, and the intensity of von Kossa staining of osteogenically differentiated MSCs.

Conclusion

HBO treatment increased osteogenic differentiation of MSCs *via* regulating Wnt processing, secretion, and signaling.

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## PP119

**Effect of osteoporosis in the transcriptional profile of osteoblastic cells from bone marrow and calvaria of ovariectomized rats**

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Changes in the ability of self-renewal and differentiation of mesenchymal stem cells may be affected by osteoporosis. The aim of this investigation was to compare the transcriptional profile of mRNAs and miRNAs of osteoblastic cells from calvaria and bone marrow of female rats after ovariectomy. Following the approval by ethics committee, 18 wistar rats were divided into control (sham) and ovariectomized groups. After 150 days, both groups were sacrificed to collect the femurs and fragments of calvaria from which cells were isolated and cultivated until subconfluence. It was performed the extraction and quantification of total RNA, followed by RNA integrity evaluation. Identification of gene modulation by cDNA microarray and evaluation of microRNAs involved in gene inhibition was carried out by means of Agilent Feature Extraction Software, and the files generated in the program were analyzed in bioinformatics platform Agilent GeneSpring GX 12.6. Data obtained showed that 5.447 differentially expressed mRNAs were detected in cells from calvaria and 4.399 in cells from bone marrow. There were found 84 miRNAs differentially expressed in osteoblastic cells from calvaria and 55 miRNAs from bone marrow. Genes found in the group of calvaria were related to ossification, bone morphogenesis and development, whereas in bone marrow genes were related to differentiation and response to estrogen stimulation. Among the miRNAs, members of 30 family were up-regulated in both ovariectomized groups, which act as negative regulators of osteoblast differentiation. The miR-17 that is associated with up-regulation of osteogenesis was down-regulated in both osteoporotic groups. These data suggest a difference in the transcriptional profile of osteoblastic cells from calvaria and bone marrow, both affecting the proper formation of bone tissue in a situation of osteoporosis.

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## PP120

**Treatment with allopurinol and oxypurinol promotes osteoblast differentiation and increases bone formation**

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Allopurinol and, and its active metabolite, oxypurinol are widely used in the treatment of gout and hyperuricemia. They act by inhibiting xanthine oxidase an enzyme in the purine degradation pathway that converts xanthine to uric acid. This oxygen-dependent reaction also results in the generation of superoxide free radicals. The aim of this investigation was to examine the effect of allopurinol and oxypurinol on osteoblast differentiation and function. Rat calvarial osteoblasts were isolated by trypsin/collagenase digestion and cultured for up to 14 days with 2mM β-glycerophosphate, 50 μg/ml ascorbate and 10 nM dexamethasone. The effect of allopurinol and oxypurinol on bone formation, cell number, cell viability, gene expression and enzyme activity was investigated in differentiating (day 7) and/or mature bone-forming osteoblasts (day 14). Xanthine oxidase expression and activity were detected in both differentiating and mature cells. Although mRNA expression remained relatively constant, xanthine oxidase activity decreased over time with cultures of mature osteoblasts displaying reduced levels of uric acid (20% decrease, $P<0.001$). Treatment with both allopurinol and oxypurinol (0.1–1 μM) reduced xanthine oxidase activity by up to 30% ($P<0.001$) in differentiating and mature osteoblasts. At these concentrations, allopurinol and oxypurinol increased bone formation ~fold and ~threefold respectively ($P<0.001$). Cell number and viability were unaffected. Both drugs increased alkaline phosphatase (TNAP) activity up to 50% in differentiating osteoblasts ($P<0.001$) and 65% ($P<0.01$) in mature osteoblasts. The expression of key osteoblast marker genes was investigated using qPCR. Osteocalcin and TNAP mRNA expression was increased, fivefold and twofold respectively. In contrast expression of NPP1, the enzyme responsible for the generation of the mineralisation inhibitor, pyrophosphate, was decreased fivefold. Col1α1 mRNA expression was unaffected. Our data suggest that inhibition of xanthine oxidase activity promotes osteoblast differentiation, leading to increased bone formation.

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## PP121

**Is the bone marrow envelope a reservoir of osteoblast progenitors?**

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It is still unknown where from osteoblasts are recruited during remodeling of adult human cancellous bone. Still, it is often suggested that they originate from perivascular osteoprogenitors in the bone marrow (BM). Here we propose also the existence of a layer of osteoprogenitors at the periphery of the BM. This proposal is based on electron and light microscopy of the bone surface and of the neighboring BM in human iliac crest biopsies, where we quantified cell densities, cell proliferation, osteoblast differentiation markers, and capillaries ($n=14$) (Danish Ethical Committee S20070121).

We found that quiescent surfaces are not only covered by bone lining cells, but also by very flat ($< 0.1$ μm) and elongated cells, which are P3NP-positive and surround the whole BM. At the level of osteoclasts, this cell envelope appears lifted forming a canopy over the remodeling site – a view which is supported by the physical continuity between this canopy and the BM envelope at the level of quiescent surfaces, and by the fact that the vast majority of osteoclast surfaces are covered by a canopy. Canopies proved to consist of osteoblast-lineage cells which are more proliferative and less differentiated than bone surface cells, as shown by the inverse levels of Ki-67 and P3NP vs osterix. Furthermore, the presence of capillary-canopy contacts peaked over osteoclast surfaces, and the number of capillary-canopy contacts correlated positively with the osteoblast density on bone-forming surfaces. Interestingly, canopy cell density was negatively affected by age, and correlated also positively with osteoblast density on bone-forming surfaces.

In conclusion, the present data point to the BM envelope as a source of osteoprogenitors. Its dual interaction with osteoclasts and capillaries upon initiation of the bone remodeling cycle fits the current knowledge of the role of osteoclasts and vasculature in triggering osteogenesis.

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## PP122

### Extracellular protein Edil3 stimulates matrix calcification *via* α5β1 and ERK pathway in MC3T3E1 cells

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Epidermal growth factor-like repeats and discoidin I-like domains 3 (Edil3) is an extracellular matrix protein that contains Arg-Gly-Asp (RGD) motif to bind to integrins. Recently it has been reported that Edil3 is involved in various biological processes, including angiogenesis and cellular differentiation. This study was to explore the role of Edil3 in osteoblast differentiation and an underlining molecular mechanism. When MC3T3E1 cells were cultured with osteogenic medium, Edil3 mRNA and protein expressions were increased in a time-dependent fashion. Treatment of Edil3 protein led to the increases in alkaline phosphatase (ALP) and osteocalcin (OC) gene expressions and phosphorylation of ERK. A neutral antibody for α5β1 and MEK inhibitor U0126 inhibited the Edil3 induction of ALP and OC expression. Overexpression of Edil3 by lentiviral system resulted in matrix calcification, and U0126 inhibited the calcification. These results suggest that Edil3 may positively regulate osteoblast differentiation and matrix calcification *via* the integrin α5β1-ERK pathway.

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## PP123

### miR-468 controls Runx2 expression during early differentiation of Runx2 in mice

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We investigated the ability of microRNAs to regulate osteoblast differentiation by controlling distinct aspects of the differentiation process. We have identified by microRNAs microarray a set of miRNAs that are differentially regulated during differentiation of the two osteoblast cell lines MC3T3-E1 and C3H10T1/2. We focused on miR-468 that is a potential regulator of Runx2.

In our microarray analysis miR-468 was expressed at a high level and was strongly down regulated during osteoblast differentiation. The down regulation of the expression of miR-468 well matches with the regulation of the expression of Runx2 that is increased during the early stage of osteoblast differentiation. After induction of osteoblast differentiation, miR-468 expression is decreasing from day 3 over time and was highly consistent with the microarray data.

One single target site for miR-468 was present in the first 500 bp of the 3′UTR of the Runx2 gene. These first 500 bp are able to mediate a 40 and 60% inhibition of the Runx2 activity in MC3T3-E1 and C3H10T1/2 cells respectively. We demonstrate that Runx2 expression was specifically down-regulated by miR-468. We showed by western blot and using reporter plasmids that miR-468 reduced Runx2 expression, inhibited its transcriptional activity and decreased expression of osteoblastic markers (SP7, BSP, type1 collagen) that are regulated by Runx2. The inhibitory effect of miR-468 on Runx2 expression was abolished when its target site on the 3′UTR of Runx2 is mutated.

All together these results indicate that Runx2 is a direct target of miR-468. Our findings suggest that miR-468 acts as Runx2 attenuators to antagonize osteoblast differentiation in pre-osteoblastic cells and possibly in BMSCs. This further indicates that a down-regulation of miR-468 may be required to release the factor Runx2 from repression and to induce osteoblast differentiation.

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## PP124

### MiR-320a and miR-483-5p are over-expressed in osteoblasts from osteoporotic fractured hips

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MicroRNAs are important regulators of gene expression with documented role in bone metabolism and osteoporosis. Moreover, the use of miRNAs constitutes potential therapeutic targets. Our aim was to identify miRNAs differentially expressed in fractured compared to healthy bone. Additionally, we performed a miRNA profiling of primary osteoblasts to assess the origin of the differentially expressed miRNAs. Total RNA was extracted from fresh femoral neck trabecular bone from women undergoing hip replacement due to either osteoporotic fracture ($n=6$) or osteoarthritis ($n=6$) in the absence of osteoporosis (according to BMD measurements), age and BMI matched, and from primary osteoblasts (at passage 0) obtained from knee replacement due to osteoarthritis ($n=4$). Samples were hybridized to the miRCURY LNA™ microRNA Array 7th (Exiqon, Denmark), in the manufacturers' facilities. QC tests, PCA plots and heat map hierarchical clustering were performed. For comparison of expression levels, the threshold was set at log fold change $>1.5$ and a $P$-value $<0.05$ (corrected for multiple-testing).

Both PCA and heat map analyses showed that the samples clustered according to their biological group (fracture vs non-fracture). However, one osteoporotic sample appeared as outlier and was excluded. Overall, 792 and 315 different miRNAs were detected in fresh bone samples and in primary osteoblats, respectively, 284 of which were shared (i.e. 35.8% of bone miRNAs were from osteoblasts).

A subset of 82 microRNAs was found to be significantly differentially expressed between osteoporotic and control samples. Upon validation of ten miRNAs with the lowest p-values, and for which an validated assay was available, using the miRCURY LNA™ Universal RT microRNA qPCR assay, two of them were confirmed: miR-320a and miR-483-5p. They are both over-expressed in the osteoporotic samples (and expressed in primary osteoblasts). In conclusion, two osteoblast miRNAs are over-expressed in osteoporotic fractures, which open novel prospects for research and therapy.

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## PP125

### The significance of Bcl-2-associated athanogene-1 (BAG-1)-mediated protein interactions in osteoblast development

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BAG-1 interacts with diverse proteins including heat shock proteins (HSC70/HSP70) and nuclear hormone receptors to modulate cell proliferation, differentiation and apoptosis. We have shown that bone marrow stromal cells (BMSCs) of *Bag-1*$^{+/-}$ female mice display reduced BMP-2-directed osteogenic differentiation potential. Estradiol binds to estrogen receptors (ERs) and is functionally involved in BMP-directed osteogenic differentiation of osteoprogenitor cells by upregulating *Bmpr2* transcription and Smad 1/5/8 signalling. BAG-1 enhances estradiol-dependent transcription and HSC70 is a likely mediator of BAG-1 function, since BAG-1 binds HSC70 and regulates refolding/activation of ERs by HSC70 when transitioning from ligand-bound to ligand-free conformations. thioflavin-S inhibits BAG-1:HSC70 binding and serves as a small-molecule chemical inhibitor to investigate the functional significance of BAG-1-mediated protein interactions.

The present study analysed differences in expression of 84 genes crucial for skeletal development between BMSCs of WT and Bag-1$^{+/-}$ female mice following BMP-2-directed osteogenic differentiation, and investigated the effect of inhibition of BAG-1-mediated protein interactions by thioflavin-S on BMP-2-directed osteogenic differentiation of WT BMSCs in presence of 17-β-estradiol.

In response to BMP-2, 30 genes were differentially expressed between BMSCs of WT and *Bag-1*$^{+/-}$ female mice. Notably, genes crucial for osteogenic differentiation (*Bmp-2*, *Bmp-4*, *Bmp-7*, *Pdgfa*, *Vegfa*, *Osterix*/*Sp-7*), matrix biosynthesis (*Col1a1*, *Col5a1*, *Serpinh-1*) and mineralisation (*Alp*, *Osteopontin*, *Osteocalcin*, *Phex*, *Sclerostin*) were expressed at significantly lower levels in *Bag-1*$^{+/-}$ BMSC cultures. 17-β-estradiol enhanced BMP-2-induced osteogenic gene expression by upregulating *Bmpr2* expression in WT BMSCs, which expressed both ERs. Addition of thioflavin-S to BMSC cultures supplemented with BMP-2 and 17-β-estradiol resulted in significant downregulation of *Bmpr2* and osteogenic gene expression. No differences in DNA content/ cell number between the two culture conditions confirmed that thioflavin-S was not cytotoxic. Thus, BAG-1 has an important role in osteoblastogenesis and inhibition of BAG-1 function by thioflavin-S reduces osteogenic differentiation of BMSCs in response to 17-β-estradiol and BMP-2.

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## PP126

### Glucose-dependent insulinotropic polypeptide directly affects collagen deposition and maturation in osteoblast cultures

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Objectives
A role for glucose-dependent insulinotropic polypeptide (GIP) in controlling extracellular bone matrix deposition and maturation has previously been evidenced in GIP receptor knock-out mice. However, as the GIP receptor is expressed in several tissues other than bone it was difficult to ascertain whether the modifications of extracellular bone matrix were due to direct effects on osteoblasts or indirect through regulation of signals originating from other tissues. The aim of the present study was to assess in osteoblast cultures *in vitro* whether GIP could affect collagen deposition and maturation.
Materials and methods
The murine MC3T3-E1 cells were used as the source of osteoblasts. A GIP mimetic, the D-ala(2)-GIP (GIP) was synthesized using standard solid phase Fmoc protocols. MC3T3-E1 cells were differentiated in the presence of ascorbic acid (50 μg/ml) and various concentrations of GIP ranged between 10–100 pM. Amine oxidase activities were assessed in supernatant of differentiated cells in response to GIP by a fluorometric assay. Collagen deposition and maturation were assessed by transmitting electron microscopy and Fourier-transformed infrared microscopy (1660/1690 per cm ratio) respectively. Non-parametric Mann–Whitney *U*-test was used to compare differences between groups.
Results
Compared to untreated cells, GIP dose-dependently stimulated the production of cAMP by osteoblasts. Furthermore, the amine oxidase activity was dose-dependently increase by GIP treatment to reach a 95% augmentation at 100 pM GIP. Furthermore, GIP affected collagen deposition as evidenced by significant decreases in collagen fibril diameters. Collagen maturity was also affected with significant augmentation of the 1660/1690 percm ratio. The use of 2′,5′-dideoxyadenosine, an adenylyl cyclase inhibitor, markedly blocked the action of GIP on amine oxidase activity, collagen deposition and maturation.
Conclusions
Overall, this study provides evidences that GIP acts on osteoblasts through an andenylyl cyclase-cAMP pathway and directly affects collagen deposition and maturation.

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## PP127

### Regulation of adipo- and osteo-genesis of multipotent cells by strontium through stimulation of small Rho GTPases: A 3D bioreactor study

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Small GTPases of the Rho family (RhoA and Rac-1) are responsible for cytoskeleton dynamics (particularly actin polymerisation) and control cellular tension. For these reasons, they are implicated in the commitment of multipotent cells (MCs). In one hand, increased tension (important RhoA activity) is commonly associated with osteogenesis (OS), in the other hand, a reduced one (low RhoA activity) is associated with adipogenesis (AD). Nevertheless, precise RhoGTPases regulations during stem cell commitment are not known. We and others observed that strontium (an anti-osteoporotic drug) promotes OS and prevents AD of MCs. We hypothesize that strontium (Sr) effects may be explained by its ability to modulate RhoGTPases activities.
To address this question, we cultivated C3H10T1/2 on microspheres in a dynamic bioreactor in conditions where OS (apatite coated beads) and AD (rosiglitazone) were promoted. 3D cultures were treated or not with 5 mM of Sr up to 6 days. OS, AD specific markers were evaluated by quantitative RT-PCR, RhoA and Rac1 activities were quantified by specific G-LISA. In our hands, OS is associated with sustained RhoA and increasing Rac1 activities whereas AD is characterized by a Rac1 activity reduction. We observe, as expected, that Sr inhibits AD (PPARγ2, C/EBPα, FABP4 & Adiponectin) and promote OS (ALP, OSX, BSP & OPN). During MC commitment, Sr increases significantly both RhoA and Rac1 activities from day 2 to 6 in OS and AD conditions. Dual activation of RhoA and Rac1 in MCs may explain why AD is blocked by strontium and fits with the promotion of fibrillogenesis and Wnt/β catenin signalling respectively controlled by RhoA and Rac1 in OS conditions.
As RhoA and Rac1 are mainly regulated by integrins and VEGFR dependent pathways, we measure various integrins, VEGF isoforms and receptors expressions. We identify matrix-bound VEGF188 and Flk1 as potential targets of Sr and ongoing studies will help understanding ability of VEGF188 to regulate RhoGTPases.
In conclusion, the ability of Sr to stimulate both RhoA and Rac1 in MCs may explain its particular action on osteoblasto- and adipo-genesis.

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## PP128

### Vitamin K2 (MK-7) is involved in bone and energy homeostasis: Effects on osteoblast, adipocyte and β-cell regulatory loops

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Uncarboxylated osteocalcin, allegedly *via* G-protein coupled receptors, interacts with adipocytes and pancreatic β-cells, thus affecting metabolic homeostasis. Here, we demonstrate that MK-7 directly and indirectly, *via* osteocalcin, modulates the endocrine functions of both β-cells and adipocytes.

- It was shown (by applying the Mir@nt@n-based emulation algorithm) that MK-7 was involved in a network of intracellular regulatory loops comprising osteoblast specific microRNAs and transcription factors, including miR-760 targeting SXR. Furthermore, it was demonstrated that MK-7, in the absence or presence of osteoclasts differentiated from human PBMCs, stimulated the secretion of osteocalcin, OPG, RANKL, and interleukins from bovine bone osteoblasts, while anti-SXR siRNA or premiR-760 did the opposite.
- MK-7 enhanced the secretion of adiponectin from adipocytes and insulin (basal and glucose/BCAA-stimulated) from β-cells differentiated from adipose tissue derived stem cells (ASCs). This effect of MK-7 was blocked by adding anti-SXR siRNA or premiR-760 to the cell cultures.
- Adipocytes and β-cells were incubated in preconditioned medium from bovine bone chips obtained in the presence of osteoclasts derived from PBMCs, and in the absence or presence of osteocalcin antibodies, in order to establish their response to G-protein mediated signalling (intracellular cAMP and $IP_3$ levels). Subsequently, the cells were exposed to siRNAs against $G_{\alpha s}$, $G_{\alpha q}$, and $G_{\alpha i2}$ in the absence or presence of MK-7 showing that: i) osteocalcin affects the G-protein coupled receptors and their intracellular signalling molecules with a certain degree of plasticity; ii) MK-7 is able to modulate this plasticity, while also stimulating egress of secretagogues from the adipocytes and β-cells respectively.

From the present experiments, it appears that vitamin K2 (MK-7) exerts strong direct and indirect hormone-like effects on inter-organ cross-talk, and may represent a key modulator of bone health, as well as energy metabolism.

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## PP129

**Modulation of sclerostin expression by estrogen *via* BMP-2 signaling in human mesenchymal stromal cells and osteoblasts**

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Little is known about the molecular mechanisms underlying the interaction between Sclerostin (SOST) and estrogen, showing an inverse relation in clinical data. Therefore, we investigated mechanisms by which estrogen modulates Sost expression at human osteoblastic cell level in association with BMP-2 signaling, which is a potential route of SOST induction. BMP-2 significantly induced *SOST* expression, which is suppressed by 17β-estradiol (E2) in human mesenchymal stromal cells (hMSCs) and mandible-derived osteoblasts from female donors, despite non-effect of E2 alone on *SOST* expression. E2 increased transcriptional activity of β-catenin/TCF/LEF responsive vector, which is suppressed by the combined treatment of BMP-2 and E2 in hMSCs. Either estrogen receptor (ER)α antagonist or silencing of ERα gene did counteract the suppressive effect of E2 on *SOST* induction by BMP-2, while it had no effect on the activity of BMP-2 alone BMP-2. However, E2 had non-effect on the transcriptional activity of Smad4, which was but dependent of BMP-2. *SOST* expression pattern from human osteoblasts was in particular correlated with the relative ratio of *RANKL* to *OPG* expression. Taken together, these findings showed that estrogen negatively modulates *SOST* expression coupled with BMP-2 signaling, which involve Wnt pathway *via* ERα and β-catenin, providing insight into how estrogen is anabolic to bone at the level of osteoblastic cells.

Keywords: Estrogen, sclerostin, BMP-2, human mesenchymal stromal cells, human osteoblasts, Wnt pathway, estrogen receptor α, RANKL/OPG

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## PP130

**Decrease of mineralization by FGF1 is linked to up-regulation of ANKH and OP in osteogenic differentiation and conversion**

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Osteoporosis is generally accompanied by the fatty degeneration of the bone marrow. The enhanced deposition of adipocytes may result from adipogenic differentiation of bone-marrow mesenchymal stem cells (MSC) and from the adipogenic conversion of osteoblastic cells respectively. Thus, whether this clinical observation is a cause of the disease or rather a reaction of the afflicted bone marrow remains to be elucidated.

Previous microarray and bioinformatic analyses showed differential gene expression patterns for members of the fibroblast growth factor (FGF) signaling pathway during the onset of adipogenic conversion of osteoblastic cells and osteogenic conversion of adipocytes. FGF1 was scored as one lead candidate gene to modulate conversion processes. This factor was shown to strongly inhibit adipogenic MSC differentiation as well as the adipogenic conversion *in vitro*. Further investigation revealed that FGF1 not only affects adipogenic commitment and differentiation but also mineralization during osteogenic MSC differentiation and the osteogenic conversion of adipocytes. Effects were reproducible in several donors and clearly concentration-dependent. Quantitative analysis showed a decrease of approx. About 60% in calcium deposition. Gene expression analyses *via* quantitative RT-PCR revealed a strong down-regulation of the adipogenic differentiation factors peroxisome proliferator-activated receptor gamma 2 (PPARγ2), lipoprotein lipase (LPL) and fatty acid binding protein 4 (FABP4) during adipogenic differentiation and conversion. Additionally, integrin-binding sialoprotein (IBSP) expression was down-regulated whereas progressive ankylosis protein homolog (ANKH), an inorganic pyrophosphate transport regulator, and osteopontin (OP) were up-regulated during osteogenic differentiation and conversion, obviously linking gene expression to the decreased mineralization outcome.

We conclude that FGF1 triggers downstream signaling molecules affecting adipogenic as well as osteogenic differentiation and conversion. Future studies will focus on intracellular pathways involved in the observed effects, which might serve as possible targets for the development of novel therapeutic approaches against osteoporosis and related bone diseases.

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## PP131

**Hyperbaric oxygen promotes osteogenic differentiation of mesenchymal stem cells by regulating sclerostin expression and Wnt/β-catenin signaling**

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Background

Hypoxia induces mesenchymal stem cells (MSCs) proliferation but results in a population with impaired osteogenic differentiation potential. Bone healing of tibial lengthening is enhanced by hyperbaric oxygen therapy. However, little is known about the effects of HBO on the Wnt signaling pathway in MSCs.

Materials and methods

MSCs were cultured in complete medium and the osteogenic groups were cultured in osteogenic induction medium. Control cells were maintained in 5% $CO_2$/95% air throughout the experiment. The hyperoxic cells were exposed to 100% $O_2$ for 25 min and then to 5% $CO_2$/95% air for 5 min at 2.5 ATA (atmospheres absolute) in a hyperbaric chamber. Cell proliferation was quantified using the WST-1 cell proliferation reagent. The mRNA and protein levels of Wnt3a, sclerostin, β-catenin, GSK-3β, Runx2, as well as alkaline phosphatase activity and calcium deposition were analyzed after HBO treatment. We investigated whether HBO affects the translocation of β-catenin between nucleus and cytosol by western blot. Alizarin Red staining was tested in osteogenetic differentiation MSCs after HBO treatment.

Results

Our data showed that HBO increased cell proliferation in osteogenically differentiated MSCs. The mRNA and protein levels of Wnt3a, β-catenin, and Runx2 were upregulated while sclerostin and GSK-3β were downregulated after HBO treatment. The relative density ratio (phosho-protein/protein) for GSK-3β was up-regulated while β-catenin was down-regulated after HBO treatment. Our western blot analysis showed increased levels of translocated β-catenin that stimulated the expression of target genes after HBO treatment. HBO increased TCF-dependent transcription, Runx2 promoter/Luc gene activity, and the expression of osteogenic markers of MSCs, such as alkaline phosphatase activity, osteocalcin, calcium, and the intensity of Alizarin Red staining.

Conclusion

Hyperbaric oxygen promotes osteogenic differentiation of MSCs by regulating sclerostin expression and Wnt/β-catenin signaling.

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## PP132

**Roles of phospholipase D during physiological and vascular calcification**

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There is a similarity of the mechanisms of physiological and pathological mineralization. Pathological calcification of soft tissues as the case of vascular calcification is characterized by the deposition of hydroxyapaptite induced by matrix vesicles at least in the initial stage as in the case of physiological calcification in skeletal tissues. Lipid metabolism is involved in the differentiation of smooth muscle cells and bone cells suggesting that phospholipases can modulate the differentiation process. Among the phospholipases, phospholipase D (PLD) action during differentiation of smooth muscle cells and bone cells are not well established. We seek to define the role of PLD during physiological and pathological mineralization. We selected a cell line human Saos-2 cells and primary osteoblasts from murine calvarias to mimic physiological mineralization and aorta culture to mimic *ex vivo* vascular calcification. The cells, in the presence of osteogenic factors ascorbic acid and β-glycerophosphate became mineral competent, having a strong alkaline phosphatase (TNAP) activity, a biomarker of mineralization process. We identified the presence of two PLD isoforms: PLD1 and PLD2 in Saos-2 cells. An increase of PLD expressions and activity were observed during Saos-2 cell differentiation which reached a maximum at day-5. To confirm the influence of PLD in the mineralization process, incubation of halopemide –a PLD inhibitor- in Saos-2 and primary osteoblast cells induced a 20% decrease of mineralization as probed by a calcification-marker Aliza-Red and by TNAP activity. Overexpression of PLD (by transfecting Saos-2 cells) stimulated the mineralization process and TNAP activity. The calcified aorta in presence of 5 mM phosphate stimulated its transdifferentiation, increasing PLD activity. Incubation

of halopemide at 10 μM in calcified aorta decreased TNAP activity by around 40%. Our findings indicated that PLD is involved during the maturation of mineral competent cells such as osteoblasts and smooth muscle cells.

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## PP133

### Chemical composition of apatites formed by matrix vesicles during bone mineralization

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Bone cells control initial steps of mineralization by forming matrix vesicles (MVs) in which calcium and phosphate ions are accumulated. Under physiological conditions, MVs are released to the extracellular matrix (ECM) and Ca2+ and Pi form hydroxyapatite (HA). Growing minerals break the MVs membrane and are deposited in ECM. Under pathological conditions the mineralization process becomes deregulated, which leads to the formation of calcium phosphate deposits. It is suggested that members of the annexins family and tissue-nonspecific alkaline phosphatase (TNAP) (activators of ossification) as well as fetuin-A (a potent inhibitor of calcification) play an important role in regulation of mineralization both in healthy and pathological conditions.
We used two human cell lines: osteoblastic hFOB 1.19 and osteosarcoma Saos-2. These cells were stimulated for mineralization for 7 days in the presence of ascorbic acid and β-glycerophosphate. Electron microscopy data revealed that MVs in osteoblastic cells are small and single-walled, whereas in osteosarcoma they are big and multi-compartmental (called then multivesicular bodies). The results from the X-ray microanalysis showed significant differences between calcium to phosphate ratio in minerals formed by both cell lines. In osteoblastic cells the atomic ratio of calcium to phosphate was close to HA (e.g. 1.67), but osteosarcoma cells formed abnormal mineral with the ratio calcium to phosphate shifted towards the phosphate. In addition enzymatic assay indicated of divergences in TNAP activity in osteosarcoma cells in comparison to osteoblasts, while western blot analysis revealed changes in distribution of AnxA2, AnxA6 and fetuin-A in both cell lines.
Concluding, not only differences between the amount and size of MVs in pathological *vs* physiological conditions, but also in the activity and distribution of vesicular proteins engaged in the process of mineralization were observed.

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## PP134

### Osteogenic markers are decreased in SHR rats during *in vitro* osteoblast differentiation

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The purpose of this study was evaluated the osteogenic differentiation *in vitro* of mesenchymal stem cells (MSC) of SHR (Spontaneously Hypertensive Rats) and Wistar (Normotensive) rats.Therefore, MSC from femoral bone marrow of Wistar and SHR rats 4 weeks old were utilized. The experimental protocols were approved by the Animal Experiments Local Ethics Committee (Process 00716-2012). The osteogenic medium (OM) consist of the proliferation medium (MEM) supplemented with 50 μg/ml ascorbic acid, 10 mM β-glicerophosphato and $10^{-8}$M dexamethasone. We evaluated the cell proliferation, alkaline phosphatase (ALP) activity and total protein by colorimetric methods; mineralization of nodules was analyzed using alizarin red staining; and osteoblasts-associated protein expression by qPCR. Statistical analysis we used two-way ANOVA test, followed by Bonferroni test. Differences were regarded as significant if $P < 0.05$. The results demonstrated reduced proliferation rate, total protein content and mineralization in osteoblast of SHR plus OM (SHROM) than Wistar plus OM (WOM). However, ALP activity has no difference between both groups. The transcription factors Osterix and β-catenin were low in SHROM, suggesting reduced differentiation in this group. The decreased expression of osteoblast-associated proteins as such OCN, BSP, COL I and OPN in SHROM, revealed the low quality of extracellular matrix (ECM). All this results described above evidence reduced osteoblast differentiation in SHROM. IL-6 and CXCL1/CINC2 production was increased in SHRC (proliferation medium) compared to WC (proliferation medium), suggesting a proinflammatory stage in SHR. In conclusion, SHR osteoblast differentiation is reduced than compared to Wistar. Lower mineralization and poor ECM quality was also observed. These data suggest that the influence of hypertensive genotype on bone metabolism of young SHR, before the hypertension development should be considered. These differences may be the reason of the bone alterations in the SHR strain. Financial support: FAPESP (Process 2012/01924-9; 2011/06070-5; 2011/19458-1).

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## PP135

### High-resolution molecular validation of self-renewal and spontaneous differentiation in adipose-tissue derived human mesenchymal stem cells cultured in human platelet lysate

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Improving the effectiveness of adipose-derived human mesenchymal stromal/stem cells (AMSCs) for skeletal therapies requires a detailed molecular characterization of mechanisms supporting cell proliferation and multi-potency of AMSCs. We compared gene expression data obtained by high-throughput RNA sequencing and RT-qPCR of actively proliferating and uninduced post-proliferative AMSCs with flow cytometry data for mesenchymal stem cell markers (e.g., CD44, CD73/NT5E, CD90/THY1 and CD105/ENG). Immuno-detection of CD markers corresponds well with mRNA expression results. AMSCs also express mesenchymal markers (NES and ACTA2) and pluripotency genes (e.g., POU5F1, NANOG, KLF4). Proliferating AMSCs express mRNAs for cell cycle-related biomarkers (e.g., cyclins CCND1 and CCNB2). These genes are down-regulated in confluent AMSC cultures. Thus, cessation of AMSC proliferation occurs by selective suppression of the cell cycle machinery. Quiescent AMSCs exhibit >tenfold upregulation of proteins involved in tissue matrix formation, including several leucine-rich repeat proteins (ASPN, ECM2, FMOD, OGN and PODN). Quiescent AMSCs also modulate expression of WNT signaling components, including the WNT-inducible gene WISP2 and the decoy receptors SFRP2 and SFRP2, while switching production of WNT5A, WNT5B and WNT7B to WNT2 and WNT2A. Furthermore, we show that post-proliferative AMSCs spontaneously transition into fibroblastic, osteogenic, chondrogenic and adipogenic cell fates. Our findings provide fundamental quality control data to support the clinical versatility of AMSCs.

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## PP136

### Transcriptional profile of osteoblastic cells cultured on titanium surfaces modified by oxidative nanopatterning

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Titanium implants have been extensively used in dentistry as a replacement of absent dental elements. The biocompatibility of a material depends on cellular response in contact with a surface. The microarray technology is a tool to obtain an overview of the cell state in terms of large-scale transcriptional expression in different situations. Thus, the objective of this investigation was to evaluate the transcriptional profile of osteoblastic cells from human alveolar bone cultured on titanium surfaces, varying the etching parameters such as temperature and composition of $H_2SO_4/H_2O_2$ solution used. Polished discs were used as control. Human alveolar bone fragments were obtained from healthy donors, using the research protocols approved by the local research ethics committee. Cells were subcultured on sterilized titanium discs at a cell density of $2 \times 10^4$ cells per disc in culture plates and divided into control (C), nanotexture (N), nano+submicro-texture (N+S) and rough microtexture (RM) groups. During the culture period, cells were incubated at 37 °C in a humidified atmosphere of 5% $CO_2$ and 95% air and the medium was changed every other day. After 10 days, total RNA was extracted using Mirvana kit®. Oligo-arrays Agilent Human GE 4×44K and microRNA microarrays 8×15K were used and data analysis was performed by

GeneSpring GX analysis software. Gene modulation was determined by ANOVA with a significant cut-off value of $P \leq 0.05$. Microarray analysis revealed 1.801 significantly regulated genes and 67 microRNAs. Differentially expressed genes were associated to osteogenesis, apoptosis and cell growth such as COL1A2, CASP4, E2F5 respectively. In the RM group, the great majority of microRNAs were expressed inversely when compared to the other experimental groups, for example hsa-miR-424-5p, hsa-miR-125a-5p and hsa-miR-125b-5p. These findings suggest that osteoblastic cells show different gene expression behavior when in contact with titanium surfaces modified by oxidative nanopatterning.

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## PP137

### IGF1 stimulates protein synthesis by enhancing mRNA translation rate in osteoblasts

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IGF1 is an osteo-anabolic factor that stimulates osteogenic precursor cell differentiation. IGF1 is produced in bone in response to mechanical stimulation, but also in mechanically-stimulated muscle cells. IGF1 enhances the rate of mRNA translation in muscle cells *via* activation of the PI3K/AKT/mTOR pathway, thereby increasing in muscle mass. Therefore we hypothesized that IGF1 not just enhances osteogenic differentiation of precursors, but also stimulates protein synthesis in osteoblasts, *via* an enhanced mRNA translation rate.
IGF1 gene expression was determined by qPCR in MC3T3-E1 osteoblasts subjected for 1 h to uni-axial cyclic strain (3 Hz, 0–5% strain). Other MC3T3-E1 osteoblasts were treated $\pm$human recombinant IGF1 (1, 10, or 100 ng/ml) for 0.5–6 h, to determine phosphorylation of AKT (upstream of mTOR) and p70s6k (downstream of mTOR) by western blot. Osteoblasts were cultured for 1 day $\pm$ IGF1 (1, 10 or 100 ng/ml), at which time total protein and DNA were quantified, as well as mRNA expression of collagen I, transcription factors Runx2 and SP7, and IGF1 and IGF1 receptor by qPCR.
Cyclic strain of osteoblasts increased IGFI gene expression by 2.3-fold. IGF1 did not affect gene expression of collagen I, Runx2, SP7, or IGF1 receptor in osteoblasts, but IGF1 (100 ng/ml) reduced IGF1 gene expression. IGF1 enhanced AKT and p70s6k phosphorylation at 2 h and 6 h in a dose-dependent manner. IGF1 enhanced total protein by $\sim$12-fold, while total DNA remained unchanged. Our results confirm that mechanical stimulation enhances IGF1 expression by osteoblasts, and show that IGF1 strongly stimulates protein synthesis in osteoblasts. The anabolic effect of mechanical loading on bone may thus, at least in part, be mediated by IGF1-enhanced protein synthesis in osteoblasts. This enhanced protein synthesis is likely the result of an increased mRNA translation rate *via* activation of the PI3K/Akt/mTOR pathway rather than *via* increased mRNA transcription.

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## PP138

### Metabolism and synthesis activity of calvaria osteoblasts from offspring of rats treated with caffeine during pregnancy

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Caffeine is a methylxanthine found in many foods and is widely consumed by the human population. Therefore, its effects and mechanisms in various tissues have been widely studied. Various bone abnormalities have been observed in fetuses from rats treated with caffeine. But the genesis of these abnormalities is not known. The objective of this study was to verify the metabolism and activity of calvaria osteoblasts from offspring of rats treated with caffeine during pregnancy. The project was *approved* by animal *ethics committee*. 24 female Wistar rats were divided into four groups: one group without caffeine (control) and three groups with caffeine in following doses 25, 50 and 100 mg/kg daily throughout pregnancy. At birth, three puppies of each dam and group were euthanized for extracting osteoblasts from calvaria for *in vitro* tests. Osteoblasts from offspring of rats treated with caffeine had significantly increased activity, represented by increased conversion of MTT in formazan crystals, alkaline phosphatase activity, collagen synthesis, and mineralized nodules synthesis, as well as, significantly increased expression of gene transcripts for osteocalcin, osteopontin, sialoprotein, alkaline phosphatase and collagen I at all doses, but specially at 50 mg/kg. These results demonstrate that the *in vivo* effect of the caffeine in the osteoblasts is different when compared to studies that evaluated the effect of caffeine added directly to the cell culture medium. In conclusion, caffeine administered *in vivo* daily throughout pregnancy increases the metabolism and synthesis activity of calvaria osteoblasts from offspring of rats, which may be related to the occurrence of fetal abnormalities caused by caffeine.

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## PP139

### Effect of lactation on the osteogenic potential of bone marrow mesenchymal stem cells of rats

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The lactation has been considered a cause of bone loss in humans and animals. Studies have shown that during lactation, there is a significant reduction of bone mineral density. Rats at the end of lactation show reduced bone mass. The objective of this study was to verify the effect of lactation under osteogenic potential of bone marrow mesenchymal stem cells (BMMSCs) of female rats. Twelve Wistar rats were distributed among the control and lactating groups. The experiment was approved by the ethics committee on animal experimentation. BMMSCs were grown in osteogenic medium. At 7, 14, and 21 days of osteogenic differentiation of BMMSCs, 3-4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) conversion, gene expression for collagen I, osteocalcin, osteopontin, BMP-2, osterix, alkaline phosphatase were analyzed. The number of mineralized nodules per field was analyzed at 21 days. The lactation increased reduction of MTT in formazan crystals and alkaline phosphatase activity in all experimental periods. The lactating group showed significantly increased the expression of gene transcripts for alkaline phosphatase, collagen I and sialoprotein at 7, 14, 21 days of differentiation. The BMP-2 expression was significantly higher in cell culture of the lactating rats only at 21 days of differentiation. The expression of osteocalcin, osteopontin and osterix did not differ between lactating and control groups. The number of mineralized nodules per field was significantly higher in the lactating group compared to the control group. It was concluded that the BMMSCs from lactating rats have higher osteogenic differentiation potential when compared to BMMSCs of the non-lactating rats (control). In addition, the bone loss observed in lactating rats is not due to reduced osteogenic differentiation of bone marrow mesenchymal stem cells.

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CNPq and FAPEMIG of the non-lactating rats (control). In addition, the bone loss observed in lactating rats is not due to reduced osteogenic differentiation of bone marrow mesenchymal stem cells.

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## PP140

### Release kinetic of prolyl hydroxylase inhibitors from collagen barrier membranes in vitro

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Collagen barrier membranes are used in guided tissue regeneration to support bone healing and periodontal regeneration. Pharmacological inhibitors of prolyl-hydroxylases (PHD) can support hard and soft tissue regeneration and are therefore a promising tool for periodontal therapy. Here we evaluate the release kinetic of the PHD inhibitors dimethyloxaloylglycine and L-mimosine from the collagen barrier membranes.

The PHD inhibitors were lyophilized onto collagen barrier membranes. Morphology of the membranes was assessed with scanning electron microscopy. The release kinetic was assessed in a bioassays with gingival and periodontal ligament fibroblasts.
We found that the membranes treated with PHD inhibitors stimulated VEGF production. Assessment of the release kinetic based on VEGF production, metabolic activity and proliferation showed that supernatants obtained from the collagen barrier membranes in the first hours had a sufficient level of PHD inhibitors to induce a cellular response. Morphology of the membranes was unchanged by the treatment with PHD inhibitors.
In conclusion collagen barrier membranes supplemented with PHD inhibitors can provoke a pro-angiogenic response *in vitro*. The membranes release PHD inhibitors in a burst like kinetic within the first hours. These findings provide the basis for preclinical studies to evaluate the regenerative capacity of PHD inhibitors in periodontal surgery.

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## PP141

### An infrared assay of the kinetics of phosphate-release from physiological substrates in living cells

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Up to now, most of standard methods for measuring inorganic phosphate ($P_i$) to determine phosphatase activity are based on coupled enzyme assays, colorimetric methods or conductance measurements. However, none of these methods can measure $P_i$, substrate and protein concentrations simultaneously, allowing direct kinetic determinations of phosphatase activity in cells in a single assay. Among the enzymes having a pyrophosphatase activity and releasing $P_i$, is tissue non-specific alkaline phosphatase (TNAP) expressed in numerous tissues with high levels in bones, liver and neurons. TNAP activity is absolutely required for human life since severe functional deficiency is perinatally lethal. Chondrocytes, osteoblasts as well as smooth muscle cells during vascular calcification are characterized by high TNAP activity. Here we developed a continuous infrared (IR) assay to determine phosphatase activity in Saos-2 cells and in primary osteoblasts from exogenous substrates such as $PP_i$, AMP, ADP, ATP, UTP, β-glycerophosphate or α-D-glucose 1-phosphate and para-nitrophenylphosphate (*p*NPP) Quantitative determinations were obtained for $PP_i$, AMP, β-glycerophosphate, α-D-glucose 1-phosphate *p*NPP using both bands of $P_i$ at 990 and 1070–1080 per cm. The substrate bands were also used to determine enzyme specific activity, as in the case of AMP (977 per cm), *p*NPP (980 per cm) and $PP_i$ (1107 per cm). At the concentrations of 5 mM, levamisole almost completely inhibited the $PP_i$ hydrolysis by Saos-2 cells ($IC_{50} = 1.16 \pm 0.03$ mM), suggesting TNAP contributed to the pyrophosphatase activity. A pyrophosphatase activity in matrix vesicles extracted from growth plates and epihyseal cartilage of 17-day-chicken embryos amounted to $1132 \pm 370$ nmol/min per mg which is 10 times higher than in Saos-2 cells (110 nmol/min per mg). Thus, the IR assay can be employed as a cost-effective label-free approach to determine the phosphatase activity from extracellular physiological substrates in whole cells. It may serve to screen the phosphatase inhibitors in TNAP-enriched cells.

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## PP142

### Cell attachment and proliferation of bone marrow-derived osteoblast on zirconia of various surface treatment

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This study was performed to characterize the effects of calcium phosphate coated and hydroxyapatite coated zirconia compared to smooth surfaced zirconia with bone marrow-derived osteoblast culture.
Bone marrow-derived osteoblasts were cultured on i) smooth zirconia, ii) zirconia coated with calcium phosphate (CaP), and iii) zirconia coated with hydroxyapatite (HA). The tetrazolium-based colorimetric assay (MTT test) was used for examining the attachment of cells. Cellular morphology was examined by scanning electron microscopy (SEM) and alkaline phosphatase (ALP) activity was measured to evaluate the cell differentiation rate. X-ray photoelectron spectroscopy (XPS) was employed for the analysis of surface chemistry. The genetic expression of the osteoblasts and dissolution behavior of the coatings were observed. ANOVA was conducted to assess the significance level of the differences between the groups.
From the MTT assay, there was no significant difference between smooth zirconia and surface coated zirconia ($P > 0.05$). From the SEM image, cells on all three groups of discs were irregularly triangular or elongated in shape with formation of filopodia. From the ALP activity assay, the optical density of osteoblasts on smooth zirconia discs was slightly higher than that of osteoblasts on surface treated zirconia discs ($P > 0.05$). Most of the genes related to cell adhesion showed similar expression level between smooth zirconia and surface treated zirconia. The dissolution rate of $Ca^{2+}$ and $P^{-}$ was higher with CaP coating than HA coating.
The attachment and growth behavior of bone-marrow-derived osteoblasts cultured on smooth zirconia and surface coated zirconia showed comparable results. However, the HA coating showed more time-dependent stability compared to the CaP coating.

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## PP143

### Utilization of L-mimosine in pulp regeneration: lessons from cell culture and tooth slice organ cultures

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After trauma or carious lesion dental pulp healing is difficult to predict. In addition systemic diseases like diabetes mellitus can impair the regenerative capacity. New regenerative strategies target prolyl hydroxylase (PHD) by pharmacological inhibitors to stimulate hard and soft tissue healing. PHD inhibitors such as L-mimosine (L-MIM) induce vascular endothelial growth factor (VEGF) production by promoting angiogenesis. However, it is unclear if L-MIM is a feasible tool to stimulate pulp regeneration.
In this study we investigated the response of the dental pulp to L-MIM in monolayer cultures based on viability, proliferation and VEGF production utilizing MTT-tests, 3[H]thymidine incorporation assays, and immunoassays respectively. In addition viability and VEGF production were assessed in tooth slice organ models. To mimic the diabetic milieu, cultures were performed in the presence of advanced glycolysed end-products (AGE).
We found that L-MIM at non-toxic concentration enhances VEGF production under basal conditions in monolayer cultures. This enhanced pro-angiogenic capacity was paralleled by an increase in VEGF production in the tooth slice model. L-MIM elevated the VEGF levels also in monolayer and tooth slice organ cultures performed in the presence of AGE.
Overall these results indicate that the dental pulp responds to L-MIM under basal and under diabetic conditions. Further studies will show if this pro-angiogenetic response to L-MIM found *in vitro* translates to enhanced pulp regeneration.

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## PP144

### Pro-osteogenic properties of nacre extract on two cell lines, primary human osteoblasts and MC3T3-E1 cell line

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Nacre, or mother of pearl, is a promising natural biocompatible biomaterial consisting of aragonite (97%) and of organics (3%) and capable to increase the cell osteogenic activity. It has been established that osteoarthritic osteoblasts

present a mineralization defect and, to date, only a few molecules (vitamin D3 and bone morphogenetic protein2) could improve the mineralization potential of this cell type. In this context, we evaluated the impact of nacreous molecules on the mineralization capacity of a pre-osteoblast lineage, MC3T3-E1, and osteoblasts from osteoarthritis (OA) patients.
For this goal, molecules were extracted from nacre with ethanol (ESM, Ethanol Soluble Matrix) and tested for 21 days at two concentrations (100 and 200 μg/ml), on MC3T3-E1, and osteoblasts of the subchondral bone from OA patients undergoing total knee replacement. Alizarin Red staining was performed to visualize capacity of mineralization and quantified at 405 nm. The Raman spectroscopy and environmental scanning electron microscopy were used to demonstrate the presence of calcium phosphate (hydroxyapatite). Additionally, the expression of bone markers such as Collagen type I, osteocalcin, osteopontin and Runx2 was monitored by quantitative PCR.
Our results with the alizarin red assay demonstrated the presence of precipitated calcium in cells treated by ESM from 7 days of culture for MC3T3-E1 and 14 days for osteoblasts. At day 7, the expression of Runx2, osteopontin andosteocalcin was increased. RAMAN and electron microscopy showed the presence of nanograins of hydroxyapatite in MC3T3-E1 and an early crystalline form of calcium phosphate in osteoblast cultures, after treatment with ESM.
In conclusion, the increased mineralization activity observed on MC3T3-E1 was also observed on OA osteoblasts. ESM would be able to restore a physiological phenomenon and induce mineralization of pre-osteoblastic cells. Thus confirming the osteogenic potential of nacre extract and place the MC3T3-E1 lineage as a promising model for screening nacreous molecules.

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## PP145

### Effects of strontium ranelate on the bone-like mineralized matrix produced in osteoblast cell cultures

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Strontium ranelate is a promising drug used in the treatment of osteoporosis. This drug has a unique dual effect on bone turnover, simultaneously increasing bone formation by osteoblasts (anabolic effect) and decreasing bone resorption by osteoclasts (antiresorptive effect). The goal of this study was to evaluate: i) anabolic effects of strontium ranelate on the formation of bone-like mineralized matrix in osteoblast cell cultures and ii) changes the drug could cause on the matrix and mineral substance. After 28 days of culture, treatment with strontium ranelate at 0.5 mM $Sr^{2+}$ increased the formation of mineralized nodules. Analysis of the intact matrix by attenuated total reflection Fourier transform infrared spectroscopy showed that the overall bone-like nature of the nodules was preserved, comprising a poorly crystalline, carbonate-containing apatite and a collagenous matrix. We found, however, using synchrotron micro X-ray fluorescence, that strontium was incorporated into the matrix produced under treatment in a dose-dependent manner. In order to further evaluate effects of the drug on the mineral substance, we isolated the minerals and analyzed them by using: Fourier transform infrared spectroscopy, solid-state $^{1}H$ nuclear magnetic resonance, micro-Raman spectroscopy, X-ray diffraction, and energy dispersive X-ray spectroscopy. Although all minerals presented typical bone-like features, the mineral produced under 0.5 mM $Sr^{2+}$ showed an increase in the relative type-B carbonate content and in the structural disorder around phosphate sites. We also found that strontium was incorporated into this mineral by replacing slightly less than 10% of calcium in the apatite crystal lattice, which led to an increase in both lattice parameters a and c. In conclusion, strontium ranelate had a clear anabolic effect on the formation of mineralized nodules in osteoblast cell cultures while preserving their overall bone-like nature. We found, however, that treatment changed the composition and crystal structure of the mineral substance.

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## PP146

### Strontium ranelate increases osteoblast cell proliferation and differentiation and the formation of bone-like mineralized matrix on different titanium substrates

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The integration of biomaterials into the calcified bone tissue is essential for the clinical success of bone implants. A particular strategy to improve such integration is the use of specific molecules to increase osteoblast cell adhesion, proliferation, and/or differentiation on the surface of the implants, aiming to enhance the interaction of cells with biomaterials. The goal of this study was to better understand the potential of the anti-osteoporotic drug strontium ranelate for improving the efficacy of bone implants. We analyzed direct effects of the drug on the interaction of osteoblast cells with different titanium substrates in cell culture. Cells were treated with 0.12 and 0.5 mM $Sr^{2+}$ of strontium ranelate and cell response to the drug was evaluated on four pure titanium substrates with different surface topographies. After 24 h in culture, treatment preserved cell morphology parameters (area, aspect ratio, circularity, and solidity) and the orientation of cells on grooved substrates, in which they were preferentially aligned with the direction of the grooves. The initial cell adhesion to the substrates after 4 h was also not changed by treatment. We found, however, that both concentrations of the drug significantly ($P<0.05$) increased cell proliferation rates in all substrates, especially from 7 to 21 days in culture. Moreover, we found a significant ($P<0.05$) dose-dependent increase in alkaline phosphatase activity after 21 days and in the formation of mineralized matrix after 28 days. This matrix resembled that described in native bone. In conclusion, we show that strontium ranelate improved the interaction of osteoblast cells with different titanium substrates, increasing cell proliferation and differentiation into mature osteoblasts and the formation of bone-like mineralized matrix in all substrates. We believe our results highlight a promising role of strontium ranelate for enhancing the clinical success of bone implants, especially in elderly patients with osteoporosis.

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## PP147

### Differential gene expression of matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) are modulated during osteogenic/odontogenic differentiation from human dental pulp stem cells (DPSCs) by BMP-7

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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 (MMP) play a key role in these processes. Mesenchymal stem cells derived from dental pulp are multipotent and have the capacity to differentiate into mesenchymal lineages. Bone morphogenetic proteins (BMP) are a family of signaling molecules critically involved at various stages in the formation of a variety of tissues and organs including bones and teeth. Recently, BMP-7 has been described to induct DPSC differentiation into odontoblastic-like cells. However, it is unknown the gene expression profile of MMPs and TIMPs during osteogenic/odontogenic differentiation induction by BMP-7. In this study, we evaluated differential gene expression of MMPs and TIMPs during osteogenic/osteogenic differentiation induction from DPSCs *in vitro* by qPCR. DPSCs isolated from extracted human third molars (collagenase/dispase digestion at 37 °C) were grown in α-MEM medium +10% FBS and differentiation induction in presence of osteogenic medium (10 mM β-glycerophosphate, 1 μM dexamethasone and 50 μg/ml ascorbate) and odontogenic medium (10 mM β-glycerophosphate, 1 μM dexamethasone and 50 μg/ml ascorbate +50 ng/mL BMP-7) for 21-days. During osteogenic differentiation, MMP-2, MMP-3, MMP-25 and TIMP-4 were downregulated upregulated throughout 21-days in relation to DPSC. During odontogenic differentiation, MMP-2, MMP-25 and TIMP-4 were upregulated from 7 to 21-days, whereas, MMP-3 was upregulated from 14 to 21-days. Our results suggest that BMP-7 may regulate MMP and TIMP gene expression during osteogenic/odontogenic differentiation *in vitro* from DPSCs.
Keywords: Dental pulp stem cells, MMP, TIMP, BMP-7, and osteoblast/odontoblast differentiation.

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## PP148

**Expression of BDNF gene in the femur of aged vs adult rats**

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Brain-derived neurotrophic factor (BDNF) has recently emerged as a factor able to concurrently target age related diseases as neurodegenerative disease, osteoporosis and metabolic syndrome. However the role of BDNF in the frailty syndrome associated with aging has never been investigated. The deletion of central BDNF gene in mice results in increased bone mass and *in vitro* experiments show that BDNF has a positive effect on bone. BDNF expression is mediated by estrogens which could explain the age-related decrease of BDNF in bone. To investigate this hypothesis we analysed the expression of the BDNF gene in the femur of aged male (18–24 months old) and adult rats (3–6 months old) using the qPCR experiments. We found that the expression of the BDNF gene was significantly reduced in the femur of the aged rats with respect to that observed in the adults (student *t*-test, $P<0.05$). The ratio BDNF/HPRT1 was $3.389\pm1.368$ in the controls (no of rats = 5) and $0.06893\pm0.0493$ in the aged rats (no of rats = 7), the ratio BDNF/2-beta globulin was $3.349\pm1.707$ in the controls (no of rats = 5) and $0.05114\pm0.0397$ in the aged rats (no of rats = 7) and the ratio BDNF/beta-actin was $2.074\pm0.9727$ in the controls (no of rats = 5) and $0.1091\pm0.088$ in the aged rats (no of rats = 7). In conclusion, the reduced expression of the BDNF gene observed in the femur of the aged rats is consistent with our previous data showing a role of BDNF in age-related diseases.

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## PP149

**Telomerase promotes osteoblast differentiation by modulating IGF-signaling pathway**

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The molecular mechanisms underlying telomere shortening and telomerase deficiency contributing to defective age-related bone formation are poorly studied. We have previously demonstrated the role of telomerase re-activation in enhancing osteoblast differentiation of BMSCs *in vitro* and *in vivo*. Here, we investigated further the signaling pathways underlying the regulatory function of telomerase in osteogenesis. Comparative microarray analysis of telomerase-over expressing hMSC (hMSC-TERT) vs primary hBMSC revealed significant up-regulation of IGF signaling in response to telomerase expression at baseline ($-\log P$ value; 5, obtained by right-tailed Fisher's exact test). Western blot analysis of IGF/PI3K/AKT signaling showed a significant increase in IGF-induced AKT phosphorylation in hMSC-TERT vs primary hMSCs. To further investigate the loss function of telomerase on IGF-induced osteogenesis *in vivo*, we employed the telomerase deficient mice ($Terc^{-/-}$). As compared to WT controls, the bone loss phenotype of $Terc^{-/-}$ mice was shown to be associated with significant reduction in serum levels of *Igf1* and *Igfbp3* and reduced mRNA expression of *Igf1, Igf2, Igf2r, Igfbp5* and *Igfbp6.* Accordingly, IGF1-induced osteogenesis was inhibited in $Terc^{-/-}$ MSCs as compared to WT controls due to the impairment of IGF-induced AKT activation. In conclusion, our data demonstrated that the anabolic effect of telomerase on bone formation is mediated at least in part by modulating IGF/IGFBPs signaling.

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## PP150

**PRKG1: A novel regulator of human skeletal (mesenchymal) stem cell differentiation**

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Protein kinases are an important class of regulatory elements and pharmacological targets for treatment of a number of diseases including cancer, heart and lung diseases. However, their role in regulation of the skeletal (mesenchymal) stem cell (MSC) functions and bone formation is not fully understood. Thus, we performed functional screening of human kinome, using three siRNAs for each of the ~700 known kinases represented in the human genome. Activity of alkaline phosphatase (ALP) was quantified to assess the effect of each siRNA on OB differentiation of human MSC (hMSC). We identified PRKG1 as a negative regulator of OB differentiation of hMSC. Follow up studies showed that siRNA-mediated loss of PRKG1 function (siPRKG1) enhanced OB differentiation of hMSC as shown by increased ALP activity, gene expression of the osteogenic markers ALP and Collagen type1 and *ex vivo* matrix mineralization and suppressed adipocyte differentiation of hMSC as shown by Oil Red O staining of mature adipocytes. Furthermore, activation of PRKG1 function using 8-pCPT-cGMP suppressed OB differentiation as shown by reduced ALP activity. We also identified the molecular mechanism of PRKG1 action mediated by phosphorylation and inactivation of the small GTPase RhoA. Thus, loss of PRKG1 function increased RhoA activity and Akt signaling, as shown by G-LISA RhoA activity assay and western blot analysis. To investigate the therapeutic benefits of targeting PRKG1 *in vivo*, we employed a small molecule kinase inhibitor of PRKG1 (H-8). Enhancement of heterotopic bone formation (96% as compared to vehicle) was observed when mice containing hMSC-loaded subcutaneous implants, received H-8 intraperitoneal injections (90 mg/kg per day for 4 weeks). Necropsy of the test animals, did not show any toxic effects of H-8 treatment. PRKG1 is a novel negative regulator of OB differentiation of hMSC and small molecule inhibition of PRKG1 enhances bone formation and has the potential to be developed as a novel anabolic therapy for treatment of bone-loss diseases.

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## PP151

**Syndecan-2 is a new negative modulator of Wnt signaling within bone marrow**

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Wnt factors bind to heparan sulfate chains of syndecans that hence control their distribution in the cell microenvironment and also contribute to the internalization and recycling of the Wnt receptors. Syndecans are therefore likely important Wnt regulators within the bone marrow. Here, we investigated the interactions between Syndecan-2 and Wnt signaling in normal bone cells. We analyzed the effects of 2.3 kb Col1A1 promoter-driven Syndecan-2 overexpression in osteoblasts. Syndecan-2 overexpression resulted in decreased Wnt/beta-catenin signaling in TG mice bearing a Wnt reporter system. Syndecan-2 overexpressing bone cells displayed lower Wnt expression and also a weaker response to recombinant Wnt. Altered levels of Wnt receptors, effectors and inhibitors were found in bone marrow cells of TG mice. Specially, R-spondins expression was decreased in bone marrow cells of transgenic mice. Indeed, Wnt3a stimulation induced a strong increase in the levels of R-spondin-2 in the cytoplasm of bone marrow cells derived from WT mouse bone and at the opposite, Wnt3a decreased R-spondin-2 in cells from TG mice. Wnt3a treatment resulted in R-spondin-2 ubiquitination in Syndecan-2 overexpression cells, suggesting that the proteoglycan was involved in Wnt signaling system internalization and degradation. Adult TG mice displayed decreased bone mass, reduced number of active osteoclasts and osteoblasts. The osteoclast alteration was clearly related to a defect in the stromal cell population. TUNEL staining revealed an increased number of apoptotic cells within the bone marrow of TG as compared to WT mice. In cultures of bone-derived cells, syndecan-2 appearance matched with cell death induction. Moreover, syndecan-2 overexpressing osteoblasts were shown to

induce apoptosis of co-cultured bone marrow cells derived from WT mice. Altogether our results revealed a new role of Syndecan-2 in the microenvironment of bone marrow cells. Syndecan-2 appeared as a key regulator of Wnt signaling and thereby of the fate of precursor cells.

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## Cell biology: osteoclasts and bone resorption

### PP152

**Involvement of LIGHT in multiple myeloma bone disease**

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Multiple myeloma (MM)-bone disease occurs in 70 to 80% of patients at MM diagnosis, and up to 90% at relapse; skeletal related events cause high morbidity and mortality. MM-bone disease consists of lytic lesions arising as a consequence of an unbalanced bone remodelling due to osteoclast (OC) activation, and osteoblast inactivation. Osteoclastogenesis may be under immune cell regulation through the production of numerous cytokines, such as LIGHT/TNFSF14, a newly identified member of the TNF superfamily, expressed by activated leukocytes. Recent literature data linked the high serum levels of LIGHT with the bone loss associated to rheumatoid arthritis. Thus, we investigated the role of LIGHT in MM-bone disease.
Interestingly, for the first time we demonstrated higher expression levels of LIGHT in T-cells, macrophages and polymorphonuclear cells from osteolytic MM patients respect to the same cells from asymptomatic MM patients as well as Monogammopathy of Undetermined Significant and healthy subjects. In particular, in osteolytic MM patients by flow cytometry we found high expression levels of LIGHT on $CD8^+$ and $CD4^+CD^{25(high)}$ T-cells, as well as on $CD14^+CD16^+$ monocytes, considered the principal source of OC precursors. We also demonstrated LIGHT expression in bone marrow biopsies from MM osteolytic patients. Moreover, to evaluate LIGHT involvement in MM bone disease, we investigated the effect of a LIGHT neutralizing antibody on bone cell differentiation *in vitro*. We found that the inhibition of the molecule significantly reduces OC formation from PBMCs of osteolytic MM patients, as well as stimulates OB differentiation in cultures derived from MM bone marrow mononuclear cells, as demonstrated by the increase of colony forming units of OBs and by the up-regulation of osterix transcription factor, bone sialoprotein and osteocalcin bone matrix proteins. In conclusion, our data demonstrated LIGHT involvement in MM bone disease and suggest it as new potential therapeutic target for MM management.

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### PP153

**Zoledronic acid differently affects long-bone and jaw bone remodeling**

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Bisphosphonates (BPs) are used to treat bone diseases characterized by excessive bone resorption. However, BPs can negatively affect the jaw bone by causing osteonecrosis of the jaw. Previously, we showed that BPs differently affect long-bone and jaw osteoclast precursors. Administration of BPs *in vivo* reduced the number of jaw bone marrow cells, without affecting long-bone marrow cells. Yet, BPs increased bone volume and mineral density of both long bone and jaw. Here, we investigated whether *in vivo* exposure to BPs has a different effect on long-bone and jaw osteoclasts and the remodeling of these two types of bone. Animal studies were approved by the Animal Welfare Committee. Female C57BL/6J mice were injected i.p. with zoledronic acid (ZA, 0.5 mg/kg weekly) for 1, 3, or 6 months. Its effect on osteoclasts and bone formation was studied. ZA did not affect number of long-bone and jaw osteoclasts *in vivo*. Interestingly, 6 months of treatment inhibited bone formation in the long bones without affecting the jaw. Finally, we showed that BPs can cause tooth root resorption. In conclusion, our results show that BPs differently affect long-bone and jaw bone marrow cells and bone remodeling in those bones. These findings provide more insight into the mechanism of bone-site-specific effects of bisphosphonates, such as osteonecrosis of the jaw. Also, we showed for the first time, that BPs can stimulate osteoclasts and their activity at the molar roots.

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### PP154

**Disruption of PLEKHM1 and TRAFD1 (FLN29) interaction impairs osteoclast resorptive activity**

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Mutations in PLEKHM1 cause bone disease in humans and animals. Truncations causedeficient bone resorption by osteoclasts leading to osteopetrosis. A gain-of-function point mutation causes increased resorption leading to osteopenia. We and others have shown that PLEKHM1, a multi-modular protein, interacts with the small GTPase rab7 and is involved in vesicle trafficking, secretion, and membrane biogenesis. To investigate other interactions of PLEKHM1 we performed tandem affinity purification and mass spectrophotometry analysis, and we found that TRAFD1 (FLN29) also interacts with PLEKHM1. Reciprocal pull-down assays confirmed the interaction and localized the regions of interaction. Trafd1 is implicated in the inhibition of innate immune responses in monocytes/macrophages *via* Toll-like receptor signaling, but a role for Trafd1 in osteoclast biology has not been described. We found Trafd1 expression and its co-localization with PLEKHM1 increased when osteoclast differentiation was induced by receptor activator for nuclear factor kappa B ligand (RANKL). Decreasing Trafd1 expression by shRNA in RAW264.7 cells dramatically attenuated osteoclast formation, acidification, and hydroxyapatite resorption. Decreased osteoclast differentiation and resorptive activity in cells with Trafd1 knockdown did not result from suppression of essential osteoclast transcription factors or from decreased cellular levels of acidification factors (ClC7 chloride channel, cathepsin K, carbonic anhydrase II, and a3- vATPase). This suggests a defect in targeting these factors to their correct locations at the ruffled border. To confirm that, for our study we used osteoclasts isolated from *incisors absent (ia)* osteopetrotic rats. *ia/ia* rats have a naturally occurring mutation in PLEKHM1 which leads to production of truncated protein. Subcellular fractionations of vesicles isolated from osteoclasts from *ia/ia* rats show that, in contrast to WT osteoclasts, Trafd1 is unable to associate with early or late endosomes. In conclusion, our data demonstrate a novel role for Trafd1 in osteoclast vesicle transport and acidification. PLEKHM1 and Trafd1 interaction is critical for their function in osteoclasts.

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### PP155

**Role of caveolin-1 in osteoclastogenesis**

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Lipid raft microdomains have important role in cellular signaling. Caveolae are a specialized type of lipid rafts that are stabilized by oligomers of caveolin protein. Here we showed that caveolin-1 (Cav-1) is a critical membrane structural protein of osteoclast. In microarray analysis, we found that caveolin-1 was significantly up-regulated by RANKL. Knock-down of Cav-1 in bone marrow derived-macrophage reduced osteoclastogenesis. Consistent with the *in vitro* results, *in vivo* injection of caveolin-1 siRNA into mice calvariae showed reduction of

RANKL-induced bone resorption. We also found Cav-1 knock-out mice showed inhibition of bone resorption and increased bone volume. Cav-1 affects cFms and RANK expression, two major receptors for osteoclastogenesis. Interestingly, cFms expression was decreased only in protein levels, not in mRNA levels. But, RANK expression was decreased both mRNA and protein levels. It seems that caveolin-1 regulates cFms protein stability. Actually, we found that cav-1 interact with cFms. Following these event, knockdown of caveolin-1 substantially suppressed RANKL-induced MAPK and Akt signaling pathways and NFATc1, the master regulator of osteoclastogenesis. Taken together, these data demonstrate that caveolin-1 has critical role for osteoclastogenesis.

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## PP156

### Interleukin-34 and macrophage-colony stimulating factor interact to form a heteromeric and functional cytokine

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Interleukin-34 (IL-34) is a newly discovered cytokine which regulates, like macrophage-colony stimulating factor (M-CSF), the differentiation/activation of the myeloid lineage. IL-34 and M-CSF are homodimers known to bind to the M-CSF receptor (M-CSFR) in a competitive manner. In this system, IL-34 can substitute for the M-CSF to induce osteoclastic differentiation. The aim of the present work was to study the functional relationships of these cytokines on cells expressing the M-CSFR.

Intracellular pathways activated by either M-CSF or IL-34 were analyzed by western Blot. Cellular proliferation/survival ($CD14^+$ cells, erythroblastic cell line TF-1 overexpressing M-CSFR) was assessed by manual counting or by measuring the cell metabolic activity (alamar blue). Protein interactions were studied by surface plasmon resonance (Biacore), *in situ* 'Proximity Ligation Assay' technology (Olink) and bioinformatic modeling. The cellular localization of the M-CSFR was followed by immunocytochemistry and confocal microscopy.

Simultaneous addition of M-CSF and IL-34 induced higher phosphorylation of the tyrosine residues of the M-CSFR, showing an additive rather than a competitive effect of both cytokines. Similar results were observed on cellular proliferation. Biacore experiments revealed the possible binding of M-CSF on immobilized IL-34, with a weak affinity around 100 nM. Molecular docking studies predicted the formation of a heteromeric M-CSF/IL-34 cytokine which was confirmed by proximity ligation assay. Moreover, the co-expression of the M-CSFR and its ligands differentially regulated the M-CSFR trafficking into the cell.

This is the first report demonstrating the direct interaction between IL-34 and M-CSF and their ability to form a new heteromeric cytokine. This heteromeric structure is biologically active and may modify specifically the tridimensional conformation of the M-CSFR chains. Furthermore, cellular expression of M-CSFR ligands may regulate the receptor glycosylation, as M-CSF expression stops the glycosylation process in the Golgi apparatus, leading to a decrease of the M-CSFR membrane expression.

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## PP157

### CCN2 induces osteoclastogenesis by regulating RANK/RANKL/OPG system

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CCN family member 2(CCN2)/Connective tissue growth factor (CTGF) is a multi-functional factor in proliferation, differentiation and migration of mesenchymal cells such as chondrocytes, osteoblasts and vascular endothelial cells. Recently, we also reported that CCN2/CTGF promotes osteoclastgenesis *via* induction of and interaction with DC-STAMP. We and other researchers reported that CCN2 binds to cytokines and receptors and modified their signaling. Therefore, we comprehensively sought additional factors binding to CCN2 and found receptor activator of NF-kappa B (RANK) as a potential CCN2-binding partner. Since RANK–RANK ligand (RANKL) signaling is indispensable for the osteoclastogenesis, we, in the present study, investigated the relation between CCN2 and RANK–RANKL system to clarify the role of CCN2 in the early stage of osteoclastogenesis.

We used a phage-display system to specify amino sequences binding to CCN2. Multiple molecular interactions were evaluated by solid-phase binding assays. The affinities of the bindings were calculated by using a surface plasmon resonance (SPR) analyzer. Nuclear translocation of NF-kappa B was monitored by fluorescence microscopy and was quantified using image analysis software. The activation of MAPKs was detected by western blotting. The differentiation of osteoclasts was monitored by TRAP staining.

CCN2 directly bound to RANK and enhanced the RANK-mediated signaling such as NF-kappa B, ERK and JNK pathways in pre-osteoclast cell RAW264.7. CCN2 did not affect on the binding of RANK–RANKL. CCN2 also bound to osteoprotegerin (OPG) *via* a common domain of CCN2. The affinity of CCN2 binding to OPG was comparable to that of RANKL to OPG. CCN2 suppressed the inhibitory effect of OPG on osteoclastogenesis. Of note, OPG inhibited the binding of CCN2 to RANK.

These findings indicate that CCN2 is a unique physiological factor, which can enhance osteoclastogenesis *via* interactions with both RANK and OPG in the early stage of osteoclastogenesis, in addition to stimulating the fusion of mononuclear osteoclast precursors.

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## PP158

### Study of the molecular effects of disease-causing mutations in RANK using human protein expression models

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The interaction of Receptor Activator of NFkB ligand (RANKL) with its cognate receptor RANK is crucial for osteoclast formation. We studied eight point mutations within human RANK associated with rare forms of osteopetrosis to gain mechanistic insights into the regulation of RANK signalling.

We investigated the role of the oligomerisation domain within the cytoplasmic region of RANK studying two mutations (W434X and G280X) identified in rare cases of osteopetrosis. Immunoprecipitation showed that, by contrast to WT RANK, W434X and G280X prevented ligand-independent but not ligand-dependent oligomerisation and, in the W434X mutant with an intact TRAF6 binding domain, NFkB signalling was normal. Hence, removal of the oligomerisation motif did not interfere with ligand-induced trimerisation and subsequent NFkB signalling.

The structural importance of the four extracellular, cysteine-rich domains (CRD1-4) in RANK has previously been highlighted in the crystal structure of the mouse RANK–RANKL complex. We studied six disease-associated point mutations within this region of human RANK. RANKL-dependent NFkB activation could only be detected in cells overexpressing WT, but not mutant proteins suggesting altered sensitivity to RANKL. Recombinant, WT and mutant his-tagged RANK proteins (residues 26–210) were expressed in mammalian cells and purified by $Ni^{2+}$-affinity, followed by size-exclusion chromatography. Surface plasmon resonance analysis confirmed the very high affinity interaction between human RANKL and WT RANK ($K_d < 1$ nM). RANKL showed slightly weaker binding to four mutants (A134V, D148V and R170G in CRD3; and C175R in CRD4) and no binding to two mutants, G53R (in CRD1) and R129C (in the linker between CRD2 and CRD3), indicating the crucial role of the latter two amino acids in human RANK. This study shows the effect of disease-associated mutations in RANK on the biophysical interaction of human RANK protein with human RANKL.

Taken together, this work provides novel insights into factors influencing RANKL–RANK interaction and signalling.

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## PP159

**Arachidonic acid and docosahexaenoic acid inhibits osteoclastogenesis and bone resorption in human CD14+ monocytes, *in vitro***

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The mature human skeleton is a metabolically active organ that is continuously resorbed and rebuilt by osteoclasts and osteoblasts. Dietary supplementation of selected long chain polyunsaturated fatty acids (LCPUFAs) has shown effects on bone turnover. Most research on LCPUFAs has been done using commercially available cell lines and further clarification of the cellular effects on models relevant to humans is required. After ethical approval, peripheral blood was collected from healthy young male volunteers to prepare a mononuclear CD14+ monocyte population to generate osteoclasts. The action of an ω-6 LCPUFA, arachidonic acid (AA), and an ω-3 LCPUFA, docosahexaenoic acid (DHA), on various parameters regarding osteoclast formation and bone resorption were investigated.

CD14+ monocytes were seeded at 130 000 cells/cm$^2$ with RANKL (30 ng/ml) and M-CSF (25 ng/ml). For experiments on differentiating osteoclasts, cells were exposed to the LCPUFAs at varying concentrations (20 μM, 40 μM, 60 μM and 80 μM) from day 3. For experiments on mature osteoclasts, cells were exposed to the LCPUFAs from the onset of resorption (day 11–14). Experiments were terminated a week thereafter. TRAP activity, osteoclast numbers, resorption and expression of prominent osteoclast markers were assessed. All experiments were conducted in triplicate and repeated three times with monocytes from three different donors.

AA (60 μM, 80 μM) and DHA (80 μM) decreased TRAP activity in differentiating osteoclasts. AA (all concentrations) and DHA (60 μM, 80 μM) decreased osteoclast numbers in differentiating osteoclasts. AA (60 μM, 80 μM) decreased bone resorption while not affecting cell numbers in mature osteoclasts. Both LCPUFAs (40 μM) decreased cathepsin K and TRAP protein expression in differentiating and mature osteoclasts. Both LCPUFAs (40 μM) decreased MMP-9 protein expression, in differentiating and mature osteoclasts, except DHA that showed no effect on mature osteoclasts. This data shows for the first time that LCPUFAs can affect osteoclast formation and function in a human primary cell line, *in vitro*.

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## PP160

**EEIG1 interacts with RANK receptor and positively regulates osteoclastogenesis**

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The receptor activator of NF-kB (RANK) and ITAM-containing adaptors have been identified as essential factors involved in osteoclast formation and bone remodeling, but their mechanisms and interacting factors have not yet been fully identified. Here we report that early estrogen-induced gene 1 (EEIG1), a novel RANK ligand (RANKL)-inducible protein, physically interacts with RANK and further associates with Gab2, PLCg2, and Tec/Btk kinases by RANKL stimulation. Overexpression of EEIG1 enhances osteoclastogenesis, whereas small hairpin RNA (shRNA)-mediated EEIG1 silencing inhibits osteoclast formation, which results from impaired RANKL-mediated PLCg2 phosphorylation and NFATc1 induction. In addition, the inhibitory peptide designed to block RANK–EEIG1 interaction showed decreased RANKL-induced bone destruction by reducing the numbers of osteoclasts. Hence, we have identified a novel RANK signaling component, which controls RANK-mediated osteoclast formation and which may provide the molecular target for a new therapeutic strategy.

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## PP161

**Ultrastructural imaging of the osteoclast secretory machinery in 3 dimensions**

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Osteoclasts secrete acid and cathepsin K to dissolve the mineral and digest the organic matrix of bone, cartilage and dentine. The secretions are by necessity destructive and potentially harmful to the cell itself and are therefore trafficked through the cell in membrane bound vesicles. Secretion takes place over a specialised membrane compartment, the ruffled border, which is only present in resorbing osteoclasts. The ruffled border membrane and the vesicles in its vicinity have been studied by classic transmission electron microscopy (TEM) and by confocal microscopy before, but the way in which vesicles dock and fuse with the membrane and how they thus contribute to maintenance of the complex membrane folds of the ruffled border have been difficult to visualise using two-dimensional microscopical methods alone.

New methods have recently become available to allow three-dimensional studies of subcellular structures at higher resolution than is possible with light microscopic techniques such as confocal microscopy. TEM tomography allows imaging of vesicle fusion events at nanometre resolution. In addition, the novel method of serial block face imaging using the Gatan 3View system allows three-dimensional reconstruction of subcellular compartments at a resolution approaching that of TEM.

Here we show, for the first time, detailed three-dimensional images of the ruffled border in resorbing osteoclasts using TEM tomography and serial block face imaging. These complementary methodologies offer new opportunities to study the osteoclast resorptive machinery, as well as other cell biological processes in bone, in great detail and will be most informative when used in combination with live cell imaging, immunofluorescence and immuno-EM methods. To allow a fuller appreciation of the capabilities and limitations of these new technologies we will show still images and (partially) reconstructed objects, but also the raw tomograms and three View files in movie format.

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## PP162

**The role of IL-6 as a modulator of osteoclastic resorption *in vitro* in acute Charcot osteoarthropathy**

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Aims

To investigate the role of interleukin-6 (IL-6) as a modulator of osteoclastic resorption *in vitro* in acute Charcot osteoarthropathy.

Material and methods

Peripheral blood mononuclear cells were isolated from six patients with acute Charcot osteoarthropathy, five diabetic and five healthy controls and cultured *in vitro* on bovine bone disks for 21 days in the presence of i) macrophage-colony stimulating factor (M-CSF) and receptor activator of nuclear factor κβ ligand (RANKL) and ii) M-CSF, RANKL and neutralising antibody to IL-6 (anti-IL-6). Bone discs in duplicate were stained with toluidine blue. Resorption was measured by two methods: i) median area of resorption at the surface by image analysis (%) and ii) median area of resorption under the surface (μm$^2$) measured by Dektak 150 surface profiler and calculated using OriginPro 8.6. Ten scans each 1000 μm long per disc were carried out.

Results

In patients with acute Charcot osteoarthropathy, the addition of anti-IL-6 to cultures treated with M-CSF+RANKL, led to a reduction in the median area of resorption on the surface by image analysis (M-CSF+RANKL *vs* M-CSF+RANKL+anti-IL-6: 39% (12.9) *vs* 31% (6.8), $P=0.028$, median (interquartile range)) but not in the median area of resorption under the surface after profilometry ($7.7\times10^3$ μm$^2$ ($6.9\times10^3$) *vs* 5.4 ($3.3\times10^3$), $P=0.2$). In diabetic patients, there was no difference in the median area of resorption on the surface ($P=0.251$) and under the surface ($P=0.249$) in cultures treated with M-CSF+RANKL compared with cultures treated with M-CSF+RANKL+anti-IL6. Similarly, in healthy subjects, there was no difference in the median area of resorption on the surface ($P=0.251$) and under the surface ($P=0.753$) between the two culture treatments.

Conclusion
In acute Charcot osteoarthropathy, IL-6 modulates osteoclastic resorption on the surface but does not influence the extent of resorption under the surface.

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## PP163

**The molecular mechanism of *n*-butanol extracts of *Panax notoginseng* on RANKL-induced osteoclastogenesis in RAW264.7 cells**

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This study examined the anti-osteoclastogenic effect of *n*-butanol extracts of *Panax notoginseng* on the receptor activator of NF-kB ligand (RANKL) induced RAW264.7 cells. *Panax notoginseng* is commonly used to treat chronic liver disease. Notoginseng has many beneficial effects, such as the suppression of liver fibrosis and anti-cancer activities. Notoginseng contains several biologically active components, such as ginsenosides $Rb_1$, $Rg_1$, Rd and Re and notoginsenoside R1.The effects of *n*-butanol extract of *Panax notoginseng* (*bPN*) on osteoclasts were examined by measuring the following: the cell viability, the formation of tartrate-resistant acid phosphatase (TRAP) (+) multinucleated cells, RANK/RANKL signaling pathways and mRNA expression of osteoclast-associated genes. *bPN* inhibited the formation of RANKL-stimulated TRAP (+) multinucleated cells. *bPN* also inhibited the RANKL-stimulated activation of ERK signaling, and inhibited the RANKL-stimulated degradation of I?B in the RAW264.7 cells. In addition, the RANKL-stimulated induction of NFATc1 transcription factors was abrogated by *bPN*. *bPN* decreased the mRNA expression of osteoclast-associated genes, including TRAP and cathepsin K in the RAW264.7 cells. Our data demonstrate that *bPN* inhibits osteoclastogenesis by inhibiting RANKL-induced activation of signaling molecules and transcription factor in osteoclast precursors.

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## PP164

**Novel highly sensitive ELISA to measure free, bioactive, human soluble RANKL**

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RANKL, the receptor activator of nuclear factor kappa B ligand, is an essential factor for the formation of mature osteoclasts. Together with its receptor RANK and its antagonist Osteoprotegerin (OPG) RANKL is a key regulator in bone metabolism[1]. RANKL is a membrane-bound protein that can be segregated to a soluble form (sRANKL), whereas only the latter has been reported to be bioactive[2]. Due to its low circulating levels and the nature of the analyte binding to OPG, free sRANKL has been proven difficult to measure: the accuracy of sRANKL measurement is compromised by the very low or undetectable levels, as observed in some patient cohorts[3].
Hence, our aim was to develop a highly sensitive and specific assay that enables the direct measurement of free, bioactive, soluble RANKL in serum and plasma samples. We have taken advantage of the high affinity and specificity protein-protein interaction between sRANKL and OPG and used immobilized, recombinant OPG to capture free sRANKL, which subsequently is detected with a biotin labeled anti-sRANKL antibody.
The data presented here, demonstrate that 98% of all samples from an unselected healthy population ($n=210$) had detectable free sRANKL values within the calibration range of the assay (0–2 pmol/l). The median of serum samples, prepared immediately after blood collection and stored at −25 °C until measurement, was 0.14 pmol/l, with a lower limit of quantification (LLOQ) of 0.01 pmol/l. Assay characteristics, such as intra/inter-assay precision, dilution linearity and spike/recovery as well as sample stability have been analysed.
Our novel ELISA provides a reliable and accurate tool for the quantitative determination of free, soluble, bioactive RANKL in human samples.
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## PP165

**Foreign body giant cells do not have the capacity to resorb bone**

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Background
Osteoclasts are unique multinucleated cells that originate from the fusion of monocytes. They are the only cells known to be capable of bone resorption. Interestingly, the foreign body multinucleated giant cell (FBGC) arises from the same lineage as the osteoclast, and they share numerous similar characteristics, among which the expression of tartrate resistant acid phosphatase (TRAcP). Yet, it is not known whether the FBGC has the capacity to resorb bone.
Objective
To compare the bone resorptive capacity of two different populations of multinucleated cells i.e. the osteoclast and the FBGC.
Methods
$CD14^+$ monocytes were isolated from human blood. Differentiation of $CD14^+$ cells into osteoclasts was induced by M-CSF and RANK-L, and for the differentiation into FBGC, M-CSF, IL-4 and IL-13 was added. Cells were seeded on bones slices. After 25 days the cells were fixed and stained for activity of TRAcP and the nuclei were stained with 4′,6-diamidino-2-phenylindool (DAPI). To analyze bone resorption, the bone slices were stained with coomassie brilliant blue.
Results
High and similar numbers of multinucleated cells were seen both in the osteoclast and in the FBGC cultures. The multinucleated FBGCs however, proved to be much larger and also the number of nuclei per cell was much higher. Bone resorption was very extensive in the osteoclast cultures; almost the entire surface of the bone slices were resorbed. In sharp contrast herewith, a complete absence of resorption was apparent in the FBGC cultures.
Conclusion
In spite of the same precursor and an obvious mulitnuclearity, there is an essential difference between both populations. The FBGCs were considerably larger and had a higher number of nuclei compared to the osteoclast culture. The most important difference was that, in contrast to osteoclasts, FBGC do not have the capacity to resorb bone.

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## PP166

**Functional read out of perturbed osteoclast behaviour in an *in vitro* model of Gaucher's disease**

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Gaucher's disease is a glycolipid storage disorder caused by an autosomally inherited deficiency of the lysosomal enzyme glucocerebrosidase. The majority of patients with Gaucher's disease develop abnormal bone remodelling with severe consequences, including osteonecrosis, bone crises, and osteoporosis related fractures. Although enzyme replacement therapy is effective at reversing many of the pathological consequences, and preventing further progression of this disease, only modest improvements in bone health can be achieved. The reason for this apparent 'bone resistance' is unclear but may reflect abnormal osteoclast function. To address this problem, we developed an *in vitro* model of osteoclast bone resorption, in which we differentiated mouse RAW264.7 cells, into functional osteoclasts using the cytokine RANKL. Multinucleated TRAP (tartrate resistant alkaline phosphatase) positive cells were apparent after 7 days of culture on uncoated tissue culture plastic.
Osteoclasts were differentiated on inorganic calcium phosphate coated plates designed to mimic the *in vivo* bone environment. Cultures were maintained for 14 days and the Gaucher's defect was modelled by culturing cells in the presence of conduritol β epoxide a potent, irreversible inhibitor of mammalian glucocerebrosidase. After the culture period, cells were removed and the number of resorption pits counted. We observed that the number of osteoclasts and resorption pits was increased by 20% following culture in the presence of 50 μM CBE and this effectively doubled at 100 μM.
Our novel *in vitro* model of Gaucher's osteoclasts recapitulates observations made in studies using osteoclasts derived from the peripheral blood of patients with Gaucher's disease. This system may provide a useful tool for understanding the mechanisms of increased bone turnover in Gaucher's disease and in particular the intracellular consequences of defective glucocerebrosidase activity within the osteoclast. This may provide insights for the development of alternative or

complementary therapies to prevent the aberrant bone turnover associated with this rare disease.

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## PP167

**Inhibitory effect of *Crossostephium chinense* extract on RANKL-activating osteoclastogenesis in patients with tophaceous gout**

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Chronic tophaceous gout is the natural evolution of untreated gouty arthritis and is characterized by the deposition of solid monosodium urate crystal aggregated in a variety of tissue including joints, bursae and tendons. Tophaceous gout is well-known to cause bone erosions and is characterized by enhanced osteoclasts development. Peripheral blood mononuclear cells (PBMCs) from patients with severe erosive gout showed the preferential ability to transform into osteoclast-like cells following stimulation with receptor activator of NF-κB ligand (RANKL). *Crossostephium chinense* (CC) is a traditional Chinese medicinal plant used to treat arthralgia and rheumatism. CC had been proved to have antioxidant, antiproliferative and hepatoprotective activities. This study is aimed to investigate the effects of CC extract (CCE) on the formation of RANKL-activating osteoclasts from RAW264.7 macrophage cells and from PBMCs in patients with tophaceous gout. The study was approved by the Human Research Ethics Committee (No. EMRP-101-059). Written informed consents were obtained from gouty patients before enrollment. CCE significantly inhibited the RANKL-induced formation of TRAP-positive multinucleated osteoclasts in a dose-dependent manner. CCE suppressed RANKL-stimulated osteoclast differentiation in RAW264.7 cells *via* down-regulating activation of TRAF6, NF-κB and MAPKs including ERK, JNK, p38 and the expression of NFATc1 and MMP-9. Additionally, CCE decreased the phosphorylation of osteoclasts survival-related signaling molecules, including TRAF6, NF-κB, ERK, JNK, p38, NFATc1 and MMP-9. Furthermore, CCE inhibited pits formation on bone slices in a dose-dependent manner that indicated the ability to inhibit the bone resorptive activity of mature osteoclasts. On the other hand, CCE was capable of suppressing RANKL-mediated differentiation and formation of TRAP-positive multinucleated osteoclasts transformed from PBMCs. CCE also decreased bone resorptive activity of mature osteoclasts derived from PBMCs. Taken together, these results demonstrate that CCE have potential in treating gouty erosions through inhibiting differentiation, formation and bone resorptive ability of osteoclasts.

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## PP168

**Effect of C-reactive protein on TRAP-positive multinucleated cell formation in RANKL-induced RAW264.7 cell culture**

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Inflammatory processes play a role in osteoclastogenesis. C-reactive protein (CRP), an acute phase reactant that reflects different degree of inflammation. More recently, accumulating evidence suggest that CRP is not only an inflammatory marker but also direct cause of diseases. Therefore, we examined the direct effects of CRP on osteoclast formation using RAW 264.7 cells. CRP significantly inhibited RANKL-induced TRAP-positive multinucleated cell formation in RAW 264.7 cell cultures in a dose-dependent manner (1 ug/ml to 30 ug/ml). We observed suppression of ERK and p38 MAPKs induced by RANKL in western blotting after CRP treatment in RAW 264.7 cells. Furthermore, CRP increased TNFa and IFNb expression in RAW 264.7 cells. OxPAPC, inhibitor of toll-like receptor signaling, decreased CRP-induced TLR signaling and expression of TNFα and IFNβ. Furthermore, OxPAPC reduced CRP-induced inhibition of TRAP-positive multinucleated cell formation. These data indicated that CRP may have a direct role on osteoclastogenesis *via* MAPK and TLR-dependent pathway.

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## PP169

**Mechanosensitive TRP channels are required for $Ca^{2+}$ signaling in osteoclastogenesis**

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Bone remodeling and maintenance require a fine balance between bone formation of osteoblasts and resorption of osteoclasts. Therefore, various skeletal disorders cause by imbalanced differentiation and activities of these cells. RANKL (receptor activator of NF-kB ligand) induces $Ca^{2+}$ oscillations and activates NFATc1 (nuclear factor of activated T cells i) during osteoclast differentiation. Although $Ca^{2+}$ oscillations play a key role for osteoclastogenesis, the molecular identification of $Ca^{2+}$ influx *via* mechanosensitive calcium channels located on the plasma membrane for the generation of $Ca^{2+}$ oscillation are not well known. We investigated the expression and functional role of mechanosensitive TRP (transient receptor potential) channels on $Ca^{2+}$ signaling during osteoclastogenesis in RAW264.7 and bone marrow macrophage (BMM) cells. $Ca^{2+}$ oscillations and entry were changed by the over-expression of TRPC3 and TRPC6. Activation of these channels had effects on the expression of NFATc1, activation of ERK pathway, and regulation of osteoclast differentiation. These results suggest that mechanosensitive TRP channels play a key role in the $Ca^{2+}$ signaling of osteoclastogenesis. This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (MEST) (2012R1A1A2007673).

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## PP170

**DBZ, a γ-secretase inhibitor, suppresses bone resorption by inhibiting c-Src activity**

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Tyrosine kinase c-Src plays an important role in actin ring formation and bone resorption activity in osteoclasts. Therefore, c-Src has been targeted for the treatment of osteolytic disorders. In the present study, we investigated anti-resorptive effect of dibenzazepine (DBZ), one of the γ-secretase inhibitors (GSIs), on osteoclast-mediated excessive bone resorption. DBZ did not affect osteoclast differentiation, but disturbed actin ring formation and inhibited osteoclast-induced bone resorption by suppressing c-Src kinase activity. In addition, the localization of c-Src exhibited scattered distribution throughout the cytoplasm by DBZ treatment. Consistent with the *in vitro* results, osteoclastic bone resorption was strongly reduced by administration of DBZ in IL-1 induced bone loss model. Collectively, we demonstrated that DBZ had a potent inhibitory activity on bone resorptive activity by Src activity in mature osteoclasts. Our results suggest that DBZ may serve as a useful agent for osteoclast-mediated excessive bone resorption.

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## PP171

**The purinergenic receptor P2Y14 is essential for RANKL-induced osteoclastogenesis**

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The P2Y14 (purinergic receptor P2Y, G protein coupled, 14) receptor for UDP-glucose and other UDP-sugars has been implicated in the regulation of the stem cell compartment as well as neuroimmune function. However, the role of P2Y14 in osteoclast formation is completely unknown. We found that RANKL selectively induced P2Y14 among seven mammalian P2Y receptors when analysed at both the mRNA and protein level, but inhibitors of the MAP pathway suppressed induction of P2Y14 proteins. Extracellular addition of UDP-sugars such as UDP-glucose, UDP-galactose, UDP-glucuronic acid, and UDP-N-acetyl glucosamine promoted RANKL-induced osteoclastogenesis, while P2Y14 down-regulation by RNA interference inhibited osteoclast formation. Taken together, these results suggest that P2Y14 may act as the receptor for UDP-sugars in osteoclast precusors and may regulate RANKL-induced osteoclastogenesis.

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## PP172

### *In vitro* inhibitory activity on RANKL-mediated osteoclastogenesis of Glossogyne tenuifolia extract

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Receptor activator of nuclear factor kappa B ligand (RANKL)-induced activation of NF-κB and MAPKs signaling pathways is critical to osteoclastogenesis. *Glossogyne tenuifolia* has been used as an antipyretic, detoxication, and anti-inflammatory herb tea in Penghu Island, Taiwan. This study investigated the effects of *G. tenuifolia* ethanolic extract (GTE) on the formation of RANKL-activating osteoclasts and expression of signaling pathways using a RAW264.7 cells model. GTE significantly inhibited the RANKL-induced formation of TRAP-positive multinucleated osteoclasts in a dose-dependent manner. GTE inhibited RANKL-induced activation of nuclear factor of activated T-cells (NFATc1) and suppressed the phosphorylation of NF-κB/p65 and JNK. Additionally, GTE reduced the phosphorylated forms of osteoclast survival-related signaling molecules, including NF-κB, JNK, p38, and Akt. Taken together, these results reveal that GTE may have potential in the development of health food against osteoclast-related diseases such as osteoporosis.

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## PP173

### Inhibition of bone remodeling by bisphosphonate displaces the plasma cell niche into the spleen

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Bone marrow is the main hematopoietic organ of adults. There, hematopoietic stem cells from which all hematopoietic lineages can be generated are preferentially homing. Importantly, bone provides niches for early B cell differentiation and survival of long-lived plasma cells that produced antibodies. Thus, that perturbing bone homeostasis might impact B cell function and antibody production is a highly relevant hypothesis for patients receiving antiresorptive drugs.
We therefore analyzed humoral immune responses of mice treated with ibandronate, a commonly used bisphosphonate. We confirmed the increased bone mass caused by inhibition of osteoclast activity in response to ibandronate and the secondary reduced bone formation due to decreased osteoblast numbers. Thus, bisphosphonate drastically inhibited bone remodeling.
We next analyzed antibodies production by stimulating B cell response with T cell-independent or T cell-dependent immunization in two different setting: i) Ibandronate was injected into mice after a primary immunization in order to mimic common anti-osteoporotic treatments. There, generation of the various B cell populations including bone marrow plasma cells and responses to booster immunization were surprisingly normal. ii) Ibandronate was chronically applied to naïve mice before immunization. Again, responses to immunization appeared normal. However, in that setting, ibandronate drastically shunted plasma cells homing to bone marrow. Interestingly, ibandronate specifically reduced the numbers of megakaryocytes, a known component of the bone marrow plasma cell niche. In line with normal antibody responses, increased plasma cells associated with increased megakaryocyte numbers were then observed in spleens of ibandronate-treated mice.
Thus, inhibiting bone remodeling by ibandronate displaces the bone marrow plasma cell niche toward a compensatory niche by relocating the megakaryocytes to spleen, thereby allowing normal B cell responses. Therefore, we identify megakaryocytes as a key regulator of plasma cell niche plasticity responsive to antiresorptive treatment, and demonstrate that antiresorptive drugs can affect plasma cells homing into bone.

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# Cell biology: osteocytes

## PP174

### Structural analysis of tooth and jawbone in a type 2 diabetes mouse model

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In type 2 diabetes mellitus (T2DM) patients, an increased fracture risk is observed, although the bone mineral density is even higher than in non-diabetic patients. This raises the question of the quality of the organic and inorganic matrix in bone[1–3]. T2DM is also known to forward dysfunctions in the development of soft tissues such as brittle skin due to cross-linking of the collagen or inflammation of the gingiva. For the latter, a possible influence of diabetes not only on the soft tissue but on the hard tissue motivated the current study. Using synchrotron small-angle X-ray scattering (SAXS) we investigated the nanostructure of the mandible and tooth of diabetic and healthy mice. Parameters to characterize bone quality are the amount, size (T- and L-parameter) and distribution (rho-parameter) of mineral particles in the organic matrix[4–5]. The samples included embedded cross-sections of the jaw bone centered around the first molar of 15 week old standard healthy controls (C57BL/) and obese diabetes mice (KKAy). Line scans with 30 μm resolutions (beam- and stepsize) over the cross-section from the lingual side towards the tooth to the buccal side were performed. Significant differences in the nanostructure of the mineral particles of the bone between the lingual and buccal side were found. In particular, the mineral platelets are thicker and well more orientated on the buccal side compared to the lingual side in the control mice. In the presence of T2DM the measurements indicate a further increase of this structural difference between both sides toward the tooth. Reasons for this high asymmetry in the nanostructure of the bone in control as well as diabetes mice could be differences in the remodeling rates related with an unequal mechanical load on both sides of the tooth.

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## PP175

### Low estrogens, weak bones: unravelling estradiol remodelling effect in bone metabolic/lipid profiles

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Introduction
For the very first time were assessed *in vivo* the metabolic and lipid profiles of osteocytes. During menopause period the appearance of an osteoporotic condition can be associated with an overall metabolic decline in bone cells, as well an increase of reactive oxygen species (ROS), and we hypothesized that it is mainly attributed to osteocytes metabolic and lipid changes, which could be apparently attenuated after raising blood estradiol (E2) levels. To test this we considered control and ovariectomized (OVX) 12 week-old female rats in order to compare bone embedded osteocytes metabolic/lipid profiles, in presence or absence of high levels of E2.
Methods
Animal groups (used accordingly with FELASA procedures) - i) controls, SHAM; ii) ovariectomized animals, OVX; and iii) OVX+E2 (single bolus injection 30 mg/kg, 24 h prior sacrifice). Animals were sacrificed 4 weeks after ovariectomy. Left and right femurs and tibias were surgically removed and freeze-clamped or preserved for DXA analysis. All bone marrow and blood were discarded, just to leave mineral embedded cells. Methanol/water extracted metabolites from those

cells were analyzed by high resolution 600 MHz $^{1}$H nuclear magnetic resonance (NMR) spectroscopy. Total lipids, extracted by Bligh and Dyer method, were quantified and analyzed by an HPLC system coupled to an electrospray linear ion trap mass spectrometer (HPLC–MS), and bone mineral content was assessed by flame atomic absorption spectrophotometry.

Results

Ovariectomy induced disproportionate increase in choline-plasmalogens content (comparatively with SHAM and OVX + E2 groups), mainly PC (O20:0/22:6), and diacyl phosphatidylcholines (PC(22:0/22:1), PC(22:0/22:0)). Also, in OVX were observed an increase of the relative proportion of long chain fatty acids, attenuated by 24 h-treatment with E2. In terms of metabolites profile, OVX presented a slight decrease of lactate/alanine ratio, but with E2, osteocytes were forced to produce high levels of lactate, increasing this ratio.

Conclusions

Here is expressively evident a change in the process of lipid biosynthesis as a result of ovaries removal, and E2 partially compensated the replacement of the control levels of long chain fatty acids. High levels of PC-Plasmalogens presented in OVX could be related to a signalling/protective action against the damaging effects of ROS triggered by the E2 decline. 24 h-E2 administration in OVX induced osteocytes to probably enhance glycolysis in an attempt to compensate for the metabolic deficit associated with the ovaries removal, considering the hypothesis that osteocytes mitochondria may present an impaired respiratory chain.

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## PP176

### Effects of osteocyte apoptosis and mechanical strain on osteoblast proliferation and migration

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There are several evidences that osteocyte plays a critical role in bone remodeling. Healthy or apoptotic osteocytes can send signals to other bone surface cells like osteoblasts, osteoclasts, osteoclast precursors and bone lining cells through their networking in canaliculi. Therefore, to determine the role for osteocytes in bone remodeling, we examined the effect of healthy and apoptotic osteocytes on osteoblasts proliferation and recruitment. In addition, the effects of mechanical strain through osteocyte on bone remodeling were examined using fluid flow system. We used the MLO-Y4 cells as *in vitro* model for osteocytes, and 2T3 cells as osteoblasts. For induction of MLO-Y4 cell apoptosis, MLO-Y4 cells were treated with 100 μM etoposide for 6 h and apoptosis was determined with increase of casapase-3 activity and ratio of trypan blue straining cells. For fluid flow experiments, MLO-Y4 cells were exposed to 2 h of fluid flow at 2, 4, 8, 16 dynes/cm$^2$ using Flexcell Streamer$^{TM}$ system. Healthy, apoptotic and shear stress-exposed MLO-Y4 cells conditioned medium (Y4-CM) was collected after 24 h culture. We did proliferation assay 2T3 cells with Y4-CM at specific time. The migration of 2T3 cells was assayed using transwells with control media or Y4-CM. Healthy Y4-CM decreased 2T3 cell proliferation and migration. However apoptotic Y4-CM increased the proliferation of 2T3 cells compared to control Y4-CM. Apoptotic Y4-CM had no effect on migration of 2T3 cell. After MLO-Y4 cells were exposed to fluid flow, Y4-CM after application of high magnitude of stress (8, 16 dynes/cm$^2$), decreased the migration of 2T3 cells. These results suggest that the osteocytes responding to apoptosis and mechanical strain might modulate the bone formation by changing the signals to osteoblasts.

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# Chondrocytes and cartilage

## PP177

### The transcription factor Foxc1 controls endochondral ossification through the direct regulation of PTHrP and Cola10a1 expression under the partnership with Gli2 and Runx2

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Endochondral ossification is regulated by various transcription factors in a temporo-spatial manner. Identification of novel transcription factors involved in chondrogenesis would facilitate to uncover the molecular basis of endochondral ossification. To approach this, we generated transgenic mice in which chondrocytes were specifically fluorescence-labeled with Venus gene using Col2a1 gene promoter, thereby allowing us to isolate Venus-positive chondrogenic cells using FACS Aria. Differential microarray between Venus-positive and -negative cells identified Foxc1 (Forkhead Box c1) as a candidate transcription factor selectively expressed in chondrogenic cells. Immunohistochemical analysis showed that Foxc1 was expressed not only in resting and proliferating chondrocytes but also hypertrophic chondrocytes. Overexpression of Foxc1 induced alcian blue-positive chondrogenesis in limb bud cells in micromass culture.

We next studied endochondral ossification in spontaneous Foxc1-inactivated mice (*Foxc1$^{ch}$*). *Foxc1$^{ch}$* mice showed shorter limbs and thinner ribs than WT mice. Of note, ossification center of sternum was completely absent and the length of Col10-positive hypertrophic zone was significantly shorter in newborn *Foxc1$^{ch}$* mice compared to WT mice.

We then examined the molecular mechanism by which Foxc1 regulates endochondral ossification. Overexpression of Foxc1 in primary chondrocytes increased PTHrP and Col10a1 expression. DNA pull-down and ChIP assays displayed direct binding of Foxc1 to the PTHrP and Col10a1 gene promoter. Foxc1 physically associated with Gli2 and cooperatively increased the expression of Ihh/Gli2 target genes including PTHrP, Gli1 and Ptch1. Moreover, Foxc1 also co-immunoprecipitated with Runx2 and synergistically increased the expression of Col10a1 and MMP13 gene, which are the target genes of Runx2 in chondrogenesis.

In conclusion, we identified a transcription factor Foxc1 which directly regulates PTHrP and Col10a1 *via* complex formation with Ihh/Gli2 and Runx2. Our findings suggest that Foxc1 is a multifaceted transcription factor that contributes to the promotion of endochondral ossification.

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## PP178

### LDL receptor-related protein 5 governs Wnt-mediated osteoarthritic cartilage destruction

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Introduction

Wnt ligands bind to LDL receptor-related protein (LRP) 5 or −6, triggering a cascade of downstream events that include β-catenin signaling. Here, we explored the roles of LRP5 in interleukin (IL)-1β- or Wnt-mediated osteoarthritic (OA) cartilage destruction in mice.

Methods

The expression levels of LRP5, Type II collagen, and catabolic factors were determined in mouse articular chondrocytes, human OA cartilage, and mouse experimental OA cartilage. Experimental OA in WT, *Lrp5* total knockout (*Lrp5$^{-/-}$*) and chondrocyte-specific knockout (*Lrp5$^{fl/fl}$*;*Col2a1-cre*) mice was caused by aging, destabilization of the medial meniscus (DMM), or intra-articular injection of collagenase. The role of LRP5 was confirmed *in vitro* by siRNA-mediated knockdown of *Lrp5* or in *Lrp5$^{-/-}$* cells treated with IL-1β or Wnt proteins.

Results

IL-1β treatment increased the expression of LRP5 (but not LRP6) *via* JNK and NF-κB signaling. LRP5 was up-regulated in human and mouse OA cartilage, and *Lrp5* deficiency in mice inhibited cartilage destruction. Treatment with IL-1β or Wnt decreased the level of *Col2a1* and increased those of *Mmp3* or *Mmp13*, whereas *Lrp5* knockdown ameliorated these effects. In addition, we found that the functions of LRP5 in arthritic cartilage were subject to transcriptional activation by β-catenin. Moreover, *Lrp5$^{-/-}$* and *Lrp5$^{fl/fl}$*; *Col2a1-cre* mice exhibited decreased cartilage destruction (and related changes in gene expression) in response to experimental OA.

Conclusion

Our findings indicate that LRP5 (but not LRP6) plays an essential role in Wnt/β-catenin-signaling-mediated OA cartilage destruction in part by regulating the expression levels of type II collagen, MMP3, and MMP13.

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## PP179

**Osteoclasts inhibit the Wnt canonical pathway in chondrocytes**

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Purpose
Osteoclastogenesis is enhanced in osteoarthritis (OA). We have demonstrated that cartilage breakdown is reduced when osteoclastogenesis is inhibited in murine models. Wnt activity, known to regulate the bone and chondrocyte cell function, might contribute to the mechanisms that promote cartilage catabolism. Our purpose was to evaluate whether osteoclast-secreted molecules affect the chondrocyte metabolism and assess the contribution of Wnt pathway.
Methods
We used osteoclasts derived from RAW cells. Primary murine chondrocyte (Ch) were cultured in the presence of osteoclast conditioned medium (Oc-CM) for 48 h. The gene and protein expressions of catabolism and anabolism were analyzed by RT-qPCR and western blot. To investigate the regulation of canonical Wnt pathway, transactivation assay was performed in primary chondrocytes derived from Topgal mice and cultured with Oc-CM. Western blot and immunocytochemistry were used to confirm the activation of β-catenin canonical Wnt signaling pathway. Finally, in order to confirm the role of Wnt canonical signaling, the β-catenin translocation was activated by lithium chloride (LiCl) in addition of the Oc-CM.
Results
Oc-CM induced a marked decrease in proteoglycan release by chondrocytes. Oc-CM reduced the expression of anabolic genes (collagen type II, Aggrecan and Sox-9) while increased the expression of catabolic genes (MMP-3, -13, Adamts-4, -5). We then monitored the nuclear translocation of β-catenin induced by Oc-CM. We observed an abolition of the translocation of β-catenin and subsequently of Topgal activity along with the reduction of Wnt target genes (Axin, Wisp1 C-Myc). LiCl reduces the expression of catabolic genes induced by Oc-CM, indicating the involvement of the Wnt-canonical pathway on the regulation of catabolic genes.
Conclusion
We here demonstrated that osteoclasts secrete soluble factors that are able to disrupt the balance of chondrocyte metabolism *via* the inhibition of the Wnt canonical signaling. Therefore, our data indicate that manipulating bone may affect chondrocyte function.

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## PP180

**Selective targeting of bone and cartilage with multivalent dendritic polyanions**

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Background and objective
Targeting bone and cartilage includes approaches using tetracyclines, peptide conjugates, as well as anionic moieties like bisphosphonates. However, the selective targeting of the different compartments still remains a challenge. In previous studies native and demineralized ovine bone was used to analyze the affinity of polyanions derived from dendritic polyglycerol (dPG) toward hydroxyapatite and collagen. Whereas the neutral polymer did not show any interaction with bone, a selective binding to hydroxyapatite and collagen was observed depending on the anionic moiety of the polymer. Based on these results the binding affinity of polyanions toward cartilage was investigated to obtain selective targeting agents.
Results
Interleukin-1 (IL-1) treated porcine cartilage explants were incubated with dye-labled dPG anions containing phosphates (dPGP), bisphosphonates (dPGBP), and sulfates (dPGS) to investigate their binding affinity by fluorescence imaging showing dPGS to efficiently penetrate the cartilage tissue, able to accumulate in chondrocytes. The non-labeled polyanions were used to determine the inhibition of IL-1 stimulated glycosaminoglycan release finding dPGS to reduce the release. Compared to this, the neutral polymer did not show any interaction with cartilage. In a collagen-induced rheumatoid arthritis rat model the polymers were investigated regarding their accumulation in inflamed joints *in vivo* using near infrared optical imaging. Both dPGBP and dPGS were found to posess an enhanced affinity to inflamed tissue with dPGS showing a higher binding affinity to inflamed cartilage as investigated by histological examinations. Moreover, dPGBP was also found in calcified areas in healthy joints in contrast to dPGS.
Conclusion
Dendritic polyglycerol based anions were found to be promising candidates for diagnostic and therapeutic applications to target bone and cartilage indicating a high selectivity toward these tissues.

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## PP181

**Hyperbaric oxygen treatment suppresses Wnt/β-catenin signaling and matrix metalloproteinases in degenerated human intervertebral disc cells**

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Background
The activation of Wnt/β-catenin signaling promotes cellular senescence and induces matrix metalloproteinases (MMPs) expression in intervertebral disc (IVD). However, little is known about the effects of hyperbaric oxygen (HBO) on the Wnt/β-catenin signaling and MMPs expression in degenerated human IVDs.
Materials and methods
Nucleus pulposus cells (NPCs) were separated from the degenerated disc nucleus tissue by performing sequential enzymatic digestion. Control cells were maintained in 5% $CO_2$/95% air throughout the experiment. The hyperoxic cells were exposed to 100% $O_2$ for 25 min and then to 5% $CO_2$/95% air for 5 min at 2.5 ATA in a hyperbaric chamber. The mRNA or protein levels of Wnt3a, LRP6, β-catenin, GSK-3β, aggrecan, type II collagen as well as MMP-3, 9, and 13 were analyzed after HBO treatment. The translocation of β-catenin from cytosol to nucleus after HBO treatment was detected by western blot. To determine the β-catenin-Tcf/Lef transcriptional activity, we measured the activity of Tcf/Lef reporter gene Topflash (optimal Tcf-binding site) or Fopflash (mutated Tcf-binding site) in NPC.
Results
The mRNA level of Wnt3a was down-regulated while that of aggrecan and type II collagen were up-regulated after HBO treatment. The relative density ratio (phosho-protein/protein) for LRP6 and GSK-3β were down-regulated after HBO treatment. Our western blot analysis showed decreased levels of β-catenin and translocated β-catenin after HBO treatment. There was decreased TOP flash activity following HBO stimulation, whereas the FOP flash activity was not affected. HBO decreased Tcf-dependent transcription and suppressed the expression of MMP-9.
Conclusion
HBO treatment suppresses Wnt/β-catenin signaling and MMPs expression in degenerated human IVDs.

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## PP182

**Hyperbaric oxygen therapy reduces the catabolic pathway mediated by toll-like receptors in human osteoarthritic chondrocytes**

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Background
Expression of toll-like receptor (TLR) 2 and 4 was up-regulated in lesional areas of OA cartilage. Hyperbaric oxygen (HBO) reduces the TLR signalling pathway in multiple organ failures. However, little is known about the effects of HBO on the catabolic pathway mediated by TLRs in human osteoarthritic (OA) chondrocytes.
Materials and methods
OA chondrocytes were separated from the knees of OA patients by performing sequential enzymatic digestion. Control cells were maintained in 5% $CO_2$ / 95% air throughout the experiment. The hyperoxic cells were exposed to 100% $O_2$ for 25 min and then to 5% CO2/95% air for 5 min at 2.5 ATA in a hyperbaric

chamber. The mRNA or protein levels of TLR2, TLR4, iNOs, aggrecan, type II collagen, MMPs, and ADAMTSs were analyzed after HBO treatment. Production of nitric oxide (NO) was analyzed by the Griess reaction. Phosphorylation of MAPKs (p38, ERK, and JNK) was evaluated by phospho-kinase array kit.
Results
The mRNA levels of TLR 2, TLR 4, ADAMTS 4, and ADAMTS 5 were down-regulated while that of aggrecan and type II collagen were up-regulated in OA chondrocytes after HBO treatment. HBO led to decreased phosphorylation of p38, ERK, and JNK. Production of MMP 3, 9, 13 and NO was significantly decreased after HBO treatment.
Conclusion
Hyperbaric oxygen therapy reduces the catabolic pathway mediated by TLRs in human osteoarthritic chondrocytes.

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## PP183

**Meniscus – Cartilage paracrine crosstalk in osteoarthritis**

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Introduction
Meniscus plays an essential role in knee joint function providing stability and load transmission. In osteoarthritis (OA), a joint disease characterized by chronic synovitis and cartilage degeneration, pathological changes in the menisci are observed. However, whether menisci contribute to the progression of OA, the underlying mechanism for meniscus-cartilage communication is still unclear. In this study we analyzed systematically the response of meniscus and cartilage explants to a number of inflammatory mediators, in order to reveal their response similarity and highlight potential crosstalks and interactions.
Methods
OA cartilage and the lateral meniscus were harvested from two patients undergoing total knee arthroplasty. Meniscus and cartilage disks (3 mm diameter) were stimulated with inflammatory mediators ((IL-1α, IL-1β, IL-12α, CSF2) (50 ng/ml), (TNF-α, IL-6, CXCL7, IL-8, CCL2, CXCL10, IFN-γ, IL-3, MIA2, IL-4) (100 ng/ml) and GROα (500 ng/ml)) for 24 h. For each condition the release of different proteins (IL-1α, IL-1β, TNF-α, IL-6, CXCL7, GROα, IL-8, CCL2, CXCL10, IFN-γ, IL-3, IL-12α, MIA-2, CSF2, IL-4) was measured in the supernatant using custom multiplexed assays on a Luminex FlexMap 3D instrument.
Results
In both tissues the major inflammatory players (IL-1α, IL-1β, TNFα) were the strongest stimuli as expected. Meniscus responses were the same up to 73 and 50% with the cartilage ones for the first and the second donors respectively. Interestingly, meniscus under certain stimuli (IL-1α, IFN-γ, CSF2, IL-8) responded differently than cartilage by releasing five different cytokines while cartilage did not.
Conclusions
Our results indicate that meniscus is affected by its inflammatory environment and responds to it as actively as cartilage. Moreover, the release of different cytokines from meniscus and cartilage suggests that meniscus can be an active player in the progression of OA. These data support the hypothesis that significant crosstalk between these two knee compartments exist and anti-inflammatory therapies should take into consideration both tissues.

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## PP184

**Therapeutic education of patients treated for osteoarthritis: medical advice and assessment of patients knowledge level**

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Introduction
Osteoarthritis is the most common and debilitating degenerative arthropathy. Prevention and patient education are part of the international recommendations. A survey was realized with 43 medical specialists at the Hospital University of Casablanca, about their point of views on the role of therapeutic education for patients with osteoarthritis of the lower limb. Doctors justified the importance of patient education.
Study purpose
Evaluate of the degree knowledge patients about osteoarthritis in order to establish a learning process and improve their care.
Materials and methods
Survey conducted with 53 patients followed in rheumatology consultation for osteoarthritis. It includes four items. Each one contains an open or a simple choice questionnaires.
Results
Among patients, 83% were female. The mean age was 65 years old. About 45.71% are treated for osteoarthritis. Its origin was overweight for 45.7% cases. The sport can be implicated for 74.3%. Duty diet is true for 74.3%. About 42.8% had no idea about the link between osteoarthritis and osteoporosis, and 34.3% with menopause. For 85.7% patients, the osteoarthritis progression is worsening whatever treatment. The pain is associated with osteoarthritis for 88.6% of cases. It is a source of aggravation for 85.7%. It requires a blood test for 54.28%, and necessity X-rays for 85.7%. Osteoarthritis is objectively better by Computer Tomography or Magnetic Resonance Imaging to 74.28%. About 71.4% believed that it is now possible to cure osteoarthriti. 62.8% of patients are using symptomatic treatment for pain. To treat joint, 40% use anti-arthritic, 10 cases local treatment. About 62.85% find no interest to use a cane.
Discussion and conclusion
Our results are consistent with expert opinion. Many data were ignored by patients. While some patients were able to correctly answer questions, others do not know. The osteoarthritis therapeutic patients should be an integral part of our practice, joint savings, and well being of the patient's autonomy.

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## PP185

**Disturbed cartilages of the mandible in achondroplasia are associated with defective mandible shape and position**

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FGFR3 activating mutations are responsible for achondroplasia (ACH), the most common form of dwarfism. ACH clinical features include short stature, midface hypoplasia, frontal bossing and prognathism and both endochondral and membranous ossifications are disturbed. It is unknown if abnormal mandibles are present in ACH. To date, it is believed that primary (Meckel's) and secondary (angular and condylar) cartilages play important roles in determining the final shape and position of the mandible. Here, we first characterized the mandibular anomalies in ACH patients and in $Fgfr3^{Y367C/+}$ mice that mimic ACH. We analyzed CT scans of 16 patients and controls and observed that the condyle, formed by endochondral ossification, was shorter ($-18\%$, $P<0.05$), broader ($+11\%$, $P<0.05$) and projected forward. These condyle shape and position are associated with mandibular prognathism. $Fgfr3^{Y367C/+}$ mice also exhibited a prognathic mandible. Morphometric analysis of uCT of the mandibles of $Fgfr3^{Y367C/+}$ mice ($n=7$) and their control littermates ($n=7$) using anatomical landmarks and geometric morphometrics showed strong differences in shape and size. We observed in $Fgfr3^{Y367C/+}$ mice that the body of the mandible was shorter from E16.5 ($-12\%$, $P<0.01$) to P21 ($-19\%$, $P<0.005$) and that the condyle, angle and mental protuberance were underdeveloped. We then explored Meckel's cartilage and mandibular secondary cartilages of these mice and observed an abnormal proliferation and differentiation of the chondrocytes revealed with Ki67 and collagen type X immunolabeling. The replacement of Meckel's cartilage by bone was delayed in these mice as showed with collagen I and TRAP labelling. Finally, we tested a FGFR3 tyrosine kinase inhibitor on *ex vivo* cultures of $Fgfr3^{Y367C/+}$ embryonic mandibles. After 6 days of culture, the cellular anomalies observed in Meckel's cartilage were partially corrected. All together these data show that cartilages of the mandible are affected in ACH and that these defects contribute to the defective mandible shape and position.

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## Genetics

### PP186

**Pharmacogenomics of bisphosphonate treatment in Paget's disease of bone: retrospective and prospective analysis**

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We previously evidenced a reduced response to i.v. pamidronate in *Q15STM1* mutation carriers (*Q15STM1*+) with Paget's disease of bone (PDB). In order to confirm and extend this observation, we investigated the effect of *Q15STM1* mutation and polymorphisms in three genes associated with PDB (*TNFRSF11A*; *OPTN*; *TNFRSF11B*) on the response to bisphosphonates. First, a retrospective study was performed in 335 patients treated with i.v. clodronate (CLN) 1500 mg/6 months ($n=84$), i.v. pamidronate (PAM) 60 mg/6 months ($n=75$), risedronate (RIS) 30 mg/day for 2 months ($n=57$), neridronate (NER) 200 mg i.m. or i.v. ($n=61$) and zoledronate (ZOL) 4 mg or 5 mg i.v. ($n=48$). Overall, *Q15STM1*+ patients had an increased disease severity and a reduced response to CLN or PAM than *Q15STM1*− patients. Despite carriers of *TNFRSF11A* rs1805034 CC genotype had increased PDB severity, there was no association between this genotype and the response to any treatment. Conversely, there was an increased prevalence of TT carriers of rs1561570 (*OPTN*) in non-responders to CLN. Normalization after the first CLN course was achieved in 69% of CC *vs* 42% of TT carriers despite no significant differences in disease extension or activity, especially in *Q15STM1*− patients. A similar but not significant trend was observed for PAM. Conversely, RIS, ZOL and NER were effective in most patients. We then designed a prospective analysis in patients treated with iv ZOL 5 mg ($n=130$) or NER 200 mg ($n=70$) and followed-up for more than 3 years. Even though most patients achieved biochemical remission after the first NER course for more than 12 months, there was a trend for an earlier relapse in *Q15STM1*+ *vs* *Q15STM1*−patients. Conversely most ZOL treated patients achieved biochemical remission independent of *Q15STM1* mutation and did not need retreatment. These results suggest that PDB patients with *Q15STM1* mutation may require a more aggressive treatment regimen for long-term disease remission.

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### PP187

**A novel mutation in IFITM5, encoding BRIL, impairs osteoblast production of PEDF and causes atypical type VI osteogenesis imperfecta**

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Osteogenesis imperfecta (OI) type V is caused by a unique dominant mutation (c.−14C>T) in *IFITM5*, which encodes BRIL, a transmembrane ifitm-like protein most strongly expressed in osteoblasts, while type VI OI is caused by recessive null mutations in *SERPINF1*, encoding pigment epithelium-derived factor (PEDF). We identified a 25-year-old woman with severe OI, whose dermal fibroblasts and cultured osteoblasts displayed minimal secretion of PEDF, but whose serum PEDF was in the normal range. Her *SERPINF1* sequences were normal despite bone histomorphometry typical of type VI OI, and elevated childhood serum alkaline phosphatase. We performed exome sequencing on the proband, both parents and an unaffected sibling. *IFITM5* emerged as the candidate gene from bioinformatics analysis. The *de novo IFITM5* mutation was confirmed in one allele of the proband, causing a p.S40L substitution in the BRIL intracellular domain, but was absent in unaffected family members. *IFITM5* expression was normal in proband fibroblasts and osteoblasts, as was BRIL protein level in proband osteoblasts on western blot and in permeabilized proband osteoblasts by microscopy. Notably, *SERPINF1* expression was decreased in proband osteoblasts and PEDF was barely detectable in conditioned media of proband cells. Expression and secretion of type I collagen was similarly decreased in proband osteoblasts, confirming that the proband's OI was collagen-related. Osteoblasts with the S40L mutation also had deceased expression of alkaline phosphatase and osteocalcin, as seen in primary PEDF defects. In contrast, osteoblasts from typical type V OI, with five residues added to the N-terminus of BRIL, have increased *SERPINF1* expression and PEDF secretion during osteoblast differentiation. Together, these data suggest i) that the type V OI and p.S40L BRIL are gain- and loss-of-function mutations respectively, and ii) that BRIL and PEDF have a relationship that connects the genes for types V and VI OI and their roles in bone mineralization.

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### PP188

**Correlation of miRNA-mRNA regulatory network profile with bone mass in inbred strains of mice**

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Growing body of evidence shows that microRNAs play an important role in regulating bone mass. We investigated the cooperative microRNA-mRNA regulatory mechanism of peak bone mass in inbred strain of mice with different bone density using microarray analysis. Femur and tibia of 12-week old C3H/He (high bone density) and C57BL/6 (low bone density) were harvested to extract total bone RNAs for microarray. A total of 30 miRNAs were differentially expressed between bone tissue of CeH/He and C57BL/6 mice; 16 miRNAs (miR-34c, 101a, 128, 135a, 181b, 181d, 210, 290, 296, 340, 425, 467e, 542, 582, 652, 705) were identified to be upregulated in C3H/He mice, while 11 miRNA (miR-26b, 29c, 34a, 101b, 204, 214, 219, 338, 342, 720, 801) were upregulated in C57BL/6 mice. Concomitant analysis of mRNA microarray revealed that 1189 genes were differentially expressed between C3H/He and C57BL/6 mice. Functional clustering analysis revealed that mRNA of the genes related with the composition of extracellular matrix, microtubule, gap junction and mRNAs of Wnt and TGF-β signaling pathways were upregulated in C3H/He mice. In contrast, the mRNA of genes involved in immune and inflammatory response as well as lipid synthesis were upregulated in C57BL/6 mice. Using the integrative analysis based on computational target prediction of differentially expressed miRNAs and concomitant mRNA profiling, we found that about 20% of differentially expressed mRNAs were putative target of differentially expressed miRNAs. The candidates for miRNA/mRNA regulatory pairs include those of the component of extracellular matrix, microtubule and gap junction, indicating that peak bone mass determination in different strains of mice is at least in part mediated by differential expression of miRNA profile. The miRNA/mRNA expression profiles reported in this study form a comprehensive transcriptome database and provide a valuable resource for understanding the role of miRNA in determining bone mass.

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### PP189

**A polymorphism in the TGF-β1 gene affects TGF-β1 secretion**

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Transforming growth factor (TGF)-β1 is the most abundant growth factor in human bone. Several polymorphisms have been described in the TGF-β1 gene (*TGFB1*). We have previously shown that individuals with the CC genotype of the $T^{29}C$ polymorphism have higher bone mass at the femoral neck. The $T^{29}C$ polymorphism causes a change from leucine to proline at codon 10, which is located in the hydrophobic α-helical part of the signal peptide. A proline at this position would be expected to disrupt the α-helical structure, thereby altering the ability of the signal peptide to direct protein transport across the endoplasmic reticulum.
To examine whether the $T^{29}C$ polymorphism affects TGF-β1 secretion in osteoblast like cell lines, we transfected the osteosarcoma cell line; U2OS and SaOS-2 with TGF-β1 expression plasmids containing different $T^{29}C$ genotypes. pcDNA3.1-TGF-β1-10L and pcDNA3.1-TGF-β1-10P were generated from cDNA encoding TGF-β1 WT and variant amplified from RNA isolated from primary human osteoblasts and the mammalian expression vector pcDNA3.1+. The two cell lines were transfected with the TGF-β1 expression plasmids using

Fugene. The influence of the $T^{29}C$ polymorphism on TGF-β1 levels in peripheral serum and bone marrow serum was examined in 185 young healthy individuals. TGF-β1 in supernatants, marrow and peripheral plasma was determined by ELISA. The study was approved by the local ethical committee.
The secretion of the variant protein was increased by 14±6% ($P=0.02$, $n=27$) compared to the WT in U2OS cells and by 80±7% ($P<0.001$, $n=27$) in SaOS-2 cells. In contrast, healthy, young individuals homozygous for the variant C allele had reduced TGF-β1 levels compared to carriers of the normal allele; 16.8±6.6 ng/ml *vs* 19.5±6.0 ng/ml, $P=0.02$ in serum and 14.0±4.3 ng/ml *vs* 15.9±4.6 ng/ml, $P=0.04$ in bone marrow serum.
In conclusion, we found that the $T^{29}C$ polymorphism was associated with TGF-β1 secretion *in vitro* and *in vivo*.

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## PP190

### Premature aging of bone is delayed by dietary restriction

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Loss of genomic maintenance contributes to aging, as exemplified by mutations in Ercc DNA repair proteins that lead to a plethora of progeroid syndromes of which some display accelerated bone loss. It is generally accepted that dietary restriction (DR) increases life span and improves organ function. We therefore assessed the impact of DR on life span and bone mass in WT and *bona fide* prematurely aging hypomorphic Ercc-deficient mice ($Ercc1^{-/\Delta}$).
Female and male WT and $Ercc1^{-/\Delta}$ mice were fed *ad libitum* (AL) until 7 weeks of age after which they were split up in 30% DR (initiated with 10% per week increments) and AL groups. Femurs were collected at several ages and micro-computed tomography was performed.
DR-fed $Ercc1^{-/\Delta}$ mice increased their life span to over 30 weeks compared to 20 weeks for AL-fed mice. At 11 weeks of age, femur length was significantly reduced in female (13.4±0.4 *vs* 15.5±0.5 mm; $P<0.001$) and male (13.5±0.5 *vs* 15.9±0.4 mm; $P<0.001$) $Ercc1^{-/\Delta}$ *vs* WT mice, consistent with reduced growth. DR did not alter femur length in these groups. AL-fed $Ercc1^{-/\Delta}$ mice had a significantly lower trabecular bone volume fraction (BV/TV) *vs* WT mice (females: 5.8±1.8 *vs* 10.7±3.2%; $P<0.001$, males: 7.4±1.5 *vs* 13.2±2.0%; $P<0.001$). In male WT mice (non-significant in females), DR reduced BV/TV (34.8%, $P=0.002$) but in $Ercc1^{-/\Delta}$ mice this was elevated (38.2%, $P=0.002$). The initial bone mass was increased in the DR-fed $Ercc1^{-/\Delta}$, but thereafter bone loss rate was similar between diets. Effects on trabecular thickness showed similar non-significant trends.
Conclusion
DR reduces bone mass in WT mice but it prolongs life span and delays bone loss in $Ercc1^{-/\Delta}$ mice. The differential response to DR in WT *vs* $Ercc1^{-/\Delta}$ mice and the DR-triggered temporal increase in bone mass in $Ercc1^{-/\Delta}$ mice deserve further mechanistic scrutiny but may be related to genotype-driven effects on bone at young age.

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## PP191

### Functional analysis of a promoter polymorphism of optineurin, a gene associated to Paget's disease of bone

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Paget's disease of bone (PDB) is the second most common metabolic bone disorder, after osteoporosis. Optineurin (OPTN) gene, which is located within the PDB6 locus, and appears to be upregulated by TNFα and NF-kB, has been found to be associated with PDB in several European populations with PDB in genome-wide association studies. Several nucleotidic variations in OPTN were previously associated with PDB and may contribute to PDB pathogenesis. Recently, we have identified in the DNA of a patient with PDB, a SNP (RV-9906 G/A) in the proximal promoter of OPTN that could affect the transcriptional activity of this gene. In this work we have analysed the basal activity of the OPTN promoter and whether RV -9906 altered the activity of that promoter, and consequently OPTN transcription. DNA fragments of 1100 bp with and without the variant, were cloned in a reporter vector, and used in transient transfection assays with HEK293 cells. We also performed co-transfections using some of the transcription factors predicted by in silico analysis to bind the OPTN promoter in the presence or absence of RV-9906 – Sp1, RAR and RXR. Our results showed that RV -9906 was responsible for a decrease in OPTN promoter associated to a new Sp1 binding site created in the presence of the A allele. We also showed that both RAR and RXR bind to the OPTN promoter construct used although this binding was not affected by the presence of the RV-9906 (either the G or A allele). Since OPTN was described as having a putative inhibitory role on NF-kB pathway, our results suggest that the A variant of this SNP, found in a PDB patient, by decreasing the activity of OPTN transcription, could activate the NF-kB signalling and contribute to PDB pathogenesis. This work also provides new insights into OPTN gene regulation.

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## PP192

### Genetic determinants of bone mineral density loss in aromatase inhibitors treatment in the B-ABLE Cohort

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Bone density (BMD) loss is a consequence of aromatase inhibitors (AI) treatment of breast cancer. B-ABLE cohort includes 391 postmenopausal women with early breast cancer starting AI therapy. Participants experienced a 1.98% (95% CI 1.54–2.42% $P<0.0001$) bone loss at lumbar spine (LS) and 1.24% (95% CI 0.81–1.67% $P<0.0001$) bone loss at femoral neck (FN) after 1 year on AI therapy and a 3.51% (95% CI 3.00–4.03% $P<0.0001$) bone loss at LS and 2.07% (95% CI 1.51–2.63% $P<0.0001$) bone loss at FN after 2 years. We aim to identify genetic variants associated with BMD loss during AI therapy.
Single nucleotide polymorphisms (SNPs) in genes involved in vitamin D and estrogen pathways were genotyped in the B-ABLE cohort. Multivariate linear regression was performed to test the association between SNPs and LS and FN BMD loss after 1 and 2 years of follow-up. All models were adjusted for age, BMI, tamoxifen, chemotherapy, 25(OH)-VITD and type of AI. $P<0.05$ was considered nominally significant.
Two SNPs in CYP11A1 (rs2959008 and rs7174179) were associated with FN BMD loss at 1 ($P=0.003$ and $P=0.012$) and 2 years ($P=0.004$ and $P=0.002$). For LS BMD loss, SNPs in HSD3B2 (rs2854964), CYP2C19 (rs12248560) and CYP2C9 (rs28371674) were associated at 1 year of follow-up ($P=0.026$, $P=0.019$ and $P=0.011$ respectively). The rs12248560 remained significant at 2 years ($P=0.014$).
The rs11907350 in CYP24A1 was associated with FN BMD loss at 1 year. For LS BMD loss, one SNP in GC (rs11907350) at 1 year ($P=0.020$) and one in VDR (rs2544037) at 2 years ($P=0.024$) reached significant *P*-values. Only the rs7174179 in CYP11A1 for FN BMD loss association at 2 years remained significant after Bonferroni correction.
Conclusion
Several genes in estrogen and vitamin D signaling appeared involved in BMD loss in AI-treated women, suggesting a complex regulation of this outcome.

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## PP193

### MK-7 enhances expression of genes related to bone, enamel and dentin, and reduces the expression of genes related to apoptosis in developing murine molars

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Introduction
The fat-soluble and vitamin K2 homologe Menaquinone-7 (MK-7) is needed for post-translational modification of proteins essential in blood coagulation, and in metabolic pathways in various tissues like bone. Recent studies found an association between long-term anticoagulant treatment (OAC) and reduced bone quality due to reduction of active osteocalcin. OAC is often linked to an undesired soft-tissue calcification in both children and adults and may lead to increased incidence of fractures, reduced bone mineral density/bone mineral content, osteopenia and increased serum levels of undercarboxylated of vitamin K-dependent proteins, known as Gla-proteins. Little is known about the effects of vitamin K2 during tooth development.
Methods
New born Balb C mice were exposed to MK-7 (0.2, 2 or 10 mg/kg body weight) (Kappa Bioscience, Oslo, Norway) using a local s.c. injection on the right side mandibula. The control group was mice injected with vehicle. At 24 h post-injection the pups were sacrificed and first right-hand side molar dissected. Total RNA was isolated from the dissected molar using the RNeasy Mini Kit and used for analysis of gene expression using deoxyoligonucleotide microarrays and RT-PCR. Biological triplicates were used. Microarray results were validated by RT-PCR. Bioinformatic analysis was performed using Ingenuity Pathways Analysis. The results are based on measurements from biological triplicates.
Results
After Treatment with 10 mg/kg body-weight MK-7, 629 genes showed altered gene expression ($P<0.05$) compared to controll with the molecular and cellular functions: carbohydrate metabolism, Cell-to cell-signaling/interaction and cellular growth and proliferation. Treatment with 0.2 and 2 mg/kg body weight MK-7 influenced the expression of genes associated with ' cell death', with a highly significant association to decreased apoptosis.
Conclusions
A clear effect on gene expression in the developing tooth germ was apparent after 24 h at all dosages. The results indicate increased transcription of genes involved in development of bone (increased biosynthesis of important carbohydrates) and of enamel/dentin, and reduced expression of apoptosis related proteins.

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## PP194

### Analysis of genetic polymorphisms in relation to bone mineral density and fracture risk in maltese postmenopausal women

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Background
Osteoporosis is a hereditary multifactorial disease characterised by low bone mass leading to an increased susceptibility to fracture. Bone mineral density (BMD) is the most widely used predictor of fracture risk. Gene variants have been found associated with a low BMD and increased fracture risk; nonetheless studies have identified the relationship between susceptibility genes and fractures independent of BMD.
Objective
Eight single nucleotide polymorphisms (SNPs) within four candidate genes were investigated for their effect on BMD at different anatomical sites and with different low-trauma fractures.
Methods
One thousand and forty-five maltese postmenopausal women were recruited and BMD measurements were performed by dual-energy X-ray absorptiometry. Women who suffered low-trauma fractures were classified as cases whereas subjects without a fracture history were included as controls. Informed consent was obtained from all participants. Genotyping was performed by PCR followed by restriction fragment length polymorphism, and RT PCR high resolution melt.
Results
Using logistic regression analysis adjusted for age, three SNPs in three genes (LRP5 (rs3736228), RANK (rs3018362) and OPG (rs2073618)) were found associated with a low BMD and increased risk of all-type of low trauma fractures ($P<0.05$). SNPs rs3736228 and rs3018362 were associated with reduced BMD at the spine and femoral neck, whereas rs2073618 was only linked to low spine BMD. Three SNPs in the OPG gene (rs3134069, rs3102735 and rs2062377) were associated with an increased fracture risk that conversely did not affect BMD. The haplotype carrying the risk alleles for rs3736228, rs3018362, rs3134069, rs3102735 and rs2062377 was associated with increased fracture risk (permutated $P$-value $= 0.01$) as opposed to the haplotype reference which was strongly linked to a high BMD and low fracture risk (permutated $P$-value $=0.0001$).
Conclusion
Results from this independent replication study indicate that a number of gene variants are associated with reduced BMD and/or increased fracture susceptibility in maltese postmenopausal women.

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## PP195

### Interactions between the effects of polymorphisms in the RANK and RANKL genes affects bone mass

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Osteoporosis is a common disorder with a partly genetic pathogenesis. Interaction between RANKL and its receptor RANK is essential in bone remodeling.
We therefore investigated the effect of polymorphisms in the *RANK* and *RANKL* genes and interaction between the effects on bone mineral density (BMD) and vertebral fractures.
The study was a case-control study with 462 osteoporotic patients and 336 controls. Ten polymorphisms in *RANK* and seven in *RANKL* were selected for genotyping. We genotyped using Taqman or sequencing and examined BMD by DXA. We examined interaction of polymorphisms on BMD or vertebral fractures using the software FAMHAP and performed other statistical analyses using SPSS.
None of the polymorphisms affected BMD or fracture risk. Interaction analyses revealed interaction between the effects of *RANK* polymorphism rs9653064 and *RANKL* polymorphisms rs2277439, rs2875459, rs922996, and rs1054016 on lumbar spine BMD (global $P<0.1$ for all). Interaction was also found between the effects of *RANK* polymorphism rs56231704 and *RANKL* polymorphisms rs2277439 on lumbar spine-, femoral neck, and total hip BMD, rs922996 and rs1054016 on lumbar spine BMD, and rs56231704 on total hip BMD (global $P<0.1$ for all). Subsequent analyses of the effect of *RANKL* polymorphisms on BMD were stratified for *RANK* genotypes and revealed several interactions between polymorphisms in the two genes, for example that BMD was higher at all sites in individuals homozygous for the normal allele at *RANK* rs9653064 and carrying the variant allele at *RANKL* rs2277439 compared with individuals homozygous for the normal allele at both polymorphisms, whereas BMD was lower at all sites in individuals carrying the variant allele at both polymorphisms compared with individuals carrying the variant allele at *RANK* rs9653064 and homozygous for the normal allele at *RANKL* rs2277439 ($P<0.05$).
This study shows that RANK and RANKL interact at the DNA level as at the protein level.

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## PP196

### Association of methylenetetrahydrofolate reductase (MTHFR) polymorphism (C677T) with clinical indicators of osteoporosis in postmenopausal Slovak women

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Objective
The enzyme methylenetetrahydrofolate reductase (MTHFR) is known to play an important role in the removal of circulating homocysteine *via* the methionine cycle. C677T polymorphism is associated with higher plasma homocysteine levels, which could affect collagen maturation. The aim of the present study was to examine possible associations of C677T polymorphism in the MTHFR gene with a variability of femoral (F-BMD), spinal BMD (S-BMD) together with circulating alkaline phosphatase (ALP), osteocalcin (OC; formation markers), beta-CrossLaps (CTx; resorption marker) and fracture incidence in 334 Slovak postmenopausal women.

Material and Methods
Postmenopausal women (62.70 ± 0.53 years) were selected according to strict inclusion criteria. Genetic polymorphism was detected by PCR-RFLP method. Genotype frequencies and frequencies of fractures were tested using the chi-square test. The differences of quantitative variables between the genotypes were analyzed by covariance analysis (GLM procedure) after correction of the measurements for age and BMI.
Results
The prevalence of each genotype was 46.41, 43.11 and 10.48% for CC, TC, and TT genotypes respectively. We reported a statistically significant effect of MTHFR/TT genotype on F-BMD ($P < 0.05$). Individuals carrying TT genotype disposed significantly lowest *T*-score values in comparison with other genotypes. Similarly, spinal BMD ($P = 0.074$) values were decreased in subjects with TT genotype but nonsignificantly. We also found that TT genotype had the highest concentrations of all analyzed bone turnover markers (ALP, OC, CTx), which could indicate a trend of increased remodelation rate in this group. Comparison of fracture incidence between the genotype groups also showed no significant differences for the polymorphisms.
Conclusion
Our results suggest that MTHFR/C677T polymorphism could contribute to the genetic regulation of BMD or bone turnover markers in population of Slovak postmenopausal women. All procedures were approved by the Ethical Committee of the Specialized Hospital of St. Svorad in Nitra (Slovakia).

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## Muscle, physical activity and bone

### PP197

**Girls with Turner syndrome have normal muscle force but decreased muscle power**

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Background
Turner syndrome (TS) is associated with decreased bone mineral density and altered bone geometry, which is assumed a risk factor leading to increased fracture rate. Although hypogonadism or SHOX gene haploinsufficiency are the probable causes, the exact mechanism remains unclarified. Particularly, the muscle function as an important determinant of bone strength has yet not been focused on in TS.
Objective
We tested the hypothesis that there is muscle dysfunction in TS. Secondary aim was to describe the influence of pubertal stage, hormone therapy, fracture history and genotype.
Design and setting
A cross-sectional study was conducted in a single university hospital referral center between March and October 2013.
Patients and methods
All TS patients consenting to the study and having no other chronic disease were included (60 patients, age 13.7 ± 4.5 years). Age- and weight-specific *z*-scores of muscle parameters were calculated based on control group of 432 healthy girls. Leonardo Mechanograph® Ground Reaction Force Platform was used to assess muscle force ($F_{max}$) by the multiple one-legged hoping test and muscle power ($P_{max}$) by the single two-legged jump test. Muscle functions were related to body weight ($F_{max}$/BW) and body mass ($P_{max}$/mass) respectively.
Results
While $F_{max}$ and $F_{max}$/BW were normal (mean weight-specific Z-scores 0.11 ± 0.77, $P = 0.27$, and 0.046 ± 0.62, $P = 0.55$), $P_{max}$ and $P_{max}$/mass were decreased in TS (*Z*-scores -0.93 ± 1.5, $P < 0.001$, and −0.45 ± 0.58, $P < 0.001$), as compared to healthy controls. The muscle functions were not significantly influenced by pubertal stage, hormone therapy, fracture history nor genotype (linear regression, adjusted for age, weight and height, all $P > 0.05$).
Conclusion
$F_{max}$ as a principal determinant of bone strength is normal in TS. The changes in bone quality and structure in TS are therefore not related to inadequate mechanical loading, but rather represent a primary bone deficit. Decreased $P_{max}$ may represent a novel indicator of impaired muscle coordination in TS.

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### PP198

**Efficacy of two exercise programs in patients with early rheumatoid arthritis: 6-month randomized controlled trial**

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Introduction
Physical exercises improve muscle strength, aerobic capacity and bone density in patients with rheumatoid arthritis (RA). However, some physicians fear to recommend the intensive exercises. The aim of the study is to compare the efficacy of two 6-month exercise programs in patients with early RA.
Methods
Fifty-one patients with early RA were randomized into 3 groups. At hospital stage 15 patients underwent ten high-intensity dynamic exercises using gym apparatus Enraf-Nonius for 45–60 min, including aerobic part (En-Cardio) and 18–20 muscle-strengthening exercises (En-Dynamic Track), 18 patients – ten therapeutic exercises for joints for 45 min under the supervision of a trainer. At outpatient stage the exercises lasted for 6 months three times a week. Eighty patients received only drug therapy (control). Tender (TJC) and swollen joint count (SJC), pain on 100 mm VAS, ESR, DAS28, HAQ, RAPID3, the average powers of knee extension and ankle flexion by EN-TreeM movement analysis were evaluated at baseline and at 6 months.
Results
After 6 months in the both exercise groups there were statistically significant differences from the control group in most parameters ($P < 0.05$). Efficacy of the intensive gym exercises was higher than the therapeutic exercises by TJC, HAQ, RAPID3 ($P < 0.05$). After 6 months in the gym group TJC decreased by 62.0%, SJC – by 56.3%, ESR – by 54.8%, pain – by 60.7%, DAS28 – by 0.99 ± 0.14, HAQ – by 0.91 ± 0.33, RAPID3 – by 5.22 ± 1.25, the extension power of a weaker knee increased by 74.7%, the flexion power of a more affected ankle joint – by 71.8% ($P < 0.01$). Most patients, who regularly did exercises, had low disease activity (66.7% in the gym group and 57.1% in the therapeutic exercises group *vs* 36,7% in the control group, $P < 0.05$).
Conclusion
The both exercise programs increase functional status, quality of life and power of motion without detrimental effect on disease activity.

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### PP199

**Anthropometric characteristics of postmenopausal women depending on appendicular skeletal mass**

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The aim of our study was to evaluate the anthropometric characteristics in postmenopausal women depending on appendicular skeletal mass.
Materials and methods
We've examined 8882 women aged 20–89 years (mean age – 56.7 ± 0.14 years; mean height – 162.5 ± 0.07 cm; mean weight – 73.5 ± 0.16 kg), among them anthropometric measures were performed in 79 postmenopausal women aged 40–82 years (mean age – 63.53 ± 1.08 years, mean height – 157.54 ± 0.79 cm, mean weight – 74.75 ± 1.68 kg). Appendicular skeletal mass (ASM) was measured in all four limbs with DXA. We also calculated the appendicular skeletal mass index (ASMI) as ASM/height ($kg/m^2$). During quartile analysis depending on ASMI the examined women were divided on following groups: Q1 – ASMI < 6.38 $kg/m^2$ ($n = 20$), Q2 – ASMI = 6.38–6.83 $kg/m^2$ ($n = 20$), Q3 – ASMI = 6.84–7.36 $kg/m^2$ ($n = 20$), Q4 – ASMI > 7.36 $kg/m^2$ ($n = 19$). Anthropometric characteristics of the women were evaluated by the method of Bunak V.V. (1941) in the modification Shaparenko P.F. (1994). Lean and fat masses were measured by DXA using a densitometer Prodigy, GE. Statistical analysis was performed using the program 'Statistica 6.0'.
Results
Frequency of sarcopenia in women aged 65 years and older was 7%. During quartile analysis depending on ASMI we found that women of Q1 and Q2 groups had significantly lower the following anthropometric characteristics: weight (Q1 – 70.90 kg, Q2 – 70.25 kg, Q3 – 74.75 kg, Q4 – 85.53 kg; F = 5.24; $P = 0.002$) , neck circumference (Q1 – 350 mm, Q2 – 357 mm, Q3 – 376 mm, Q4 – 393 mm; F = 5.68; $P = 0001$), abdomen circumference (Q1 – 846 mm, Q2 – 936 mm, Q3 – 1008 mm, Q4 – 1106 mm; F = 11.52; $P < 0.0001$), shoulder width (Q1 – 903 un., Q2 – 963 un., Q3 – 1029 un., Q4 – 1078 un.; F = 2.22; $P = 0.09$),

narrow tibia circumference (Q1 – 221 mm, Q2 – 227 mm, Q3 – 244 mm, Q4 – 248 mm; F=6.44; $P$=0.0006). We also observed the significantly lower thorax circumference in women of Q1 group (Q1 – 903 mm, Q2 – 963 mm, Q3 – 1029 mm, Q4=−1079 mm; F=3.82; $P$=0.01) in comparison with women of Q4 group (Q1 – 903 mm, Q2 -963 mm, Q3 – 1029 mm, Q4 – 1079 mm; F=3.82; $P$=0.01).

Conclusion

Women with lower ASMI (Q1 and Q2 groups) had the significantly lower following anthropometric characteristics: weight, neck circumference, abdomen circumference, shoulder width, narrow tibia circumference. Thus, we can use the anthropometric measures for determining the groups with the relative risk of sarcopenia and its complications.

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## PP200

**Body composition and bone mineral density in postmenopausal women**

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The aim of the study was to evaluate the peculiarities of body composition in postmenopausal women depending on bone mineral density (BMD).

Materials and methods

We've examined 8882 women aged 20–89 years (mean age – 56.7±0.14 years; mean height – 162.5±0.07 cm; mean weight – 73.5±0.16 kg). Anthropometric measures were performed in 80 postmenopausal women aged 40–82 years (mean age – 63.53±1.08 years, mean height – 157.54±0.79 cm, mean weight – 74.75±1.68 kg), who were divided on the following groups depending on BMD: i) (N) – 32 women with normal BMD, ii) (OSN) – 28 women with osteopenia; iii) (OSP) – 20 women with osteoporosis. Anthropometric characteristics of the women were evaluated by the method of Bunak V.V. (1941) in the modification Shaparenko P.F. (1994). Lean and fat masses, BMD were measured by DXA using a densitometer Prodigy, GE. Statistical analysis was performed using the program 'Statistica 6.0'.

Results

Frequency of sarcopenia in women aged 65 years and older was 7%. We found that women with osteoporosis had significantly lower the following anthropometric characteristics: weight (N – 81.50 kg, OSN – 72.5 kg, OSP – 69.4 kg; F=5.62; $P$=0.005), head circumference (N – 558 mm, OSN – 558 mm, OSP – 541 mm; F=4.59; $P$=0.01), circumference of the forearm widest part (N – 272 mm, OSN – 252 mm, OSP – 246 mm; F=9.41; $P$=0.0002), calf diameter (N – 110 un., OSN – 107 un., OSP – 98 un.; F=3.90; $P$=0.02), shoulder width (N – 89 un., OSN – 82 un., OSP – 80 un.; F=4.09; $P$=0.02), transverse diameter of the chest (N – 310 un., OSN – 292 un., OSP – 278 un.; F=4.69; $P$=0,01). Using DXA we observed that women with osteoporosis had significantly lower lean (N – 43382 g, OSN – 40042 g, OSP – 40702 g; F=3.73; $P$=0.03) and fat (N – 36826 g, OSN – 31160 g, OSP – 27323 g; F=6.03; $P$=0.004) masses.

Conclusion

We have found that women with osteoporosis had significantly lower anthropometric characteristics (weight, head circumference, circumference of the forearm widest part, calf diameter, shoulder width, transverse diameter of the chest) and lean and fat masses in comparison with women with normal BMD. Thus, we can use the received results for form the 'anthropometric portrait' of women suffering from osteoporosis and sarcopenia.

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## PP201

**Prevalence of 'dysmobility syndrome' in community dwelling older adults: findings from the Hertfordshire Cohort Study**

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Sarcopenia and osteoporosis are common in later life, often coexist, and increase the risk of adverse health outcomes such as disability, falls and fracture. Binkley and colleagues recently devised a score-based system for the diagnosis of 'dysmobility syndrome' in an attempt to combine adverse musculoskeletal phenotypes and indentify older individuals at particular risk. We applied these criteria to a larger cohort of participants from the Hertfordshire Cohort Study (HCS) to define dysmobility prevalence in this unselected cohort of UK community dwelling older adults.

Dysmobility syndrome was defined as three or more of low appendicular lean mass ratio, low grip strength, low gait speed, low leg mass:fat mass ratio, osteoporosis, and fall in the last year. Body composition and BMD were measured using dual-energy X-ray absorptiometry (DXA), gait speed was determined by 3 m walk test and grip strength was assessed with a Jamar hand-held dynamometer. Participants completed a questionnaire detailing self-reported falls and fracture history.

Data were available from 156 men and 142 women. The mean age of participants was 76.1 (s.d. 2.56) years with a mean BMI of 27.4 (s.d. 4.08) kg/m$^2$. The prevalence of sarcopenia in this cohort was 7 and 8.3% using the European Working Group on Sarcopenia in Older People (EWGSOP) and International Working Group on Sarcopenia (international) definitions respectively. Dysmobility syndrome was more common in women than in men (33.10 and 17.31% respectively, $P$<0.05). While dysmobility was more prevalent in overweight or obese subjects than normal weight individuals, these trends were statistically non-significant. As expected those with dysmobility reported significantly higher number of falls (last year and ever) ($P$<0.01) than counterparts without dysmobility, but no increased fracture rate was observed in the dysmobility group ($P$=0.96).

Dysmobility syndrome is common in UK community dwelling older individuals, with higher rates observed in women than men.

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## PP202

**Vertical trabeculae are thinned more rapidly than horizontal trabeculae in skeletal unloaded rats**

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Most studies using botulinum toxin A (BTX) to achieve skeletal unloading use a study period of 3–4 weeks. The aim was to prolong this study time and to investigate the influence on the bone microstructure including the relationship between the thickness of horizontal and vertical trabeculae. Fifty-seven 16-week-old female Wistar rats were randomized into six Groups: Base; Ctrl 4w; and BTX4 4w; Ctrl 8w; BTX4 8w; BTX4+2 8w. The BTX animals were injected with 4 IU at baseline or 4 IU at baseline and 2 IU again after 4 weeks. The animals were killed after 0, 4, or 8 weeks. The distal femoral metaphysis was μCT scanned at a resolution of 6 μm and the bone strength was ascertained. After 4 weeks there was a significant loss of BV/TV (BTX4 4w: −24%) and of Tb.Th* (BTX4 4w: −11%). After 8 weeks there was a significant loss of BV/TV (BTX4 8w: −21% and BTX4+2 8w: −13%) and of Tb.Th* (BTX4 8w: −13% and BTX4+2 8w: −12%). After 4 weeks the horizontal:vertical trabecular thickness ratio Tb.Th.horz/Tb.Th.vert did not differ between immobilized and control animals, while after 8 weeks it was significantly higher in the immobilized animals (Ctrl 8w: 0.93±0.009, BTX4 8w: 0.97±0.018 and BTX4+2 8w: 0.98±0.013) indicating a more rapid thinning of the vertical trabeculae. Thus, the load-bearing vertical trabeculae at the distal femoral metaphysis are more susceptible to skeletal unloading than the horizontal trabeculae. There was a significant loss of bone strength after 4 weeks (BTX4 4w: −19%) and after 8 weeks (BTX4 8w: −22% and BTX4+2 8w: −17%).

Conclusion

No additional BTX is needed to extend the study period from 4 to 8 weeks. Horizontal trabeculae are thinned more rapidly than vertical trabeculae during disuse in rats. The experiment was approved by the Danish Animal Experiments Inspectorate.

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## PP203

**Sarcopenic obesity worsens bone strength: hip strength analysis in post-menopausal women**

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Background
Among body composition phenotypes there is no consensus on definition and consequences of sarcopenic obese phenotype. The recommended method to quantify the muscle mass is the dual energy X-ray absorptiometry (DXA), based on measurement of total fat mass and appendicular lean mass. A high BMI may provide a great bone mineral density (BMD) in many individuals, but it does not guarantee optimal bone strength. The aim of our study is to investigate the influence of sarcopenic obesity on hip bone strength indices: femoral strength index (FSI), Cross-Sectional Moment of Inertia (CSMI), cross-sectional area (CSA), section modulus (Z) and buckling ratio (BR).
Methods
In this retrospective case–control study, participants were recruited among patients who were assessed for osteoporosis, using DXA method, from January 2011 to December 2013. Inclusion criteria were: post-menopausal women aged 50 years or older; BMI $\geq$ 30 kg/m$^2$. We classified all the included patients in sarcopenic obeses and non sarcopenic obeses following Newman's criteria, based on appendicular lean mass adjusted for height and body fat mass (residuals). We performed a hip structural analysis (HSA) from hip DXA images to measure FSI, CSMI, CSA, Z and BR.
Results
We evaluated 127 women mean aged 63.50 years $\pm$ 8.69 S.D. (min. 50 years and max. 84 years) with a mean BMI of 34.27 kg/m$^2$ $\pm$ 4.01 S.D. (min. 30.04 and max. 53.97). Forty-five sarcopenic obeses patients (35.43%) had a mean FSI of 1.17 $\pm$ 0.33 S.D., a mean CSMI of 9613 mm$^4$ $\pm$ 2403 S.D., a mean CSA of 134 mm$^2$ $\pm$ 23 S.D., a mean Z of 556 mm$^3$ $\pm$ 113 S.D. and a mean BR of 9.11 $\pm$ 4.23 S.D. Eighty-two non sarcopenic obeses (64.57%) had a mean FSI of 1.30 $\pm$ 0.30 S.D., a mean CSMI of 9886 mm$^4$ $\pm$ 1970 S.D. and a mean CSA of 135 mm$^2$ $\pm$ 21 S.D., a mean Z of 571 mm$^3$ $\pm$ 102 S.D. and a mean BR of 7.11 $\pm$ 2.67 S.D.
Conclusions
In our cohort of post-menopausal women, sarcopenic obeses had worse bone quality and strength.

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## PP204

**Sarcopenia in patients scheduled to undergo orthopaedic surgery**

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Purpose
The purpose of the present study was to characterize the extent of sarcopenia among orthopaedic surgery patients.
Methods
This retrospective cross-sectional study identified 222 patients immediately after or before an orthopaedic surgery including elective total hip and knee arthroplasty and hip fracture surgery at a single institution. 364 patients from an outpatient department who did not have an orthopaedic surgery were also examined as a control group. We checked whole-body dual energy X-ray absorptiometry to analyze body composition. Sarcopenia was classified into class I defined relative skeletal muscle mass loss within 1–2 S.D. of the gender-specific mean for healthy young adults and class II below 2 S.D.
Results
The prevalence of class II sarcopenia in patients undergoing orthopaedic surgery was 25.7% by height-adjusted definition and 44.1% by weight-adjusted definition. The prevalence in patients from outpatient department was 6 and 33.2% respectively which was significantly smaller than surgery group ($P < 0.0001$ and $P = 0.011$). The highest rates of sarcopenia with height-adjusted definition were seen in patients who were diagnosed as femur neck fracture. In multivariate analysis, female sex, older age, and lower BMI were associated with the presence of class II sarcopenia adjusted by height (OR 27.588, $P < 0.0001$; OR 1.035, $P = 0.024$; and OR 0.542, $P < 0.0001$ respectively).
Conclusion
Present study showed a higher prevalence of sarcopenia in individuals undergoing orthopaedic surgeries.

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## PP205

**Volumetric bone mineral density at the distal radius in premenopausal Spanish women and grip strength: role of calcium intake**

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Peripheral computed tomography (pQCT) can estimate volumetric bone mineral density (vBMD) and distinguish trabecular from cortical bone. Few comprehensive studies have examined correlates of vBMD in Spanish premenopausal women.
The aim was to study the association between bone microarchitecture, muscle mass and strength in premenopausal Spanish women and to evaluate the role of calcium intake. vBMD was assessed in 164 premenopausal women at the distal radius by pQCT. BMD was assessed by dual-energy X-ray absorptiometry (DXA). Grip strength in the left arm was measured by dynamometry and lean mass in the left arm was assessed by electric bioimpedance. Calcium intake was quantified using a dietetic scale.
Age was not correlated with vBMD in our sample. Based on the *T*-score WHO criteria, women were classified as healthy. Women were further classified according to the grip strength quartile (highest quartile $>$ 22.03 N ($n = 115$) and lower quartile $<$ 15.83 N ($n = 49$)). There were no differences in age and BMI between groups (44.04 $\pm$ 4.51 vs 42.52 $\pm$ 6.36 years and 25.03 $\pm$ 3.65 vs 25.45 $\pm$ 3.76 kg/m$^2$ respectively ($P > 0.05$ in both cases)). In multivariable models, there were no difference in the measurements of vBMD and BMD among women in the two groups. Bone area measurements were higher in women within the highest quartile vs women in the lower quartile of grip strength ($P < 0.05$ for total, trabecular and subcortical areas). In the highest quartile group, calcium intake correlated (partial correlation) with total vBMD after adjustment for age, weight, height and lean mass in the left arm (kg) ($r = 0.198$; $P < 0.05$). No correlations of calcium intake with the vBMD were found in the lowest quartile group. No partial correlations were found with the trabecular or the cortical density in both groups ($P > 0.05$).
In conclusion, in premenopausal Spanish women, we speculate that a high grip strength associates with greater bone size additionally, an increase in the vBMD might be calcium intake dependent after adjustment for potential confounders. Further investigations are needed to evaluate if physical performance evaluation may help with osteoporosis prevention when bone density scores have not been obtained or are unavailable.

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## PP206

**Influence of sarcopenic obesity on osteoporosis and vertebral fragility fractures in post-menopausal women**

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Background
Sarcopenic obesity is usually defined by the combination of sarcopenia and obesity but there is not a standard definition yet. The recommended method for the quantification of muscle mass is dual energy X-ray absorptiometry (DXA), based on assessment of total fat mass and appendicular lean mass. Low Body Mass Index (BMI) used to be considered as a risk factor for fragility fracture. However, recent evidences have demonstrated a correlation between BMI and vertebral fractures in post-menopausal women. The aim of our study is to evaluate the influence of sarcopenic obesity on osteoporosis and vertebral fragility fractures in post-menopausal women.
Methods
In our retrospective case–control study, participants were recruited among post-menopausal women aged 50 years or older with a BMI $\geq$ 30 kg/m$^2$ from January 2011 through December 2013. According to Newman *et al*. definition, based on appendicular lean mass adjusted for height and body fat mass (residuals), these

patients were classified in two groups: sarcopenic obeses and non sarcopenic obeses. We evaluated bone mineral density at lumbar spine, femoral neck and total body DXA scans. Vertebral fragility fractures were identified using the technique of lateral vertebral assessment (LVA) of DXA scan.

Results

We evaluated 133 women mean aged 63.71 years ± 8.59 (min. 50 years and max. 84 years) with a mean BMI of 34.31 ± 3.96 kg/m$^2$ (min. 30.04 and max. 53.97). Forty-seven patients (35.33%) of our population had sarcopenic obesity, 19 of these patients (40.43%) were osteoporotic and 15 (31.91%) had a vertebral fracture: 8 (17.02%) with a single vertebral fracture and 7 (14.89%) with multiple vertebral fractures. Sarcopenic obesity was associated with a higher risk of osteoporosis (odds ratio, (OR) 1.20; 95% Confidence Interval, CI 0.25 0.58) and a higher risk of vertebral fractures (OR 1.21; 95% CI 0.56–2.62).

Conclusions

In our cohort of post-menopausal women, sarcopenic obesity was associated with a higher risk of osteoporosis and vertebral fragility fractures.

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## PP207

**Epidemiology of hip fractures in over 50 years old in merida venezuela 2008–2011**

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Method

The aim of this retrospectively study was to ascertain trends in the incidence of hip fracture, resulting from traumas of low intensity (fall of same high), in age over 50 years old, using register information from University Hospital of Los Andes (Merida Venezuela) theatre records. There was a total of 404 fractures of the proximal femur, which affected 293 women's and 111 men's, the overall female:male ratio was 2.64:1. The average standardized incidence per 100 000 population per year was estimated.

Results

The mean standardized female incidence was 68.4/100 000 and male's 47.0/100 000 inhabitant. Trochanteric fractures affect 73% of patients and the femoral neck fractures 27% of the patients (ratio 2.7:1). The case numbers increases sixfold, exponentially with age groups between 50 and more than 80 years, to go from 36 to 204 cases, and when we analyzed the incidence by the aged population is more evident to female trochanter fractures. According sex trochanteric fractures affects more women in a ratio of 2.5:1 and in the same way to the neck cervical 3.0:1. According to age, trochanteric fractures in women with a mean age of 80.8 and 74.8 years for men and femoral neck fractures 78.8 and 73.7 years respectively. If the stability criteria are used, stable fractures occurred in 30% of cases and unstable fractures in 70% of them.

Conclusions

In Merida (Venezuela) the hip fractures in older 50 years, affect more ladies (2.64:1). Mean age 78.7 years old. Between 50 and more than 80 years old the hip fractures increase sixfold, specially the female trochanteric fractures.

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## PP208

**A new functional test for the evaluation of the erector muscles of the spine: the Back Extensor Test (BET)**

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Vertebral fractures are one of the most common and *disabling* manifestation in osteoporosis. The relationship between bone mass and muscle strength has already been shown in the literature, but only a few studies argues the relationship between BMD and erector spinae muscles. Simple biomechanical considerations suggest that the loss of trunk muscle mass may contribute in vertebral fractures. In the literature, the Soerensen and isokinetic tests are often reported for the evaluation of the spine extensors strength, but their there are doubts about safety, usability.

The BET (Back Extensor Test) is a test, created in 2005. The protocol execution has been standardized: it consists in flexing your fully extended torso forward with an angle of 45° with the thighs and in measuring, in seconds, the time that the patients can maintain that position. The test ends when the patient reports pain and when he flexes the torso more than 10°. The extensor muscles are in anti-gravitary position, and are evaluated without hazards for osteoporotic patients.

To validate the BET different studies have been performed in the last 8 years. The subjects under investigation (229) were volontary subjects randomly selected (in several sports clubs, at the S.Paolo Hospital Osteoporosis clinic, at the S. Paolo Hospital volunteers AVO) and divided in three categories: healthy, low back pain and osteoporosis.

We found good reproducibility but the variability interoperator, even thought seems to be statistically acceptable ($P = 0.002$), needs further study. Good sensitivity and significance ($P < 0.001$) and statistically correlations with other common tests (Shoeber, Owestry, VAS, Back Pain Function Test) were found.

The BET proved to be a good predictor to identifying pathologies of the spine and also a good indicator of athletic training ($P < 0.01$). We demonstrated a correlation between time and the BET area of the multifidus sonographically determined ($P < 0.001$).

Future developments concern more studies of BET in osteoporotic patients and the resolution of some BIAS emerged regarding the interoperator reproducibility.

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## PP209

**Does bone density, bone strength, sarcopenia or dynapenia explain greater risk of fracture in obesity?**

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Obesity is associated with greater risk of proximal humerus and ankle fracture, despite greater areal BMD (aBMD). We aimed to investigate whether greater risk of some fractures in obesity was due to skeletal or non-skeletal determinants. 100 individually-matched pairs of normal weight (NW) (18.5–24.9 kg/m$^2$) and obese (OB) (BMI $>30$ kg/m$^2$) individuals, aged 25–40 and 55–75 years underwent DXA to determine hip and lumbar spine aBMD, whole body lean mass (WBLM) and appendicular lean mass (ALM). We used HR-pQCT to determine volumetric BMD (vBMD) at the distal radius and tibia and micro-finite element analysis to estimate bone strength. Falls in the 6 months prior to recruitment were reported by questionnaire. Grip strength was measured with a dynometer. Short physical performance battery (SPPB) score was derived from gait speed, balance (from 6 m narrow walk), and repeated chair-stand time quartile. OB had greater hip and spine aBMD, tibia and radius vBMD (all $P < 0.001$) and estimated failure load (Table 1). 15% of OB reported having fallen, compared to 5% of NW ($P = 0.07$). OB had greater WBLM, ALM and skeletal muscle index (all $P < 0.001$). Despite greater LM, SPPB score was poorer in OB, due to slower gait speed ($P < 0.001$), slower chair-stand time ($P < 0.001$) and poorer balance ($P < 0.01$). There was no difference in grip strength between OB and NW. Obese individuals have higher BMD at all sites, so excess of some fractures in obesity may not be due to lower bone strength. Obesity appears protective against sarcopenia due to greater LM, but physical function is impaired. Poor physical performance in obesity could contribute to greater risk of falls and some fractures.

**Table 1** Comparison of estimated failure load, skeletal muscle index and SPBB scores between normal weight and obese individuals.

| | NW | OB |
|---|---|---|
| Radius estimated failure load, kN | 4.13 (1.2) | 4.79 (1.2)*** |
| Tibia estimated failure load, kN | 11.14 (2.6) | 12.71 (2.6)*** |
| Skeletal muscle index, kg/m$^2$ | 7.03 (1.1) | 9.01 (1.3)*** |
| SPPB score | 8.29 (2.2) | 6.51 (2.3)*** |

Significant at: ***$P < 0.001$ mean (s.d.)

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## PP210

**No beneficial effects of vitamin D supplementation in muscle function or quality of life in primary hyperparathyroidism: results from a randomized controlled trial**

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Context
Impairment of muscle function and strength in patients with primary hyperparathyroidism (PHPT) is rarely addressed although decreased muscle function may contribute to increased fracture risk in PHPT.
Objective
We aimed to assess changes in muscle strength, muscle function, postural balance, quality of life (QoL), and well-being during treatment with vitamin D or placebo before and after parathyroidectomy (PTX) in PHPT patients.
Design and setting
Randomized placebo controlled trial at a single center.
Patients
We included 46 PHPT patients, mean age 58 (range 29–77) years and 35 (76%) were women.
Interventions
Daily treatment with 70 µg (2800 IU) cholecalciferol or placebo for 52 weeks. Treatment was administered 26 weeks prior to PTX and continued for 26 weeks after PTX.
Main outcome measures
Changes in QoL and measures of muscle strength and function.
Results
During 26 preoperative weeks of medical treatment, we did not measure any beneficial effects of supplementation with vitamin D compared with placebo regarding QoL, postural balance, muscle strength, or function. In the entire group of patients, we measured marked improvements in QoL, well-being ($P<0.01$), muscle strength in the knee flexion and extension ($P<0.001$), and muscle function tests ($P<0.01$) after surgical cure by PTX. Postural balance improved during standing with eyes closed ($P<0.05$), whereas patients had a decreased stability with eyes open ($P<0.05$).
Conclusions
Patients with PHPT and vitamin D insufficiency did not benefit from vitamin D supplementation concerning muscle strength, muscle function, postural balance, or quality of life. Independent of preoperative vitamin D levels, PTX improved these parameters.

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## PP211

**The peroxisome proliferator-activated receptor α agonist fenofibrate improves the effect of high-impact exercise on bone and muscle mass in ovariectomized rats**

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Estrogen deficiency promotes bone loss and skeletal muscle dysfunction. Peroxisome proliferator-activated receptor (PPAR) agonists and high-impact exercise both affect musculoskeletal properties. Whereas PPARδ agonists ameliorate training response in muscle, PPARα agonists exert beneficial effects in bone and muscle. In the present study we investigated the effects of the PPARα agonist fenofibrate and high-impact jumping exercise alone and combined in ovariectomized (OVX) rats. Female Sprague–Dawley rats, 12 weeks of age, were allocated to a sham-operated group (SHAM), and four OVX groups: fenofibrate (OVX-Fen), exercise (OVX-Ex), combined fenofibrate and exercise (OVX-FenEx) and a control group (OVX-Ctr), respectively ($n=12$ per group). Fenofibrate (90 mg/kg per day) or vehicle (methylcellulose) were given by gavage for eight weeks. Whole body, spine and femur bone mineral density (BMD), and body composition were assessed by dual X-ray absorptiometry (DXA). Trabecular microarchitecture was analyzed using micro-computed tomography, and bone metabolism markers in plasma by immunoassays. Femoral BMD increased more in all intervention groups relative to OVX-Ctr ($P<0.01$), and was also normalized in the OVX-Ex and OVX-FenEx groups relative to SHAM. The BMD gain at the femur was higher in the OVX-FenEx group than in OVX-Fen and SHAM (7.4 vs 2.4 and 2.7%, $P<0.05$). Trabecular number was higher in the OVX-FenEx group relative to OVX-Ctr and OVX-Fen (1.79 vs 1.15 and 1.26 1/mm, $P<0.05$), while trabecular separation was lower in the OVX-FenEx group compared to OVX-Ctr (0.44 vs 0.63 mm, $P<0.05$). Higher levels of bone formation marker in plasma were observed in all intervention groups compared to OVX-Ctr ($P<0.05$). After the intervention, lean mass was maintained at sham level in the OVX-FenEx group, whereas all other groups had lower lean mass than SHAM ($P<0.05$). In conclusion, combination of fenofibrate and jumping exercise seems to increase femoral BMD more than each intervention alone, while preserving lean mass in OVX rats.

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## PP212

**New insights in the bone-muscle axis: the novel myokine irisin is involved in skeletal metabolism**

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It has been recently reported that, after physical exercise activity, the skeletal muscle releases Irisin, the newly identified myokine able of driving transition of white adipocytes to brown, following a phenomenon known as the browning response. This result has suggested that skeletal muscle is crucial in the regulation of energy homeostasis, supporting the idea that it can be considered an endocrine organ that targets adipose tissue by promoting energy expenditure. In accordance with these new findings, we hypothesized that a direct involvement of Irisin in bone metabolism could also exist, demonstrating its ability to enhance differentiation of bone marrow stromal cells into mature osteoblasts (OBs) by increasing the expression of alkaline phosphatase and collagen I.
In view of further proving the involvement of Irisin in bone metabolism, we validate its direct effect on OBs by using recombinant Irisin (r-Irisin). Phosphorylation of MAPK ERK and expression of Atf 4 ($P<0.001$), the key trascription factor of OB differentiation, were significantly increased after Irisin treatment. Furthermore, ALP and pro-collagen I mRNA resulted up regulated ($P<0.001$), as we already demonstrated by treating OBs with conditioned medium from primary myoblasts of exercised mice.
To recapitulate *in vivo* the effect of physical exercise, with regard to the specific action of the myokine Irisin on the skeleton, we injected mice with r-Irisin. Here we show that BV/TV of Irisin-treated mice was higher than vehicle-injected mice and this positive effect on bone was also confirmed by reduction of RANK-L serum concentration in treated mice that, given the unchanged OPG levels, resulted in a decreased RANK-L:OPG ratio.
These data demonstrate for the first time that Irisin could be a new anabolic molecule to the bone and add new evidence to the positive effect on skeletal homeostasis exerted by physical exercise, highlighting a novel link in the bone–muscle axis.

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## Osteoporosis: evaluation and imaging

### PP213

**HIV patients have deteriorated bone material properties assessed by *in vivo* microindentation**

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There is a growing evidence of the association between HIV infection and fracture risk. Independently of its cause (antiretroviral therapy (ART) or HIV), what remains most important is a prompt diagnosis. Although densitometry is the gold standard, sometimes this technique is not as accurate as necessary in clinical practice. A new validated tool for early and more accurate diagnosis is presented.

Methods

In a HIV group of patients, we analyzed the bone properties by microindentation, studying bone strength *in vivo* in $<5$ min. Its results are comparable and reproducible. Two groups of patients were included: HIV+ (with and without ART), and controls without HIV and no bone disease. ART naïve patients had at least 5 years of HIV diagnosis. Bone material strength (BMS) was measured with osteoprobe applying a 20 N force on the anterior tibia. Age and gender-adjusted ANOVA was used for comparisons.

Results

23 HIV patients (nine on ART and 14 without) and 43 controls were included. Age ($42+9.9$ vs $69+13.4$) and gender (14 men and nine women vs four men and 39 women) for HIV+ and controls respectively. After adjusting by age and gender, HIV+ patients showed worse bone quality (lower BMS) BMS ($79.6+12.7$) than controls ($84.9\pm6.2$, $P=0.013$) (mean$\pm$S.D.). HIV+ patients receiving ART had lower BMS than controls ($76\pm8$) ($P<0.001$ vs controls) and also compared with HIV+ without HAART (BMS $81\pm14$) although the difference was not significant. *T*-scores in lumbar spine ($-1.5+1.7$) and total hip ($-1.2+1.7$) were, on average, in the range of osteopenia in the HIV+ patients. No differences in BMD were observed between HIV+ and controls ($P=0.38$ and $P=0.67$ for lumbar spine and total hip respectively). There was no correlation between BMS and lumbar spine BMD ($R^2=0.002$, $P=0.80$) or total hip BMD ($R^2=0.002$, $P=0.81$). In a subset of four HIV patients with vertebral fractures BMS was $72\pm4$ significantly lower than controls ($P<0.001$) and also than the rest of HIV group $P<0.05$. In this subgroup there were no differences in BMD (lumbar spine) with the rest of HIV group, nor with controls.

Conclusions

Microindentation is a new feasible technique that seems to be superior to BMD to study bone quality, and therefore bone toxicity. HIV infected patients have deterioration in bone quality measured by microindentation and this effect seems more pronounced in those receiving ART.

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### PP214

**Bone marrow densitometry by clinical high resolution computed tomography of human vertebrae**

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Bone mineral density (BMD) as measured by quantitative computed tomography (QCT) is biased by the variable marrow composition since marrow fat reduces the apparent BMD. We developed a marrow densitometry (MD) method that identifies subvolumes in the vertebrae that consist of bone marrow only (bone voids) and determines their average mineral equivalent density values. The method was developed for use with *in vivo* high resolution QCT (HR-QCT). HR-QCT still has limited spatial resolution and thus we aimed to determine the accuracy of our MD method by comparison with HR-pQCT as gold standard using the Scanco Medical XtremeCT device.

11 excised frozen human $T_{10}$ vertebrae analysed within the BioAsset study were scanned using the standard HR-pQCT protocol and by HRQCT (Siemens Somatom, 120 kVp, 360 mAs, 0.6 mm slice thickness and 0.3 mm slice increment). HR-pQCT images were filtered with an anisotropic diffusion filter (Matlab, Natick, MA). Both HR-pQCT and HR-QCT images were binarized to exclude air-voxels. Morphological filters using a binary, sphere as structuring element were applied to find spherical subvolumes of bone voids with increasing radii (0.1–1.2 mm, 0.1 mm increment). Mineral equivalent marrow density (MEMD) within the bone-void regions was estimated for each sphere-radius. A convergent MEMD value was observed for larger radii. The corresponding detected regions in both techniques were co-registered (ITK implementation under Structural Insight, in-house software). BMD and tissue mineral density (TMD) measurements within the same search-region were obtained as well. Significant correlations were obtained between the HR-QCT and HR-pQCT based MEMD: ($R^2=0.42$, RMSE$=9.9$ mg/cc, $P<0.03$). Strong correlations between both techniques were observed for BMD ($R^2=0.97$, RMSE$=3.0$ mg/cc, $P<0.0001$) and TMD of HR-QCT vs HR-pQCT ($R^2=0.86$, RMSE$=12.0$ mg/cc, $P<0.0001$).

Our results demonstrate that marrow composition can be estimated by HR-QCT with a residual error of about 10 mg/cc mineral equivalent density despite the limitations due to partial volume effects. This MD method is applicable to *in vivo* assessments of treatment effects and thus can be used to distinguish between true mineral and apparent changes induced by changes in marrow composition.

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### PP215

**Bone quality in diabetes mellitus type 2**

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Diabetes is associated with increased risk of fracture, although type 2 diabetes mellitus (T2DM) is characterized by normal bone mineral density (BMD). Thus, diabetes may be associated with a reduction of bone strength that is not reflected in the measurement of BMD. It is very problematic to measure the bone quality in daily practice. Trabecular bone score (TBS) iNsight® is one of these tools, now available for routine clinical practice, that allows for refinement of micro architecture and fracture risk, independent of BMD.

Objective

TBS evaluation of increased fracture risk in T2DM vs control group of patient without DM.

Patients and methods

Retrospective study, 56 postmenopausal women patients with T2DM and 61 women patient without DM or IGT. We evaluated the ability of lumbar spine TBS to account the increased risk of fractures in T2DM.

Results

Mean age of the patients with T2DM was $67.5\pm9.1$, mean BMI $29.7\pm6.1$. Diabetes was associated with higher BMD – the *T*-score of LS was $-0.8\pm1.5$ and of the HIP $-0.7\pm1.3$, while in the group without DM *T*-score of LS was $-1.2\pm1.5$, and of the HIP $-1.1\pm1.1$. Fractures occurred in 16.3% patients with T2DM (13.3% in patient without DM, $P<0.05$). The diabetic patient has lower lumbar spine TBS ($1.172\pm0.133$). Least squares mean (LSM) for BMD were significant greater in women with T2DM than without, whereas LSM for TBS-LS was significant lower in T2DM. The adjusted odds ratio for a measurement in the lowest vs highest tertile was $<1$ for BMD (all $P<0.001$), but was increased for TBS-LS (OR 2.41). TBS was a BMD-independent predictor of fracture in those with diabetes.

Conclusion

TBS together with BMD helps to provide better information about real bone status and bone micro architecture in patients with T2DM. TBS is a promising tool to identify patient with T2DM in higher risk of osteoporotic fracture. To identify these patients in daily clinical practice is the basic for effective treatment.

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## PP216

### Correlation between osteoporosis and dental caries in Korean population

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Aging is associated with a loss of systemic bone mass and an increased risk of especially the spine and hip. Therefore bone loss of teeth and ridge resorption can occur in the mouth. However, results of several studies investing association between systemic bone density and oral health have varied. In this study, we investigated the relationship between osteoporosis and dental caries with the Korea National Health and Nutrition Examination Survey (KNHANES) conducted in 2008–2010 in the Korean population.

This cross-sectional study is based on the body composition data and dental examination data acquired in the KNHANES. Among them, we analyzed 1803 women aged over 65 years old selected in all the 16 administrative districts of South Korea. Bone mineral density (BMD) at the spine was measured using dual X-ray absorptiometry. And a dentist conducted a complete oral health examination.

The prevalence of dental caries in osteopenia (27%, $P<0.001$) and osteoporosis groups (26%, $P<0.001$) is higher than the prevalence of dental cries in normal BMD group (14%). Results were adjusted for age, body weight, cigarette smoking, and alcohol. Comparing with normal group, osteopenia and osteoporosis group increased dental caries risk (respectively, odd ratios (ORs)=2.178, 95% CI=1.266–3.747 and ORs=2.075, 95% CI=1.209–3.561). Our results provide evidence of an association between osteoporosis and dental caries in aging women (>65 years). The associations between bone density, and dental health require further research, particularly in longitudinal studies.

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## PP217

### An observational study investigating the prevalence of anaemia in hip fracture patients

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Introduction

The blood loss sustained during hip fracture repair can be significant and is associated with a high-risk of post-operative anaemia. Anaemia may exacerbate chronic cardiac and pulmonary conditions suffered by this aging population leading to increased morbidity and mortality.

Objective

To assess the prevalence and severity of anaemia throughout hospital admission in patients admitted with a fractured neck of femur.

Method

Data for haemoglobin levels of 100 consecutive patient admissions to the acute hip fracture ward in a UK district Hospital (Carmarthen, Wales, UK) were collected from a computer blood system. Patients' haemoglobin levels (g/l) were collected; on admission, post-operatively and pre-discharge. The WHO guidelines for diagnosis of anaemia were used: <120 g/l for females and <130 g/l for males; severe anaemia (<80 g/l), moderate (between 80 and 100 g/l), mild (below normal range but >100 g/l).

Results

Sample: 100 hip fracture patients, 65% females. Average age: 80 years. The table shows the prevalence of anaemia. Average haemoglobin reductions of 26.4 g/l post-operatively and 12.9 g/l on discharge were recorded compared to admission. At discharge, 65 patients had a haemoglobin drop of at least 10 g/l compared to their admission level, 35 suffered a 20 g/l reduction or greater.

| Degree of anaemia | On admission | Post operatively | On discharge |
|---|---|---|---|
| Mild | 41 | 33 | 55 |
| Moderate | 9 | 46 | 24 |
| Severe | 0 | 15 | 0 |

Conclusions

Anaemia on admission is common in hip fracture patients. Post operative anaemia following hip fracture repair is common, especially moderate anaemia. At time of discharge, high percentages of patients remain anaemic with some suffering substantial reductions in their haemoglobin level compared to admission. Further analysis to assess the effect of anaemia on morbidity, mortality and length of stay is required.

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## PP218

### Sacral insufficiency fracture associated with osteoporotic spinal compression fracture: analysis of incidence and clinical factors

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Sacral insufficiency fracture is known as a common cause of lumbosacral pain developed without evident trauma in patients with osteoporotic spinal compression fracture. The purpose of study was to investigate the incidence and predisposing factors of sacral insufficiency fracture accompanied by osteoporotic spinal compression fracture.

We carried out a retrospective study of 949 patients who were measured bone mineral densitometry (BMD) and showed osteoporotic spinal compression fracture on MRI between January 2008 and December 2012. Sacral insufficiency fracture was diagnosed by MRI and whole body bone scan. We analyzed the correlations between sacral insufficiency fracture and demographic, clinical factors such as sex, age, BMI, BMD, comorbidities (hypertension, diabetes, rheumatoid arthritis, and thyroid disease), the number of osteoporotic spinal compression fracture.

Among 949 patients with osteoporotic spinal compression fracture (80 (8%) males and 869 (92%) females), 40 (4.2%) had sacral insufficiency fractures (7 (17.5%) males and 33 (82.5%) females). There were significantly differences in age, the number of compression fracture and the osteoporosis severity between the groups. Mean age was significantly different between the patient groups with or without sacral insufficiency fracture (71.0 vs 65.6 years; $P<0.05$). The mean number of osteoporotic spinal compression fracture was significantly different between the patient groups with or without sacral insufficiency fracture (2.75 vs 1.87; $P<0.05$). The severity of osteoporosis was significantly different between the patient groups with or without sacral insufficiency fracture($-3.05$ vs $-2.19$; $P<0.05$).

Sacral insufficiency fracture should be assessed carefully in patients with lumbosacral pain. High index of suspicion is needed for old age, multilevel osteoporotic spinal compression fractures, and severe osteoporosis.

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## PP219

### Experimental glucocorticoid-induced bone loss in mice is strongly influenced by the strain

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Glucocorticoid (GC)-induced osteoporosis (GIO) occurs in patients undergoing therapeutic intervention for inflammatory disorders such as rheumatoid arthritis and lupus. The effect of long-term GC administration on bone turnover was investigated in two frequently used mouse strains; C57BL/6 and CD1 in order to assess the influence of genetic background.

GIO was induced in 12 weeks old female C57BL/6 and CD1 mice by s.c. insertion of long-term (60 days) release prednisolone or placebo pellets. Animal care and experimental procedures were approved and conducted in accordance with the Home Office Animals Act of 1986 (UK).
Biomechanical properties of the long bones as assessed by three point bent testing revealed that femoral elasticity and strength significantly decreased in CD1 mice receiving CG, whereas C57BL/6 mice showed no differences between placebo and prednisolone treatment.
Bone turnover was assessed by microcomputer tomography (micro-CT). Contrary to C57BL/6 mice, prednisolone treated CD1 mice developed osteoporosis and presented reduced trabecular bone volume and trabecular number. Serum carboxy-terminal telopeptide of type I collagen (CTX-I) measured by ELISA validated the effects of GC on 12 weeks old CD1 mice that had significant bone degradation, whereas bone turnover was not affected by long term GC administration in 12 weeks old C57BL/6 mice.
While administration of long term release prednisolone pellets provides a robust GIO animal model in 12 weeks old CD1 mouse strain, 12 weeks old C57BL/6 mice were not susceptible to GIO due to long term prednisolone administration. This study indicates that for the induction of experimental GIO, the mouse strain choice should be carefully evaluated.

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## PP220

**The fracture outpatient clinic: what is the additional value of vertebral assessments and which individuals should be more actively recruited?**

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Fracture outpatient (FO) clinics aim to identify individuals at high fracture risk. Identification of individuals at high fracture risk has been improved since the introduction of vertebral fracture assessment (VFA). Unfortunately, participation rates in FO clinics are often low.
The aim of this study is to i) assess the contributory value of VFA in addition to BMD measurements in identifying individuals with high fracture risk; ii) assess characteristics of individuals with as compared to those without vertebral fractures; and iii) determine characteristics related to non-participation to the FO clinic.
Data from the FO clinic of the VU University Medical Center were used. Individuals aged 50 years and over who presented with a fracture at the emergency ward between 2006 and 2012 were invited and underwent fracture risk assessment including BMD measurements and VFA with dual-energy X-ray absorptiometry. High fracture risk is defined as $T$-score $\leq -2.5$ and/or one or more vertebral fractures (loss of vertebral height $\geq 25\%$).
Participation rate of the FO clinic was 1664 (39%) of 4204 invited individuals. Individuals who did not participate were older, were more often male, more often had two or more fractures at a time and more often had a hip fracture. They less often had a wrist fracture.
Of screened individuals 709 (42.6%) participants had a high fracture risk, 327 (46.1%) on the base of vertebral fractures without the presence of low BMD ($T$-score $> -2.5$). Of 466 patients with a vertebral fracture, 233 (50.0%) had osteopenia and 93 (20.0%) had a normal BMD. Patients with a vertebral fracture were older, had lower BMD, had more often been immobilized, more often reported loss of height and body weight $<60$ kg than patients without a vertebral fracture.
To conclude, almost half of individuals with high fracture risk in the FO clinic was identified on the base of vertebral fractures in the absence of low BMD. Individuals with vertebral fractures are characterized by higher age, more frequent immobilisation, loss of height and/or body weight $<60$ kg. Finally, recruitment of FO clinics should be more actively aimed at older males, individuals with more than two fractures and/or hip fractures.

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## PP221

**Comparison of diagnostic strategies to detect prevalent vertebral fracture for adults over age 50: use of vertebral fracture assessment or spine radiography**

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Background
The prevalent vertebral fracture (VF) is a risk factor for future VF, which can be decreased with drug therapy. However, most VFs are not recognized clinically. VFA by DXA and spine X-ray can be performed to detect these prevalent VFs.
Objectives
This study aimed to estimate the costs, effectiveness, and radiation exposure of VF diagnostic strategies.
Methods
Markov model over a 10-year period was used to calculate the medical costs for diagnostic tests and VF treatment, the reduction of incident VFs of patients who have experienced a VF, and the radiation doses in population aged over 50. We compared three strategies: 'VFA followed by confirmatory radiography (VFA screening)', 'only VFA' and 'only X-ray' every 2 years, to 'no test before recognition'. We assumed that all patients tested positive for VF received drug therapy. A discount rate of 5% was applied in cost.
Results
The results showed the incremental costs for women over age 50 who had VFA screening, only VFA, and only X-ray were €838, €1165, and €957 per person respectively. Future VF incidence was reduced in all strategies by 29% in both VFA screening and only VFA and 35% in only X-ray as compared with no test for 10 years. Also, the level of radiation in X-ray was 2647 μSv and 3253 μSv higher than in VFA screening and only VFA. For men, both the effects and costs were decreased, but trends were similar to the results of women. The sensitivity analyses showed that these results are robust to variety assumptions including cycle length (1 year), costs, and diagnostic accuracy.
Conclusion
VFA screening strategy can be relevant option for future VF prevention as considering lower cost, less radiation, and patient convenience. This study is expected to provide useful information as establishing the VF diagnostic strategy in clinical practice.
Conclusion
The VFA using DXA appeared to have a moderate sensitivity and a high specificity for detecting vertebral fracture.

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## PP222

**Association of CT-based finite element estimates of femur strength with fracture status in three clinical studies on post-menopausal women**

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Introduction
The first clinical applications of FE-strength estimates to classify osteoporotic fractures showed inhomogeneous results. We developed a FE model that correlated well with femur strength *in-vitro* ($R^2 = 0.9$, 14 femurs). This work aims to verify if our model can classify osteoporotic fractures in three case-control studies: a retrospective and a prospective study on proximal femur fracture, and a retrospective study on prevalent osteoporotic fractures.
Methods
Femur retrospective study: 22 proximal femur fractures, 33 controls, all osteopenic or osteoporotic. Femur prospective study: 21 incident proximal femur fractures, 45 age-matched controls. Prevalent fractures study: 35 women

with prevalent osteoporotic fracture (e.g. radius and vertebrae), 40 aBMD-matched controls. CT-based FE-strength estimates: FE-strength was defined as the load inducing εmax > εlim on the femoral neck surface (0.73% tensile and 1.04% compressive limit). For each patient, FE-strength was evaluated for a range of loading directions mimicking the *in-vivo* variability of hip reactions. The minimum FE-strength among all directions was retained for patient classification. Fracture classification: Odds- or hazard-ratios and area under roc curve (AUC) were derived for FE-strength and aBMD.
Results
Femur retrospective study: FE-strength was 33% lower in cases (vs 12% for aBMD). FE-strength classified fractures better than aBMD (AUC = 0.88 vs 0.71) and remained associated with fracture in age- and aBMD-adjusted models. Femur prospective study: FE-strength was 19% lower in cases (vs 15% for aBMD). FE-strength showed higher fracture classification than aBMD (AUC = 0.78 vs 0.72) and remained associated with fracture in aBMD-adjusted models. Prevalent fractures study: fractures and controls were aBMD-matched by design. FE-strength was 5% lower in fracture cases (non-significant difference).
Discussion
In postmenopausal women, i.e. the population at the highest risk of bone fracture, our linear FE model was highly associated with femur fracture. We confirmed that site-specificity is important since femur models can predict femur fractures in addition to aBMD, but not all prevalent osteoporotic fractures.

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## PP223

**Bone mineral density and bone turnover markers in patients with thyroid cancer and L-$T_4$ suppressive therapy after 25 years of follow-up**

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Background
Patients with differentiated thyroid cancer (DTC) are treated with L-thyroxine (L-$T_4$) in relatively hight doses to suppress endogenous TSH levels. Very long-term effect of thyroid hormones supplementation on bone are controversial.
Objetive
To study the possible negative effects on bone mineral density (BMD) and bone markers in DTC patients followed in our center.
Methods
A 46 postmenopausal women (age 62.85 ± 11.29 years) with DCT on TSH suppressive treatment for 27.72 ± 4.04 years were classified at baseline in: Group 1 = premenopausal and Group 2 = postmenopausal. BMD was measured by DXA (Hologic QDR 1000) at baseline (year 1992) and at the present time at lumbar spine (LS),total hip (TH), femoral neck (FN) and ultradistal radius (UDR). Serum bone turnover markers (β-CTX and osteocalcin) and calcitropic hormones (PTH and 25OH vitaminD) were measured.
Results
At the 25 years follow-up study: Group 1: $n = 27$, age 56.3 ± 8.4 and Group 2: $n = 19$, age 72.1 ± 7.8 years. Compared with baseline, we found a statistically significant decrease in BMD at LS ($P = 0.000$) and FN ($P = 0.000$) in Group 1. No significant changes were found in Group 2. Percentage of BMD change were: Group 1: LS −6.23 ± 11%; FN −8 ± 12%; TH 3.2 ± 12%; and UDR 2.7 ± 7.7%.Group 2: LS 3.6 ± 19%; FN −8.5 ± 12.2%; TH −5 ± 16.8%; and UDR 7.5 ± 3.5%. At the second DXA, group two had lower BMD than group one at all localizations (at TH and UD statistically significant). Osteoporotic women were 39.1% (total), 22.2% (Group 1) and 63.2% (Group 2). No correlation was found between BMD and TSH, L-$T_4$ dose, PTH, β-CTX or vitamin D. Only osteocalcin showed a negative correlation with BMD at UDR ($r = -0.58$, $P < 0.01$) and TH ($r = -0.51$, $P0.04$) in Group 1.
Conclusion
Very long-term L-$T_4$ suppressive therapy in women with DTC does not induce severe bone loss. Patients that began therapy in postmenopausal status had lower BMD compared to premenopausal women at the end of the study.

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## PP224

**Discordance between bone mineral density and speed of sound measures of bone: the Canadian Multicentre Osteoporosis study**

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The objective of this investigation was to compare bone mineral density (BMD) attained from dual-energy X-ray DXA with speed of sound (SOS) data attained from a Beam-Med Omnisense multisite quantitative ultrasound (mQUS) in a large sample of randomly-selected community-based individuals from the Canadian Multicentre Osteoporosis Study (CaMOS). A total of 1177 men and 2949 women were assessed with mQUS at the distal radius, tibia, and phalanx sites as part of the Canadian Multicentre Osteoporosis Study (clinical sites included Saskatoon, Calgary, Hamilton, Quebec City, Halifax and St Johns). Estimated stiffness was provided at each site as SOS (m/s). BMD (g/cm$^2$) was assessed at the lumbar spine (L1–4; LS), femoral neck (FN), total hip (TH), and the greater trochanter (GT) by DXA. Pearson product-moment correlations were performed between measures of BMD and SOS for men and women separately. Alpha was set at $P < 0.05$ for all analyses. In this subset of CaMOS data, the mean (S.D.) age for the women was 66.5 (11.49) years and 63.7 (13.04) years for men, with a range of 30–96 years of age. Correlations between BMD and SOS were low (0.02–0.31), irrespective of QUS site or DXA site investigated (all correlations for women were statistically significant, numerous correlations for men were statistically significant). Controlling for height and weight (partial correlation) did little to change the correlations. The greatest r2 attained indicated that there is at best 10% of the variance shared between the measures; most associations were far weaker. While the power of this sample allowed for statistically significant results to be found between the DXA BMD and mQUS it can be safely assumed that they were measuring different attributes of bone. Therefore, these analyses demonstrated that BMD and SOS are relatively independent from one another and therefore may both contribute independent information to fracture risk assessment tools.

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## PP225

**Utilizing bisphosphonate binding kinetics and soft tissue-derived input functions to differentiate changes in long bone and vertebra bone metabolism using *in vivo* fluorescent molecular tomography**

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Bone resorption and formation occur in a tightly regulated fashion reflecting the coupled activities of osteoclasts and osteoblasts. Several pathological conditions perturb this balance, including osteoporosis and skeletal metastases. In the case of metastases, the uncoupling of resorption and formation activities contributes to disseminated tumor cells homing to the bone and to tumor growth within the bone in highly localized regions. Therefore, a site-specific marker of bone remodeling is required to provide a reliable assessment of skeletal disease severity, to evaluate the efficacy of anti-resorptive interventions and to distinguish benign from malignant tumors. The purpose of this study was to determine if the fluorescent bisphosphonate imaging probe Osteosense could model changes in bone metabolism. Evaluation of the kinetic uptake of bisphosphonates in ovariectomized mice revealed significant changes in binding rate constants, as well as the binding plateau of bone. Binding kinetics also revealed differential binding kinetics of bisphosphonate to the knee and spine regions and was able to monitor the individuals region's bone loss resulting from ovariectomy. The utility of binding kinetics was further confirmed in a bone-gain model in which ovariectomized mice were treated intermittently with parathyroid hormone (PTH). Kinetic analysis revealed a significant increase in binding rate constant of fluorescent bisphosphonate in mice treated with PTH as compared to control mice. Localization of bisphosphonate binding at early and late time points after injection revealed initial binding adjacent to the growth plate, associated with high levels of osteoblast and osteoclast activity, and later throughout the bone at regions undergoing bone remodeling. Measurements of the soft tissue region, which show strong correlation to serum level bisphosphonates, were also used to generate $K_{Bone}$ values with results consistent with those stated above. Kinetic modeling was also applied to mice with lytic bone tumor. Analysis revealed a strong correlation between kinetic binding capacity and the size of the tumor as assessed by bioluminescence. Our data suggests a highly sensitive method for monitoring changes in bone metabolism using the binding kinetics of fluorescent bisphosphonates and may serve as a useful tool to monitor pharmacological intervention and tumor progression.

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## PP226

**Combining BINDEX and FRAX in treatment decision pathway for osteoporosis**

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Objective
According to National Osteoporosis Foundation (NOF) guidelines, treatment is recommended for osteoporotic patients and patients with osteopenia and high fracture probability (FRAX with BMD over 3% for hip and/or over 20% for other fractures). In this study, a pocket size pulse-echo (PE) ultrasound (US) device and FRAX with BMI is used in treatment pathway analysis and compared to NOF guidelines.
Materials and methods
Elderly Caucasian woman ($n=427$, age $=69\pm9$ years) were examined using Bindex device. Bindex reports a diagnostic parameter, density index, DI. Previously, the 90% sensitivity and specificity thresholds for DI were determined along ISCD guidelines in diagnostics of osteoporosis. By using these thresholds, subjects were classified as healthy (green), osteoporotic (red) or in need of DXA examination to verify diagnosis (yellow). Osteoporosis was assessed by proximal femur axial DXA. In addition, FRAX scores with BMD ($FRAX_{BMD}$) and with BMI ($FRAX_{BMI}$) were determined.
Results
A total of 173 subjects (73 osteoporotic) were selected to be treated along NOF guidelines. $FRAX_{BMI}$ was analyzed for patients with DI value in yellow or green area. Subjects with red DI value and yellow DI value with $FRAX_{BMI}$ over 20% were selected to be treated. Subjects with yellow DI value and $FRAX_{BMI}$ under 20% or green DI value and $FRAX_{BMI}$ over 20% were selected for additional DXA measurement. The patients with green DI value and FRAX under 20% were considered healthy. The sensitivity and specificity of treatment decisions were 84 and 93%, respectively. Only 31% of the patients were found to require additional DXA measurement to verify the treatment decision.
Conclusion
The present results demonstrate that the ultra-portable US instrument with $FRAX_{BMI}$ shows strong agreement (89%) with treatment decisions using NOF guidelines.

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## PP227

**New point of care method for osteoporosis diagnostics in us**

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Objective
Currently, majority of the osteoporotic patients are not diagnosed i) a new ultrasound based device (Bindex) has been recently introduced for osteoporosis (OP) screening and diagnostics at primary healthcare ii) Bindex measures cortical thickness and determines parameter called density index (DI). Thresholds for DI in OP assessment have been determined in Finnish-Caucasian (F-C) population ($n=448$) along the International Society of Clinical Densitometry (ISCD) guidelines iii) In this study, these thresholds are tested in American-Caucasian (A-C) population.
Materials and methods
A total of 221 A-C females participated the study (age $69.7\pm9.4$ years). Subjects were measured with DXA to determine BMD at proximal femur. Further, the cortical thickness was measured at three locations (distal radius, distal and proximal tibia) with Bindex. Subjects were diagnosed with OP when *T*-score at femoral neck or total proximal femur was below $-2.5$ (NHANES III). DI was calculated either by using measurement at one location ($DI_1$, proximal tibia) or all three locations ($DI_3$). By using the diagnostic thresholds, subjects were classified as healthy, osteoporotic or in need of DXA examination to verify diagnosis.
Results
A total of 74.2 and 73.8% of the subjects could be directly diagnosed by using Bindex measurement, with $DI_1$ and $DI_3$ respectively. Both parameters showed significant linear correlation with total proximal femur BMD ($r=0.62$–$0.70$). Sensitivity in OP diagnostics was 80.9 and 89.7% for $DI_1$ and $DI_3$, respectively. Specificity was 86.9 and 84.3% for $DI_1$ and $DI_3$, respectively. OP was diagnosed in 68 subjects in total.
Conclusion
In this study, fewer subjects would have needed additional DXA examination to verify diagnosis when compared to previous findings. The correlation between BMD and DI was similar than previously observed. These results suggest that F-C thresholds may be applicable for A-C population.

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## PP228

**Changes of health-related quality of life 36 months after vertebral and distal forearm fracture: results from Icuros in Lithuania**

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Objective
To evaluate the changes of health-related quality of life (HRQoL) 36 months after fracture in patients from Lithuania following vertebral or forearm fractures.
Material and methods
Patients aged 50 years and older, with low energy trauma clinical vertebral fracture (VFx) or distal forearm fracture (FFx), enrolled and observed for 18 months in the International Costs and Utilities Related to Osteoporotic fractures Study (ICUROS) in Lithuania, were further interviewed 24 and 36 months after the fracture. HRQoL was measured using EQ-5D. Exclusion criteria were significant changes of health status which could influence the quality of life during the follow up. Multivariate regression analysis was performed to evaluate the impact of sex, age, education and income to EQ-5D index.
Results
In total, 256 persons were included in this study: 65 with VFx (51 women and 14 men) and 191 with FFx (179 women and 12 men). Results of the evaluation at 36-month after the fracture showed that the patients with FFx had better HRQoL compared to the VFx group, although the quality of life did not achieve the pre-fracture level in either group. Pain/discomfort and usual activities were mostly affected dimensions of EQ-5D, in both fracture sites. Before VFx, 85.6% of patients had no back pain, compared to 13.8% 36 months after fracture ($P<0.001$). In patients with VFx, anxiety was reported more frequently 36 months after than before fracture (by 57 and 18.9% of subjects, respectively; $P<0.001$). At 36 months after FFx, the mobility decreased to 67.5 and 77% of subjects were able to look after themselves without problems. In patients with FFx, higher age and lower educational level were significantly associated with lower EQ-5D index scores.
Conclusion
Health-related quality of life did not achieve the pre-fracture level 36 months after a vertebral or a distal forearm fracture.

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## PP229

**Assessment of the possible use of the Austrian model of frax algorithm to predict fragility fracture risk factors in Ukrainian women**

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In the recent years, worldwide the FRAX algorithm is used more extensively to estimate the risk of fragility fractures. Since 2009, the Ukrainian Scientific Medical Centre on Osteoporosis has been extensively using the FRAX algorithm to estimate the fragility fracture risk.
The aim of the study was to establish the normative data for the Austrian model of FRAX algorithm. We examined 3405 women aged 40–89. For the statistical analysis of results the STATISTICA-7.0 and SPSS-17 software were used.
Results
Analyzing the relationship between FRAX-1 and FRAX-2 scores for all fragility fractures and hip fractures we found strong correlations for different subgroups, the results suggest that the FRAX model without the BMD scores has a sufficient informative value and could be used in the decision making on treatment initiation.
The analysis of FRAX scores revealed that FRAX-all and FRAX-hip scores of 11.5 and 2.5 respectively, when the Austrian model is applied to the postmenopausal women, are the criteria for the osteoporotic treatment initiation. FRAX-all and FRAX-hip scores of 7.0 and 1.5 (when the Austrian model is applied to the postmenopausal women), respectively, are the criteria for a further investigation with DXA scan.
This set of criteria could be used in decision making on the antiosteoporotic therapy initiation, especially if DXA scan is not available. However, the researchers should remember that the BMD score is a significant characteristic of the bone tissue state that could be used in monitoring effectiveness of the treatment.
Finally, the results of our study have defined a new approach toward an antiosteoporotic treatment initiation in the Ukrainian women, however, the limitation of this study is explained by using the models developed for different populations, and it could be a source of system error due to the regional features of

osteoporosis and its complications in the Ukrainian population. It was a reason to start a multicenter epidemiological survey on fragility fractures prevalence.

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## PP230

**Combination of spine TBS and clinical risk factors (CRF) can be used to detect women with osteoporotic fracture: a US study**

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BMD alone is not sufficient to predict the fracture risk for an individual. Others parameters, such as microarchitecture play a key role in bone fragility. Several cross-sectional studies have shown the ability of TBS to discriminate fractured from healthy subjects in European populations. The aim of our study is to assess the ability of TBS, evaluated at the lumbar spine, to discriminate subjects with and without fracture in a large white US population.

We present a case–control study on white non Hispanic US women aged 40 and older. Patients who had prior exposure to corticosteroids, systemic illness or who were taking medications known to affect bone metabolism were not included. Fractured subjects had a history of at least one low energy fracture (all osteoporotic fractures). BMD was measured at the lumbar spine (L1–L4) using a Prodigy densitometer (GE-Lunar, Madison, WI, USA). TBS was calculated at L1–L4 directly on the same image as the BMD using the TBS iNsight software (Medimaps, Pessac, France). Descriptive statistics and tests of difference were used. Univariate and multivariate logistic regressions (backward) were used to investigate possible correlations between independent variables (weight, height, BMI, BMD and TBS) and the status of fracture. Odds ratio per SD decrease (OR) and area under the ROC curve (AUC) of discriminating parameters were calculated.

After applying the selection criteria of subjects, 2182 were eligible. This group consisted of 305 fractured subjects (age $=59.7\pm8.3$ years, BMI $=25.4\pm3.8$ kg/m$^2$) and 1877 control subjects (age $=57.4\pm7.3$ years, BMI $=25.0\pm3.9$ kg/m$^2$). Weak correlations were obtained between TBS and BMD and between TBS and BMI ($r=0.327$ and $r=-0.167$ respectively, $P<0.01$). The average value of age, weight, BMD and TBS between the control and fractured group were significantly different ($P<0.0001$, $P=0.02$, $P=0.0004$, $P<0.0001$ respectively), whereas no difference between groups is obtained for BMI and height ($P>0.05$). The OR per SD decrease and the AUC for age, BMD and TBS were presented in the table below. After adjustment for age, weight, BMD, smoking, maternal and family history of fracture, TBS remained significant (but not BMD) with an OR of 1.18 (1.02–1.35). This study confirms the potential of TBS to discriminate subjects with and without fracture and thus even after adjustment for several clinical risk factors.

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## PP231

**The impact of physical activity on estimated bone stiffness as assessed by multisite quantitative ultrasound: the Canadian Multicentre Osteoporosis study**

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This investigation sought to assess the relationship between physical activity (PA) and estimated bone stiffness as assessed by multisite quantitative ultrasound (mQUS) in a large cohort. A total of 1177 men and 2949 women were assessed with mQUS (Beam-Med Omnisense quantitative ultrasound; Isreal) at the distal radius, tibia, and phalanx sites as part of the Canadian Multicentre Osteoporosis Study (clinical sites included Saskatoon, Calgary, Hamilton, Quebec City, Halifax and St Johns). Estimated stiffness was provided at each site as speed of sound (SOS, in m/s). Mean estimated weekly hours of moderate PA, vigorous work and strenuous sport in the past year (converted to kcal energy expenditure/week) and mean estimated daily hours of sedentary activity in the past year were gathered by interview. Weekly moderate PA was stratified into lowest ($<3000$ kcal/w), low (3000 to $<6000$ kcal/w), moderate (6000 to $<9000$ kcal/w), high (9000 to $<12\,000$ kcal/w) and highest ($\geq12\,000$ kcal/w). Moderate PA, vigorous work and strenuous sport were combined and energy expenditure stratified as above. Lastly, average sedentary hours per day in the past year were stratified into lowest ($<10$ h/d), low (10 to $<15$ h/d), moderate (15 to $<20$ h/d) and high ($\geq20$ h/d). Analysis of variance and Tukey HSD established whether statistical differences among and between groups existed. While there were no significant differences in SOS among groups for mean moderate physical activity per week and mean daily sedentary hours per day, there were significant differences when vigorous work and strenuous sport were added to moderate physical activity.

| Distal radius measure | Lowest mean SOS | Low mean SOS | Moderate mean SOS | High mean SOS | Highest mean SOS |
|---|---|---|---|---|---|
| Sedentary | NA | 4046 | 4038 | 4045 | 4066 |
| Moderate PA | 4035 | 4040 | 4051 | 4056 | 4064 |
| $\geq$ Moderate PA | 4025 | 4045* | 4055* | 4062* | 4071* |

*Significantly different ($P<0.05$) from lowest mean SOS group.

A low amount of high-intensity physical activity may increase estimated bone stiffness.

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## PP232

**Serum creatinine profiles in hip fracture patients, an observational study**

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Introduction

Kidney dysfunction is common in the elderly and has been proven to have an association with increased hip fracture incidence. Acute dysfunction may also delay surgery. Serum creatinine, a product of metabolised muscle tissue is a marker used to determine changes in renal function.

Objective

To assess the renal function of hip fracture patients throughout their hospital stay using serum creatinine as the marker of renal function.

Method

Data on serum creatinine levels was collected from records for 100 consecutive patients admitted to an acute hip fracture ward in a UK district Hospital (Carmarthen, Wales). Patients' serum creatinine levels (in μmol/l) were recorded; pre-admission (from previous blood tests), on admission and before discharge. Laboratory specific creatinine reference ranges were used; 44–80 μmol/l (females), 62–106 μmol/l (males). Comparisons were made between levels pre-admission to discharge (PA-DC) and admission to discharge (OA-DC).

Results

100 hip fracture patients, 65% females. Average age: 80 years. The table shows creatinine levels in hip fracture patients.

The average creatinine reduction PA-DC was 10.2 μmol/l, OA-DC it was 19.7 μmol/l. A creatinine reduction of over 20 μmol/l was noted in 26 patients OA-DC but in only 11 patients PA-DC.

| Creatinine level | Pre-admission | On admission | On discharge |
|---|---|---|---|
| Low | 11 | 10 | 18 |
| Normal | 61 | 51 | 67 |
| High | 28 | 39 | 15 |

Conclusions

This study suggests significant percentages of patients presenting to hospital with a hip fractures have some degree of renal impairment as defined by a high creatinine level. Many of these patients' creatinine levels normalise during their hospital stay. The greatest reductions in creatinine were observed between admission and discharge; this suggests a substantial number of hip fracture patients have acute kidney injury on admission. As such, reversible causes of renal impairment should be actively sought and treated in hip fracture patients. The contribution of renal impairment towards post operative outcomes and bone fragility needs further investigation.

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## PP233

### aBMD at the hip measured by DXA overestimate cortical vBMD assesed by QCT scans

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Background
DXA scan has for many years been gold standard when analyzing areal bone mineral density (aBMD) in osteoporotic patients. Risk of fractures increases as aBMD declines. However, although risk of fracture is highest in patients with osteoporosis, it is well known that the majority of fragility fractures actually occur in patients with osteopenia rather than osteoporosis. As aBMD is a projected two dimensional imaging technique it does not differentiate between cortical and trabecular bone. 3D measures of bone by quantitative computed tomography (QCT) is considered to measure true volumetric BMD (vBMD, mg/cm$^3$). Furthermore, this technique can distinguish between cortical and trabecular bone. Therefore, we looked at correlation between aBMD by DXA and vBMD by QCT measured at the hip.
Patient and method
In a cross-sectional study, 125 postmenopausal women mean age 63 (ranges 56–82) were scanned by DXA and QCT at the hip.
Results
As expected linear regression of total hip measured by DXA and QCT showed a positive correlation between aBMD and vBMD at trabecular and integrated site varying between 0.63 and 0.75 ($P<0.01$). However, linear regression of aBMD and cortical vBMD showed a significant negative correlation ($\beta=-0.56$, $P<0.01$). The inverse relation persisted even after adjusting for confounders such as BMI.
Conclusion
Our results indicate that 2D imaging by DXA provides a true measure of trabecular bone density, whereas the inverse correlation between aBMD and cortical vBMD suggest that cortical bone strength may be overestimate by aBMD. As most fractures occur in peripheral bones which are mainly composed of cortical bone, these patients may actually have week cortical bone despite not being osteoporotic as assessed by DXA.

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## PP234

### Influence of age factor on the structural and functional organization of dentoalveolar system bone tissue in rats

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Objective
The purpose of this study was to determine the impact of age on the ultrastructural, histological, biomechanical, and biochemical changes of bone tissue in rats.
Methods
The study included 24 female rats of two age groups: Group A (Young) of 5-month old rats and Group B (Old) of 18-month old rats. Radiomorphometric assessment of the hydroxyapatite was used to investigate the ultrastructure of the alveolar bone tissue. Biomechanical and biochemical changes were measured; histological and histomorphometrical evaluations were made.
Results
There was an increase in the size of the hydroxyapatite crystals and their shape as well as the bonds with collagen type I molecules and the organic matrix of bone tissue in older rats compared to young rats. The biomechanical characteristics of the alveolar process and the mandible deteriorated as evidenced by the decrease in ultimate strength ($374.94\pm27.99$ GPa compared to $407.44\pm17.97$ GPa) and elasticity coefficient ($16.3\pm1.5$ GPa compared to $21.2\pm1.2$ GPa; $P<0.05$). Index work of bonre destruction was $75.24\pm2.28$ mJ in old rats compared to $70.66\pm3.09$ in the young rats. The height of the jaw and alveolar process were higher in old rats compared to young animals ($7.18+0.07$ mm vs $6.18+0.07$; $P<0.01$). The thickness of alveolar process in cervical area were significantly higher in old rats $302.19\pm1.02$ microns, compared to $262.22\pm1.62$ microns in young rats ($P<0.01$).
Conclusion
Ultrastructural organization of bone tissue of the alveolar process and the jaw deteriorate with age, which leads to a weakening of the biomechanical characteristics of bone tissue. The degradation of bone tissue with age may contribute to the development of adaptive reactions in bone tissue and is aimed at increasing the volume of bone tissue, increasing the height of the jaw and alveolar process and thickness in the counterfort areas, and maintaining its function (chewing).

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## PP235

### The impact of vitamin D and calcium intake on estimated bone stiffness as assessed by multisite quantitative ultrasound: the Canadian Multicentre Osteoporosis study

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This investigation sought to assess the relationship between vitamin D and calcium intake and estimated bone stiffness as assessed by multisite quantitative ultrasound (mQUS) in a large cohort. A total of 1177 men and 2949 women were assessed with mQUS (Beam-Med Omnisense quantitative ultrasound; Isreal) at the distal radius, tibia, and phalanx sites as part of the Canadian Multicentre Osteoporosis Study (clinical sites included Saskatoon, Calgary, Hamilton, Quebec City, Halifax and St Johns). Estimated stiffness was provided at each site as speed of sound (SOS, in m/s). The average daily amount of vitamin D (IU/d) and calcium (mg/d) ingested from all sources (dietary and supplemental) over the past year were estimated via food frequency questionnaire. Daily vitamin D intake was stratified into lowest ($<500$ IU/d), low (500 to $<1000$ IU/d), moderate (1000 to $<2000$ IU/d), high (2000 to $<4000$ IU/d) and highest ($\geq4000$ IU/d). Mean daily calcium intake was similarly stratified: lowest ($<400$ mg/d), low (400 to 800 mg/d), moderate (800 to $<1200$ mg/d), moderate-high (1200 to $<1600$ mg/d), high (1600 to $<2000$ mg/d) and highest ($\geq2000$ mg/d). Analysis of variance and Tukey HSD established whether statistical differences among and between groups existed. While there were no significant differences in SOS among groups for mean daily vitamin D or mean daily calcium intake, there were clear trends.

| Distal radius | Lowest intake SOS | Low intake SOS | Moderate intake SOS | Moderate-high intake SOS | High intake SOS | Highest intake SOS |
|---|---|---|---|---|---|---|
| Vit. D | 4045 | 4035 | 4018 | NA | 4113 | 3980 |
| Ca. | 4040 | 4051 | 4034 | 4053 | 4044 | 4030 |

Note: all measures mean SOS and no groups statistically different from one another.

For mean daily vitamin D intake, there was a trend for SOS to decrease in the highest group. For mean daily calcium intake, there was a trend for increasing calcium ingestion to have a lower SOS at all three sites. The relationship between daily vitamin D and calcium intake with bone is complex and requires further study.

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## PP236

### Improved assessment of vertebral cortex thickness by means of analytical deconvolution of radial bone mineral density distributions

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New treatment agents against osteoporosis may not only lead to an improved trabecular structure, but can probably also strengthen the cortex. To assess this treatment effects by means of QCT, one has to deal with significant partial volume effects observing this very thin, but compact structure. A new method for cortical thickness estimation has been developed using an analytical deconvolution approach. After estimating the point-spread-function (PSF) of a scanner/kernel combination, the associated line-spread-function (LSF) and edge-spread function

(ESF) have been derived. Moreover, using sophisticated 3D segmentation, a radial bone mineral density (BMD) distribution was calculated. By fitting a combined LSF/ESF functional to these distributions assuming full mineralization (1100 mgHA/cc) in a compact cortex structure, we directly get an averaged de-convolved cortical thickness dcCt.Th for the cortex fraction of interest. The spongiosa plateau is reflected by the ESF, whereas the Cortex is translated into the LSF. The nonlinear fit (JMP, SAS Institute, Inc,. USA) directly delivers the spongiosa plateau BMD and the cortical thickness and its relative position to the initial segmentation. We did the HRqQCT processing using software provided by Scanco Medical (Switzerland) for a vertical cortex segmentation and compared the calculated cortical thicknesses with HRQCT derived data using StructuralInsight (in-house, ITK-based). Besides a normal maximum-sphere based variable (Ct.Th), we also calculate a density-weighted variable (wCt.Th). In addition to the already mentioned dcCt.Th another variable dcCt.Th* is introduced respecting the unusual embedding material of the ten analysed human vertebral bones (ethical approved during Eurogiops/Bioasset studies). Bland-Altman plots show mean differences to the HRpQCT based estimation of ($1.17 \pm 0.24$), ($0.16 \pm 0.08$), ($0.13 \pm 0.03$) and ($0.09 \pm 0.03$) mgHA/cc for Ct.Th, wCt.Th, dcCt.Th and dcCt.Th* respectively. In the first two cases, significant slopes of ($1.35 \pm 0.21$) and ($0.59 \pm 0.18$) are observable, but not for the latter two. Comparing the mean values of the estimates directly, we find ($0.36 \pm 0.07$) for the HRqQCT and ($1.52 \pm 0.27$), ($0.52 \pm 0.13$), ($0.49 \pm 0.09$) and ($0.46 \pm 0.08$) mgHA/cc.again for CT.Th, wCt.Th, dcCt.Th and dcCt.Th* respectively. Therefore we use basic image analysis to improve the estimation of cortical thickness despite the partial volume effect.

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## PP237

### Reference intervals for bone turnover markers in Spanish premenopausal women

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Background and aims
Bone turnover markers (BTMs) are used in clinical practice for assessing patients with osteoporosis and their treatment. In Spain it is necessary to fine-tune the reference intervals, since they were established years ago in a low number of individuals. The aims of this study were to establish robust reference intervals for BTMs in healthy young premenopausal Spanish women and to investigate the factors influencing BTMs.
Methods
185 women aged 35–45 years. from 13 centres in Catalonia were recruited. Blood and second void urine samples were collected between 0800 and 1000 h after an overnight fast. Serum PINP and βCTX were measured by two automated methods (Elecsys, Roche[a] and IDS-ISYS, Immunodiagnostic Systems[b]), bone ALP by ELISA (IDS, Vitro), osteocalcin by IRMA (Cis Bio) and urinary NTX by ELISA (Osteomark, Vitro). PTH and 25OHD levels were measured in all participants, who completed a questionnaire on lifestyle factors. A quantile regression was fit to estimate the 5, 50 and 95% percentiles for the BMTs, and the Fisher's exact test and non-parametric tests were used to assess the influence of factors on BTMs.
Results
Reference intervals are shown in the Table 1.
Oral contraceptive pills (OCPs) were reported in 10.9% of participants, mean BMI was 23 and 60% had 25OHD levels lower than 20 ng/ml. Women on OCPs had lower PINP levels ($P = 0.007$). 25OHD levels didn't influence BTMs, but low BMI was associated with higher levels of almost all BTMs.
In conclusion, robust reference intervals for BTMs in a southern European country are provided.

**Table 1** Reference intervals

| Bone ALP (ng/ml) | PINP[a] (ng/ml) | PINP[b] (ng/ml) | NTX (nM/mM) | CTX[a] (ng/ml) | CTX[b] (ng/ml) | Osteocalcin (ng/ml) |
|---|---|---|---|---|---|---|
| Median 9.3 | 35.9 | 35.8 | 32.7 | 0.250 | 0.246 | 14.0 |
| P5 6.0 | 20.8 | 20.8 | 19.3 | 0.137 | 0.107 | 8.0 |
| P95 13.8 | 60.6 | 64.9 | 68.9 | 0.480 | 0.541 | 23.0 |

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## PP238

### Correlation between localized femoral BMD *T*-scores and fractures site of hip, and evaluation of the sensitive of FRAX probability in hip fracture patients

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Objection
We compared *T*-scores of each femoral neck and trochanteric area in the neck fracture patients (NFP) and intertrochanteric fracture patients (IFP). Our hypothesis is that *T*-score of neck portion in NFP is lower than *T*-score of neck portion in IFP, and *vice versa*. We evaluate how FRAX probability is meaningful and sensitive in hip fracture patients.
Meterials and methods
From April, 2003 to September, 2012, 180 hip fracture patients (98 for NFP group, 82 for IFP group were included, and all patient was evaluated BMD within 2 weeks after trauma. we calculated FRAX probability in each patients. We evaluated the correlation between localized femoral BMD *T*-scores and fractures site of hip. We checked how many patients were included in high risk group by FRAX, defined as 10-year major osteoporotic fracture (MOF) probability ($\geq 20\%$) or hip fracture probability ($\geq 3\%$). Our study was approved by IRB.
Results
In NFP, average of *T*-scores in neck portion ($-3.23$) was lower than IFP ($-2.93$, $P = 0.029$). In IFP, average of trochanteric *T*-score ($-2.56$) was lower than NFP ($-2.54$, $P = 0.95$). Average of total femoral *T*-scores in NFP ($-2.92$) was lower than IFP ($-2.75$, $P = 0.28$). FRAX probability of MOF in NFP (14.4%) was higher than in IFP (11.1%, $P = 0.009$). FRAX probability of hip fracture in NFP (8.6%) was higher than IFP (5.9%, $P = 0.008$). 19.5% of NFP and 10.1% of IFP were classified as high risk group for MOF. 77.3% of NFP and 80.8% of IFP were classified as high risk group for hip fracture.
Conclusion
Localized femoral *T*-scores were valuable predictive factor for hip fracture in osteoporotic patients. FRAX probability is meaningful and sensitive tool to evaluate the hip fracture in osteoporotic patients.

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## PP239

### Impact of metal artefacts in QCT of the hip caused by a contralateral Gamma type nail: an *ex vivo* study

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Introduction
Gamma type nails are routinely used for the fixation of intertrochanteric fractures of the proximal femur. QCT at the hip is an advanced method that is increasingly being used to estimate BMD and determine the future fracture risk. Yet, implants cause metal artefacts (MA) in CT images, even at the contralateral side due to the high absorption of metal, which may impair BMD accuracy.
Methods
Three human cadavers were scanned first without and then with an implanted Titanium intertrochanteric Gamma type nail. CT scans were carried out on a GE Lightspeed VCT using two scan protocols: 140 kV, 120 mAs and 120 kV, 170 mAs (kernel Standard, 1.25 mm slice thickness). At higher kV, MA should be smaller because of the lower X-ray absorption. A QRM BDC phantom was used

for in-scan BMD calibration. BMD was measured in the contralateral hip using MIAF-Femur.

Results

Table 1 shows BMD differences in mg/cm$^3$ and % between scans with and without the nail in integral, cortical and trabecular BMD (120 kV 170 mAs protocol). Values in the intertrochanter and trochanter were similar. At 140 average differences were reduced for trabecular BMD of the neck (3.4%), all other differences were slightly increased. The difference between native and intertrochanteric Gamma type nail scans were slightly higher than *in-vivo* precision errors of MIAF-Femur (2.2, 3.2 and 3.9% for neck integral, cortical and trabecular BMD respectively).

**Table 1** Differences in BMD between scans

| | Neck BMD (mg/cm$^3$/%) | | | Trochanter BMD (mg/cm$^3$/%) | | |
|---|---|---|---|---|---|---|
| Cadaver | Integral | Cortical | Trabecular | Integral | Cortical | Trabecular |
| 1 | −8.3/−3.3% | −8.8/−1.5% | −6.2/−7.5% | 2.9/1.5% | 6.3/1.6% | 1.5/1.5% |
| 2 | 3.4/1.3% | 13.8/2.5% | −8.6/−7.8% | −3.9/−2.2% | −11/−2.6% | 0.2/0.2% |
| 3 | −8.1/−3.9% | 0.5/0.1% | −3.5/−4.1% | 0.7/0.4% | −2.1/−0.5% | 2.0/2.4% |
| Average of abs values | 6.6/2.9% | 7.7/1.4% | 6.1/6.5% | 2.5/1.4% | 6.4/1.6% | 1.2/1.4% |

Conclusion

Artefacts of titanium implants at the contralateral hip are small in the neck and negligible otherwise and it is questionable whether the added use of metal artefact reduction software may further reduce this BMD error.

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## PP240

**Glucocorticoids aggravate reduction in mineral and lean mass in active juvenile idiopathic arthritis**

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The study is aimed to evaluate body composition and bone status in adult patients with active juvenile idiopathic arthritis (JIA) untreated with tumor necrosis factor α inhibitors. Adult patients (12 males and 19 females) with JIA and 84 healthy age- and gender- matched controls were enrolled into the study. Body composition and areal bone mineral density (aBMD) at the lumbar spine, proximal femur, femoral neck, distal radius and total body were assessed using dual energy X-ray absorptiometry, and correlated with clinical characteristics of the disease and physical performance tests. Disease activity was assessed using high-sensitivity C-reactive protein (hsCRP) and disease activity score 28 (DAS 28). The study has complied with the World Medical Association Declaration of Helsinki regarding ethical conduct of research involving human subjects. In patients with active JIA (DAS 28, 6.36±0.64, hsCRP, 18.36±16.95 mg/l), aBMD at all measured sites, bone mineral content (BMC) and lean mass were reduced, and fat mass was increased in the JIA patients as compared with healthy controls. Significant negative correlations were observed between BMC and disease duration, use of glucocorticoids (GCs), and fat mass, respectively. A positive correlation was found between BMC and lean mass, and between the body fat fraction and the use of GCs. Using multiple linear regression analysis, lean mass was the only significant predictor of BMC of total body both in men and women, and of BMC of legs (only in men). Lean mass was also the only predicting factor of total proximal femur BMD and femoral neck BMD. No significant correlations have been determined among the body composition parameters and DAS 28 or hsCRP endpoints. In conclusion, in adult patients with active JIA, lean mass was the main determining factor of total body and leg BMC, and total proximal femur and femoral neck aBMD.

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## PP241

**Peripheral densitometry in the assessment of fracture risk in adults with cerebral palsy**

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A clinical and densitometric study was performed in the group of 62 adults with cerebral palsy (CP) under institutionalized care in the district of Przemyśl (Southern Poland), 12 of whom had the history of 15 radiologically confirmed non-phalangeal limb fractures. The study assessed correlation between the prevalence of fractures and age, body mass, locomotor abilities determined by Gross Motor Function Classification System (GMFCS), presence of epilepsy, antiepileptic drugs treatment and the values of *T*-score of bone mineral density (BMD) in the heel and forearm (distal radius), measured by DXA method with PIXI device. The study found significantly lower results of the BMD measurement in patients with past history of the fragility fractures and reduced body mass and also in the heel of the individuals with worse locomotor abilities assessed with the GMFCS. ROC analysis did not show statistically significant difference between the heel and forearm BMD in the efficiency of identifying the individuals with the history of fractures. Body mass in the group with prior fractures was higher than in the one without fractures.

Conclusions

Peripheral DXA (pDXA) allows the identification of individuals with increased risk of fracture among adults with CP. Description of locomotor abilities with GMFCS scale allows the estimation of BMD value, suitability of the scale in the assessment of fracture risk has not been demonstrated in the study.

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## PP242

**A systematic review of diagnostic accuracy of VFA using DXA in people aged over 50**

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Objective

The postmenopausal women and elderly men have a big burden for osteoporosis related vertebral fracture and the frequency of clinically unrecognized vertebral fracture is known to be increasing. Vertebral Fracture Assessment (VFA) using DXA is a convenient and safe method to patients together with bone density test and is reported to be proper as a screening method to prevent additional vertebral fracture as diagnosing the vertebral fracture with unrecognized symptoms beforehand. This study aimed to perform a systematic review to examine the overall diagnostic performance of VFA using DXA in identifying the vertebral fracture.

Methods

We searched potentially relevant studies using electronic databases, such as Ovid-Medline, Ovid-EMBASE, Cochrane library, KoreaMed, Kmbase, KISS, RISS from their inceptions to May 2013. Two independent reviewers extracted data using a standardized form. The quality of the selected studies was assessed using the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2). To perform the data composition of the diagnostic accuracy, we constructed the forest plot, SROC curve and bivariate random models. The unexplained heterogeneity among each study existed. Therefore, it was decided to be not proper to combine sensitivity and specificity, and we performed the qualitative data analysis.

Results

Overall, the studies had 'poor' methodological quality. In the study with the best quality evaluation result, the sensitivity in the vertebra level was 0.70–0.73, the specificity was 0.96–0.99 and the sensitivity in the patient level was 0.82 and the specificity was 0.93. Generally, the sensitivity in the patient level showed the higher figure than that in the vertebra and the figure of specificity was higher than that of sensitivity.

Conclusion

The VFA using DXA appeared to have a moderate sensitivity and a high specificity for detecting vertebral fracture.

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## PP243

**Trabecular bone score, bone mineral density and body composistion in men of different ages**

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The aim of this study was to evaluate the trabecular bone score (TBS), bone mineral density (BMD) and body composition in men of various ages.
Materials and methods
300 men aged 40–87 years (mean age – 60.5 ± 0.6 years; mean height – 1.61 ± 0.003 m; mean weight – 84.1 ± 0.9 kg) were examined. The patients were divided into the following age-dependent groups: 40–49 years ($n=52$), 50–59 years ($n=90$), 60–69 years ($n=88$), 70–79 years ($n=58$), 80–87 years ($n=12$). The BMD of total body, PA lumbar spine and proximal femur were measured by the DXA method (Prodigy, GEHC Lunar, Madison, WI, USA) and PA spine TBS were assessed by the TBS iNsight software package installed on our DXA machine (Med-Imaps, Pessac, France).
Results
We observed a significant decrease of TBS (L1–L4) as a function of age (40–49 years – 1.161 ± 0.022; 50–59 years – 1.108 ± 0.018; 60–69 years – 1.114 ± 0.016; 70–79 years – 1.061 ± 0.024; 80–87 years – 1.105 ± 0.049; $F=2.49$; $P=0.04$). We also found the decrease of BMD of lumbar spine (40–49 years – 1.186 ± 0.003 g/cm$^2$; 50–59 years – 1.128 ± 0.021 g/cm$^2$; 60–69 years – 1.224 ± 0.026 g/cm$^2$; 70–79 years – 1.247 ± 0.034 g/cm$^2$; 80–87 years – 1.131 ± 0.064 g/cm$^2$; $F=3.25$; $P=0.01$) and proximal femur (40–49 years – 1.050 ± 0.021 g/cm$^2$; 50–59 years – 0.996 ± 0.018 g/cm$^2$; 60–69 years – 1.032 ± 0.018 g/cm$^2$; 70–79 years – 1.004 ± 0.021 g/cm$^2$; 80–87 years – 0.879 ± 0.050 g/cm$^2$; $F=3.34$; $P=0.01$) with age.
Significant correlation was observed between TBS and BMD of lumbar spine (TBS = 1.017 + 0.079 × BMD (L1–L4); $r=0.11$; $t=1.90$; $P<0.05$) and lean (TBS = 1.441 − 0.000006 × Lean mass (g); $r=-0.25$; $t=-4.50$; $P=0.00001$) and fat (TBS = 1.33 − 0.000009 × Fat mass (g); $r=-0.54$; $t=-11.04$; $P<0.001$) masses.
Conclusion
TBS and BMD in examined men significantly decreased with ageing. We have also found a significant correlation of TBS and BMD of lumbar spine, lean and fat masses.

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## PP244

**Trabecular bone score, bone mineral density and body composistion in women of different ages**

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The aim of this study was to evaluate the trabecular bone score (TBS), bone mineral density (BMD) and body composition in women of various ages.
Materials and methods
494 women aged 41–89 years (mean age, 63.6 ± 0.4 years; mean height, 1.61 ± 0.003 m; and mean weight, 74.0 ± 0.6 kg) were examined. The patients were divided into the following age-dependent groups: 40–49 years ($n=35$), 50–59 years ($n=130$), 60–69 years ($n=177$), 70–79 years ($n=128$), and 80–88 years ($n=24$). BMD of total body, PA lumbar spine and proximal femur were measured by the DXA method (Prodigy, GEHC Lunar, Madison, WI, USA) and PA spine TBS were assessed by the TBS iNsight Software package installed on our DXA machine (Med-Imaps, Pessac, France).
Results
We observed a significant decrease of TBS (L1–L4) as a function of age (40–49 years, 1.321 ± 0.021; 50–59 years, 1.245 ± 0.012; 60–69 years, 1.189 ± 0.011; 70–79 years, 1.166 ± 0.001; and 80–88 years, 1.114 ± 0.033; $F=14.28$; $P<0.001$). We also found the lumbar spine BMD (40–49 years, 1.156 ± 0.038 g/cm$^2$; 50–59 years, 1.068 ± 0.018 g/cm$^2$; 60–69 years, 1.022 ± 0.016 g/cm$^2$; 70–79 years, 1.003 ± 0.001 g/cm$^2$; and 80–89 years, 1.007 ± 0.037 g/cm$^2$; $F=5.11$; $P=0.0005$) and proximal femur BMD (40–49 years, 1.012 ± 0.037 g/cm$^2$; 50–59 years, 0.940 ± 0.013 g/cm$^2$; 60–69 years, 0.923 ± 0.011 g/cm$^2$; 70–79 years, 0.843 ± 0.012 g/cm$^2$; and 80–89 years, 0.741 ± 0.020 g/cm$^2$; $F=20.09$; $P<0.001$) decrease with age.
Significant correlation was observed between TBS and BMD of lumbar spine (TBS = 0.93 + 0.26 × BMD (L1–L4); $r=0.37$; $t=8.61$; $P<0.001$), proximal femur (TBS = 0.97 + 0.27 × BMD (L1–L4); $r=0.29$; $t=6.61$; $P<0.001$) and lean (TBS = 1.34 − 0.000003 × lean mass (g); $r=-0.11$; $t=-2.47$; $P=0.01$) and fat (TBS = 1.25 − 0.000003 × fat mass (g); $r=-0.100$; $t=-2.200$; $P=0.03$) masses.
Conclusion
TBS and BMD of the examined women significantly decreased with ageing. We have also found a significant correlation of TBS and BMD of lumbar spine and proximal femur, lean and fat masses.

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## PP245

**Prevalence of co-morbid conditions among fracture neck of femur patients in an acute hip unit**

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Objective
To assess the prevalence of co-morbidities in fracture neck of femur patients.
Methodology
Study design: Retrospective cohort study.
Setting: District General Hospital in United Kingdom.
Study subject: a total of 75 patients with fracture neck of femur from 2012 to 2013 were ascertained for any associated co-morbid conditions by case note study.
Results
Out of 75 patients, 61 (81.3%) were females (age ranges 60–102) with a mean age of 84 years and 14 (18.6%) were males (65–89) with a mean of 80 years.
70 (93.3%) of them had one or more co-morbid conditions with an average number of comorbidity of 3.56% (ranges 0–10).
Hypertension was the most common condition seen – 30 patients(40%) followed by cardiac disease – 24 (32%),dementia – 22 (29%) and osteoporosis – 15 (20%).
The remaining being thyroid disease (14.6%), stroke (12%), renal disease (12%), malignancies, depression, COPD.
The above conditions were seen in isolation or in combination with other diseases.
The average age adjusted Charlson Comorbidity score was 6.14% (ranges 2–13).
Conclusion
The majority of fracture neck of femur patients are elderly with multiple co-morbid conditions including osteoporosis. Increased awareness and management of co-morbidities are essential in the functional recovery and prevention of further fractures. Our findings support the need for holistic management of these vulnerable patients with close medical care from admission till discharge.

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## PP246

**Do osteophytes protect femoral neck against fracture in osteoarthritis?**

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Introduction
The FRAX tool can be used to assess absolute fracture risk, but the majority of treatment decisions are still based on measurements by DEXA. In remote rural Greek areas, after the socioeconomy crisis, access to the nearest DEXA facility became more difficult.
Purpose
Role of combined QUS and FRAX in the identification of low risk patients who do not require DEXA applying a FRAX threshold of 4% for the 10-year major osteoporotic fracture probability (10YMOFP) like in UK studies, where the incidence and prevalence of osteoporosis is higher than Greece.
Methods
Population study 214 women aged 40–60 years. Clinical risk factors evaluated with FRAX (FRF), BMD measured using heel QUS.
Results
Mean age was 51, 52 years and mean BMI: 27.28 kg/m$^2$. We found that 52 and 33 out of 90 and 124 women (57.77 and 26.61%) who had (10YP) <4% had also $T<-1$. In total 12 out of 214 were found eligible for treatment after DEXA measurement (four and eight for the age groups: 40–49, 50–60, respectively). Our results:

| Age | (10YMOFP) <4%, NO (FRF) | | (10YMOFP) <4%, 1–2 (FRF) | | (10YMOFP) >4%, 1–2 (FRF) | |
|---|---|---|---|---|---|---|
| | $T<-1$ | $-1<T<-2,5$ | $T<-1$ | $-1<T<-2,5$ | $T<-1$ | $-1<T<-2,5$ |
| 40–49 ($n=90$) | 20 | 10 | 32 | 10 | 4 | 11 |
| 50–60 ($n=124$) | 22 | 26 | 11 | 3 | 14 | 40 |
| Total ($n=214$) | 42 | 36 | 43 | 13 | 18 | 51 |

Conclusions
We went onto determine if FRAX and QUS could be employed to 'pre-screen' patients at low osteoporosis risk. In our referral population 42 out of 214 women (19.6%) who had (10YMOFP) <4% and no (FRF), all had $T<-1$ (QUS and DEXA).
We conclude that furher studies are needed for the identification threshold of low risk patients. This would have resulted in fewer scans representing a reduction in workload and cost.

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## PP247

**Evaluation of bone mineral density at the inhabitants of Karelia**

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Age is one of the most important factors that influence bone condition. At the age of 40–45 years for women (F) and 50 years for men (M) processes of bone formation and resorption are balanced, then bone resorption predominates.
We examined 103 M (20–78 years) and 360 F (20–87 years), residing in the Republic of Karelia. Densitometry was performed for all patients to assess age-related changes in bone mineral density (BMD) at the lumbar spine. At F from 20 to 40 years and M up to 50 years, averages of vertebral BMD had no significant differences. A significant decrease in BMD was observed after 40 years. At F reduction in BMD for every 5 years were: aged 40–50 years – 1.7% (0.3% per year), 50–60 – 3.8% (0.8% per year), after 60 – only 1.7–2.5% (0.3–0.5% per year), for 75 – 20% (0.7% per year), to 81–87 – 25.2%. At M BMD reduction were: aged 45–60 years – 2.7% (0.5% per year), after 60 – 1.5% (0.3% per year), to 71–78 – 11.1%. Statistically significant ($P<0.05$) decrease in BMD in F detected in 41–45 years, and in M – in 51–55 years. 89% of F under the age of 50 years had normal BMD, and aged 51–60 years – 44.4%. Osteoporosis (OP) was detected in 55.6% F aged 50–60 years, and in aged 71–80 years – 42.3%, in aged 81 years and older in 57.1%. In males under 40 years, 70% of the BMD values were normal, at 61–70 years in 44.7% of M was revealed osteopenia and OP was observed in 23% of M over 70 years.
Thus, a statistically significant decrease in BMD in female found in the age period of 41–45 years, in male – in 51–55 years. OP was detected in women after age 50, men – after 70 years.

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## PP248

**Effect of minodronate on vertebral bone microarchitecture *in vivo* assessed by computed tomography**

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Minodronate has been reported to increase bone mineral density (BMD) and reduce fracture risk. The purpose of this study was to clarify the effect of minodronate on the three-dimensional vertebral microarchitecture *in vivo*.
Minodronate-treated osteoporosis female patients ($n=9$) and non-treated age-matched historical control ($n=5$) were retrospectively evaluated. After areal BMD (aBMD) evaluation by DXA, 3rd lumbar spine was scanned by quantitative computed tomography (qCT) at a spatial resolution of $351\times351\times500$ μm, and were re-evaluated after 1-year. Through the compilation of consecutive 2D data set of the whole bone, a 3D reconstructed data providing an isotropic voxel was obtained after appropriate interpolation. Bone volume fraction (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tn.N), and connectivity density (CD) were calculated by custom-made software. In addition, bone changes in each of the patients were visualized by subtraction method, which superimpose and subtract longitudinal 3D-reconstructed data sets.
After 1 year, non-treated control lost −2.1% of aBMD, and the minodronate group gained 5.4%, while BV/TV changed −7.1 and +17.2%, respectively. Tb.Th and Tb.N in non-treated control were severely decreased over 1-year period (−6.3%, −0.3% respectively), whereas minodronate treatment could reverse changes in those parameters (+6.3%, +10.0% respectively). CD, however, could not be improved by the treatment (−2.3 vs −3.6%). Subtraction method revealed severe bone loss in the non-treated control and bone formation achieved in the minodronate group.
Our results indicate that evaluation of aBMD by DXA would underestimate both volumetric bone loss and gain of the vertebra. Despite the apparent increase in bone volume by minodronate treatment, it might be difficult to reestablish trabecular connectivity. In order to prevent connectivity loss, earlier therapeutic intervention would be necessary before the connectivity has been lost.

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## PP249

**Prevalence of post-operative medical and surgical complications among fracture neck of femur patients in an acute hip unit**

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Objective
To assess the prevalence of post-operative medical and surgical complications among fracture neck of femur patients.
Method
Study design: retrospective cohort study.
Setting: District General Hospital in UK.
Study subject: a total of 75 patients with fracture neck of femur from 2012 to 2013 were ascertained for any post-operative complications by case note study.
Results
Out of 75 patients studied, 61 (81.3%) were females (age range 60–102) with a mean age of 84 years and 14 (18.6%) were males (65–89) with a mean of 80 years. 44 (58.6%) of them developed post-operative complications. 20 (26.6%) of them developed post-op anaemia (Hb <8 g/dl) and required blood transfusion. 14 (18.6%) developed urinary tract infection and treated with antibiotics. 12 (16%) developed lower respiratory tract infection and were treated with antibiotics.
Other less common complications seen were hypotension, cellulitis, surgical wound infection (three cases), worsening confusion, diarrhoea, seizures and one each case of new onset atrial fibrillation and stroke.
Conclusion
Most of the hip fracture patients are elderly with osteoporosis and a significant percentage of them are prone to post operative complications suggesting the need for specialised dedicated hip fracture unit with close collaborative medical care throughout the perioperative period to reduce the morbidity and mortality.

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## PP250

**Vitamin D levels in male patients with systemic lupus erythematosus**

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Introduction
Although systemic lupus erythematosus (SLE) has traditionally been considered a disease of women, men may also be afected. Male osteoporosis is increasingly recognised. Several studies have reported that mean bone mineral density is significantly reduced in SLE women patients and most of them have low levels of vitamin D. The aim of our study is to analize this situation in men.
Objectives
Determine 25-hydroxyvitamin-D (25OHD) serum concentrations and the presence of osteoporosis in male patients with SLE.
Methods
Eight male patients SLE diagnosed and controlled in our department were included. In all patients were analyzed clinical and laboratory features (including PTH, 25OHD, 24 h urinary calcium excretion, complement (C3, C4), P1NP and βCTX); bone mineral density of lumbar spine (L1–L4) and femur was measured and spinal X-rays.

Results
Eight male patients with SLE with a mean age of 45.8 years (23–65). 75% receive calcium plus vitamin D supplements (500 mg of calcium and 400 UI of vitamin D (cholecalciferol)). Seven patients receive corticoids as a part of their SLE treatment and two of them receive biologic therapy.
Patients with SLE have average levels of 25OHD of 23.2 ng/ml (14.2–34.2). Insufficient vitamin D levels (25OHD $<30$ ng/ml) were observed in most patients (87.5%), 37.5% showed deficient levels (25OHD $<20$ ng/ml). Bone remodeling markers were within normal values P1NP 36.7 (18–134) ng/ml and βCTX 412 (143–797) pg/ml.
Three patients have decreased bone mass according to densitometric criteria. one vertebral fracture was registered.
Conclusions
High prevalence of 25OHD insufficeincy/deficiency was found in SLE male patients, despite treatment with calcium and vitamin D supplements, which indicates that these doses are insufficient. Almost 40% of these patients have decreased bone mass. These findings underline the importance of prevention and treatment of vitamin D deficiency and osteoporosis in male patients with SLE.

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## PP251

**The ability of quantitative ultrasound variables to identify osteoporosis in various regions of interest in female corticosteroid users**

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The objective of this study was to evaluate the ability of various quantitative ultrasound variables to discriminate osteoporotic female corticosteroid users. Thirty-two women aged between 26 and 79 years who have been receiving corticosteroids equivalent to a minimum of 5 mg daily prednisolone for more than 3 months underwent bone mineral density (BMD) measurements at the lumbar spine, hip, and one-third radius (33% radius) using dual-energy X-ray absorptiometry (DXA). Acoustic parameters of bone were also obtained using a calcaneal quantitative ultrasound (QUS) (Quantitative Ultrasound Index (QUI), Broadband Ultrasound Attenuation (BUA), and Speed of Sound (SOS)) and a multi-site QUS device (radial and tibial SOS). Osteoporosis was defined according to the WHO criteria (a $T$-score $\leq -2.5$) at the regions of interest (ROIs) as measured using DXA in postmenopausal women. Whereas in premenopausal women, $Z$-scores of $\leq -2.0$ were used for defining osteoporosis or BMD below the expected range for age. For predicting osteoporosis or BMD below the expected range for age at the lumbar spine, only radial speed of sound (SOS) showed a fair area under the receiver operating characteristic curve (AUC) (0.754, $P=0.028$), while AUCs for other QUS parameters represented poor or failing accuracy ranging from 0.534 to 0.638. As for discriminating subjects with femoral neck, total hip, and radius osteoporosis or BMD below the expected range for age from those without, all calcaneal QUS variables could be considered to be good with AUCs ranging from 0.848 to 0.875 ($P$ values ranging from 0.017 to 0.026) for the first ROI and AUCs calcaneal QUS variables ranging from 0.747 to 0.769 (for QUI: 0.769; $P=0.043$, for BUA:0.750; $P=0.060$, and for SOS: 0.747; $P=0.063$) for the second ROI, and AUCs for calcaneal QUS variables (for QUI: 0.814; $P=0.005$, for BUA: 0.816; $P=0.005$, and for SOS: 0.789; $P=0.010$) for the third ROI showed fair to good accuracy. However, AUCs for multi-site QUS variables which ranged from 0.526 to 0.709 ($P$ values ranging from 0.847 to 0.061) revealed failing, poor, or marginally fair accuracy. The preliminary results of this study with a very small sample size have implications that various calcaneal QUS variables, but not multi-site QUS variables, may be useful in discriminating corticosteroid-induced osteoporosis at the hip and the radius.

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## PP252

**The development of a mathematical model to predict the time to osteoporosis using DEXA scanning**

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Background
Dual-energy X-ray absorptiometry (DEXA) is the gold standard used for measuring bone mineral density and such readings are currently used to predict osteoporosis and osteoporotic fractures. However, no similar prediction model has been developed to identify the age that a patient will become osteoporotic based on DEXA scanning.
Objective
The aim of this study was to develop a mathematical model to determine the TTO based on two or more DEXA scans with TTO defined as the age at which the patient will enter the osteoporotic $T$-score range.
Methods
Fifty patients who had previously undertaken two or more DEXA scans were identified from the DEXA database. $T$-scores were graphed against patient age using GraphPad Prism Software. Straight line curves for the most recent scans were generated with the age at which the curve intersects $T=-2.5$ being classed as TTO. A multiple-point best fit curve was used to generate an overall osteoporotic trajectory over time.
Results
The mathematical model developed successfully predicted the age to osteoporosis for each patient, as well as creating a cumulative osteoporotic trend based on total DEXA scans performed. Additionally, if the patient was classified as osteoporotic following DEXA scanning, the model also successfully predicted the age that the patient would leave the osteoporotic range.
Conclusions
The TTO provides a simple and informative parameter of DEXA scanning that a patient can immediately comprehend and understand, while also providing a more simple measure to monitor response to therapy. This parameter has the potential to further empower patients and revolutionise compliance of treatment strategies. Based on the results presented TTO can be incorporated into future DEXA scans result summaries.

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## PP253

**Bone mass in HIV male patients undergoing antiretroviral therapy**

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Multiple factors of risk have been described to the osteoporosis (OSP) and fractures in people infected by human immunodeficiency virus (HIV). Antiretroviral treatment has changed the vital prognosis of these patients, nevertheless seems that antiretroviral treatments can cause a greater loss of bone mineral density (BMD). Experts support the use of densitometry screening for HIV-infected postmenopausal women and men older than 50 years.
Objectives
To evaluate BMD and risk factors for OSP in HIV-infected male patients undergoing antiretroviral therapy with tenofovir and/or an inhibitor of the protease that attended an outpatient clinic.
Materials and methods
23 patients were included. Clinical factors: age, BMI, previous fractures, habits were investigated and serum calcium, phosphorus, 25(OH)vitamin D, HIV RNA, and CD4 lymphocyte were measured. Densitometry (Lunar iDXA GE c.v. in alive 1.2%) was made at lumbar spine L2–L4 (LS), femoral neck (FN) and total hip (TH). Diagnosis of low bone mass was made using International Society for Clinical Densitometry $Z$-score criteria in the 40–49 years age group and WHO $T$-score criteria in the $>50$-year age group.
Results
Median age was 47 years (range 27–60). All patients had a indetectable viral load and CD4 $518.52\pm251.24$. Twelve patients presented coinfection by VHC. Median BMI was 25.4. No referred personal or familiar previous fracture. Other factors of risk for fracture were: tobacco (five patients) and use of corticoids (one patient). Biochemical levels (mg/dl): calcium $9.31\pm0.44$, phosphorus $2.36\pm1.56$, GOT $42.41\pm32.24$, and GPT $60.32\pm51.66$. Seven patients presented a deficiency of 25 (OH) vitamin D ($<25$ ng/ml) mean: $18.71\pm7.77$.
BMD were: LS $1.077\pm0.194$ g/cm$^2$, $T$-score $-0.7\pm1.9$ $Z$-score $-1.0\pm1.5$, FN $0.935\pm0.201$ g/cm$^2$, $T$-score $-1.1\pm1.5$, $Z$-score $-0.6\pm1.3$, TH $0.928\pm0.192$ g/cm$^2$, $T$-score $-1.2\pm1.4$, and $Z$-score $-0.7\pm1.2$. Four patients had OSP and other four patients osteopenia.
Conclusion
To evaluate the BMD and status of vitamin D in patients with HIV can be important to identify those patients in risk of developing OSP and to need an antiosteoporotic treatment

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## PP254

**Ten-year absolute fragility fracture risk and central DXA assessment: does hypovitaminosis D matter in menopausal women?**

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Introduction
The FRAX model is a new method to assess fracture risk and this aspect is important in menopause. The DXA is still the golden standard but the relationship between these two instruments is complex.
Aim
We correlate the 10-year absolute fracture risk of hip (10-yH) and major fractures (10-yM) based on FRAX with or without bone mineral density (BMD) with lumbar DXA in menopause women, and we use a statistical model to find out whether the vitamin D status influences these correlations.
Materials and method
This is a cross-sectional study design. The inclusion criteria were: i) women in menopause for minimum 1 year; ii) central DXA scan (GE Lunar Prodigy) at Parhon National Institute of Endocrinology, Bucharest, Romania and iii) FRAX risk calculation (for Romania). The exclusion criteria were: i) anterior medication for osteoporosis or fragility fractures prevention and ii) active metabolic bone diseases. 25-Hydroxy vitamin D (25-OHD) was assessed (chemiluminescence; normal: 30–100 ng/ml).
The SPSS21 soft calculated linear regression, backward stepwise regression. Statistical significance (SS) was at $P<0.05$.
Results
197 women (mean age of 57.7 years) were enrolled. The 25-OHD median was 15 ng/ml (5% had $<30$ ng/ml). Mean 10-yM was 4.5% and mean 10-yH was 1.12%. Mean lumbar BMD was $1.101\pm0.2$ g/cm$^2$. The linear correlation coefficient was between lumbar BMD and 10-yM was: $r=-0.25$ respective, 10-yH $r=-0.23$ ($P<0.005$). Similar data were obtained when inputting neck BMD in FRAX calculation. The backward stepwise regression coefficients (adding 25-OHD) were for lumbar BMD and 10-yM $r=0.308$, $r^2=0.095$, respective for 10-yH $r=0.295$, $r^2=0.087$, and after adjusting for 25-OHF $r=0.29$, $r^2=0.089$, respective $r=0.285$, $r^2=0.08$ ($P<0.05$).
Discussion
The studied menopausal population was not preselected based on 25-OHD but a general prevalence of D hypovitaminosis is registered.
Conclusion
The correlation between BMD and 10-year absolute fracture risk is not influenced by the D vitamin status, including deficient levels.

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## PP255

**Dynamic changes in bone marrow adiposity during the menstrual cycle**

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Background
Bone marrow (BM) adiposity is inversely related to bone mineral density and increases with ageing and menopause. We previously observed that the variation in BM fat fraction was more pronounced in premenopausal women compared to men and postmenopausal women. We hypothesized that the variation in BM fat fraction in premenopausal women is associated with hormonal variations during the menstrual cycle.
Objective
To investigate the dynamic changes in BM adiposity during the menstrual cycle.
Methods
In ten healthy premenopausal women, we measured vertebral BM adiposity using Dixon's quantitative chemical shift imaging twice a week during one menstrual cycle. In addition, we measured serum concentrations of FSH, LH, progesterone, estradiol and the bone turnover markers C-terminal crosslinking telopeptides of collagen type I (CTx) and procollagen type I N propeptide (P1NP). The study was approved by the Medical Ethics Committee. Data were analyzed by a linear mixed model.
Results
The average change in fat fraction was 5.4% with an increase during the follicular phase ($P=0.03$) and a decrease during the luteal phase ($P=0.09$). Progesterone ($P=0.03$) and LH ($P=0.03$) had a significant overall association with the fat fraction. During the follicular phase, FSH ($P=0.05$, effect estimate per unit increase (EE) $-0.36$, 95% CI ($-0.72$ to 0.00) and LH ($P=0.02$, EE 0.3, 95% CI 0.05 to 0.57) were significantly associated with the fat fraction and during the luteal phase progesterone ($P=0.01$, EE $-0.04$, 95% CI $-0.07$ to 0.01), LH ($P=0.03$, EE 0.09, 95% CI 0.01 to 0.1) and CTx ($P<0.01$, EE 0.015, 95% CI 0.01 to 0.02) had a significant association.
Conclusion
This study indicates that the BM fat fraction is at least in part determined by the phase of the menstrual cycle, suggesting that BM fat is not merely the reciprocal of bone mineral density and can be regulated independently of bone mass.

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# Osteoporosis: pathophysiology and epidemiology

## PP256

**Sarcopenia increased mortality among elderly men**

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Sarcopenia has been indicated as one of the important markers of frailty among the elderly. This study aims to evaluate mortality risk among Japanese elderly persons with sarcopenia, taking into account lifestyle and physical factors.
From among a population-based community cohort, the Adult Health Study conducted at RERF, 1880 subjects (626 men and 1 254 women) aged 47–95 years old were enrolled in a prospective and longitudinal cohort study in Hiroshima, Japan. These individuals underwent physical examinations including lean body mass using a dual X-ray absorptiometry (DXA) as well as a questionnaire during the period 1994–1995, and were followed for mortality status through 2007. Sarcopenis was defined as having skeletal muscle mass index (SMI) of 6.87 kg/m$^2$ or less for men and 5.46 kg/m$^2$ or less for women. Mortality risk was estimated using an age-stratified Cox proportional hazards model. In addition, sex, radiation dose, lifestyle and physical factors such as smoking status, alcohol intake, total cholesterol, blood pressure, and diagnosed diseases were included as adjustment factors for analysis of total mortality and mortality from each cause of death.
There were a total of 496 deaths, 42 coronary heart disease deaths, 67 stroke deaths, 116 respiratory disease deaths including 90 pneumonia deaths, and 183 cancer deaths during the follow-up period. Sarcopenia showed a significant association with all-cause mortality among men (HR $=1.9$, 95% CI 1.3–2.9, $P=0.0006$), after adjustment was made for atomic-bomb radiation exposure, and lifestyle and physical factors in men. Sarcopenia was also significantly associated with respiratory deaths (HR $=2.6$, 95% CI 1.2–5.4, $P=0.01$) in men. There was no significant association between sarcopenia and increased mortality among women.
In conclusion, sarcopenia predicted all-cause deaths and respiratory deaths in men, but not in women.
This study was supported by the research funding for Longevity Sciences from the Ministry of Health and Welfare.

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## PP257

**Molecular evidence of osteoblast dysfunction in elderly men with osteoporotic hip fractures**

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Fractures of the hip are serious complications associated with osteoporosis. In previous work we found evidence of osteoblast dysfunction in middle aged men with idiopathic osteoporosis. The aim of this study was to investigate gene

expression and bone microarchitecture in bone samples derived from elderly men with osteoporotic hip fractures. Femoral heads and adjacent bone tissue from 12 men with low-trauma hip fractures (mean age 82±7 years) and consecutive surgical hip replacement were collected. Femoral heads from age matched men with osteoarthritis served as controls. One half of the femoral head was subjected to gene expression analysis of osteoblast related genes by RT-PCR. From the second half of the femoral head bone samples from four regions (central and subcortical region of the femoral head and neck) were analyzed by static histomorphometry. We could show a significantly decreased expression of the osteoblast related genes runx2 (−2.43-fold, $P=0.004$), osterix (−7.63-fold, $P<0.001$), and sclerostin (−7.74-fold, $P=0.001$) in bone samples from hip fracture patients ($n=9$, mean age 82±7 years) compared to controls. Osteoporotic bone samples were characterized by a significant decrease of bone volume (BV/TV: 40.1%, $P=0.008$) and a significant increase of trabecular separation and marrow cavity volume in the subcortical region of the neck (TbSp: 76.6%, $P=0.031$ and MaV/TV: 37.2%, $P=0.005$)) ($n=10$, mean age 83±6 years). In conclusion, decreased local gene expression of runx2 and osterix in men with hip fractures strongly supports the concept of osteoblast dysfunction in male osteoporosis. Microstructural changes in the trabecular structure associated with osteoporotic hip fractures in men are localized in the subcortical region of the femoral neck and are characterized by loss of trabeculae.

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## PP258

### Socio-economic status and hip fracture risk: a region-wide ecological study

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Purpose

To determine the association between socio-economic status (SES) and risk of hip fracture.

Methods

Retrospective cohort study. We used a population database which contains primary care and hospital inpatient records of over >5 million people. Anyone registered in this database in 2009–2012 and resident in an urban area was eligible. Main measures: a validated SES composite index (accounting for proportion of unemployed, insufficiently educated, temporary workers, manual workers, and insufficiently educated youngsters) was estimated for each area based on census data. Outcome measure: hip fracture as coded (ICD-10) in 2009–2012. Statistics: Zero-inflated Poisson models were fitted to study the association between SES quintiles and hip fracture rates after adjustment for age, gender, obesity, smoking and alcohol drinking.

Results

Compared to the most deprived, wealthy areas had a older population (46.83 (18.49) vs 43.29 (17.59), mean years (s.d.)), had more women (54.8 vs 49.1%), as well as a lower percentage of obese (8.4 vs 16.2%), smokers (11.9 vs 16.9%) and alcoholics (1.3 vs 1.5%). The most affluent areas reported a higher incidence of hip fracture compared to the most deprived (Age–sex-adjusted incidence 38.57 (37.14–40.00) and 34.33 (32.90–35.76)/10 000 persons-year respectively). When compared to the wealthiest, deprived areas had lower hip fracture rates (unadjusted RR 0.71 (95% CI 0.65–0.78)), although age-gender (RR 0.90 (95% CI 0.85–0.95)) adjustment and further adjustment for obesity prevalence (RR 0.96 (95% CI 0.90–1.01)) significantly attenuated this association. Further adjustment for smoking and alcoholism did not make a difference (RR 0.96 (95% CI 0.91–1.02)).

Conclusion

Subjects living in wealthy areas had a 30% increased risk of hip fracture. Differences in age-gender composition, maybe due to a previously described higher mortality associated with deprivation, and a higher prevalence of obesity explain the observed risk reduction in these deprived areas. This information should be used for health-care planning and commissioning.

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## PP259

### High sclerostin levels in primary biliray cirrhosis: relationship with cholestasis and bone remodelling

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Background and aims

Low bone formation is the main pathogenic mechanism of osteoporosis in primary biliary cirrhosis (PBC). Sclerostin, an inhibitor of the Wnt pathway, is involved in the regulation of osteoblastogenesis and little is known about its role in the development of bone disease in PBC. Thus, we evaluated the circulating levels of sclerostin and its relationship to bone mass, the parameters of mineral metabolism and liver disease severity.

Methods

Serum sclerostin levels were measured in 83 women with PBC (mean age: 60± 12 years) treated with ursodeoxycolic acid and 101 control women of the same age. In patients with PBC, we assessed the degree of cholestasis, lumbar and femoral BMD (DXA), as well as parameters of mineral metabolism (Ca/P, PTH, 25OHD, PINP, bone ALP, sCTX, NTX, and osteocalcin). In 20 PBC patients sclerostin levels were measured again after 5 years.

Results

77% had low BMD (22% osteoporosis and 55% osteopenia). Patients with PBC showed higher sclerostin levels than controls (76.7±38.6 vs 32.5±14.7 pmol/l, $P<0.001$). Sclerostin levels were higher in patients with less severe cholestasis. A direct correlation between sclerostin and lumbar ($r=0.354$, $P=0.002$) and femoral BMD ($r=0.336$, $P=0.003$) and age ($r=0.256$, $P=0.002$) was observed. In the 65 patients not receiving bisphosphonates at the time of the evaluation, there was an inverse correlation of sclerostin with bone formation markers, PINP ($P=0.05$) and osteocalcin ($P=0.037$) and bone resorption, NTX ($P=0.01$) and sCTX ($P=0.03$). Sclerostin was significantly decreased in patients who were evaluated at 5 years (99.5±35.9 vs 60.1±30.9 pmol/l, $P=0.001$).

Conclusion

In primary biliary cirrhosis there is an increase in sclerostin, related to bone mass and liver disease severity. The inverse association with bone formation markers indicates that high sclerostin plays a role in the decreased bone formation in this liver disease.

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## PP260

### Dietary phosphorus intake is positively associated with some radial and tibial pQCT bone traits in premenopausal females with seemingly high calcium intake

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Phosphorus intake in western countries is high due to abundant consumption of animal protein and also because of the expanding use of highly absorbable food additive phosphate (FAP) salts in food processing. P is essential for hydroxyapatite in bone, but short-term studies have shown that high P, especially FAP, intake has acute negative effects on bone metabolism. In this study, we investigated the associations between dietary P intake and bone turnover markers by using a cross-sectional design in 37- to 47-year-old premenopausal Caucasian females ($n=370$). Serum intact pro-collagen type I amino-terminal propeptide (iPINP), collagen type 1 cross-linked C-terminal telopeptide (CTX), total osteocalcin (totOC), carboxylated osteocalcin (cOC), and some other biomarkers related to bone metabolism were analyzed. Data were examined by ANOVAS and covariance in tertiles of both total P (TP) and FAP intake, and molar calcium:phosphorus (Ca:P) ratio, and subjects were stratified by contraceptive

use. Among contraceptive users ($n=123$), high TP intake was associated with lower bone formation in terms of low cOC ($P=0.034$), and a tendency for an association with decreased turnover as lower totOC ($P=0.094$) was found. In subjects with a high Ca:P ratio, a tendency existed for an association with decreased bone resorption (i.e. lower CTX) ($P=0.081$). Among those who did not use contraceptives ($n=247$), high TP intake was associated with decreased bone formation (i.e. low iPINP, $P=0.034$), and a borderline association with decreased resorption (i.e. low CTX, $P=0.056$) was present. High FAP intake was associated with low cOC ($P=0.008$) and a tendency for low totOC ($P=0.087$). In summary, high P intake was weakly associated with lowered bone turnover, and the association was modulated by hormonal contraceptive use, but the exact role of high P intake in bone health remains to be elucidated.

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## PP261

**The relationship between pulmonary function and bone mineral density in healthy non-smoking women: The Korean National Health and Nutrition Examination Survey (KNHANES) 2010**

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Introduction
It has been reported that low bone mass is common in patients with pulmonary disorders such as chronic obstructive pulmonary disease. However, in healthy non-smoking women, the relationship between bone mass and pulmonary function has yet to be clarified. The object of this study was to determine whether pulmonary function is related to BMD in healthy non-smoking women based on menopausal status.
Methods
This study was a cross-sectional study based on data obtained from the Korean National Health and Nutrition Examination Survey (KNHANES), a nation-wide representative survey conducted by the Korean Ministry of Health and Welfare in 2010. This study included 506 subjects who had never smoked and analyzed data concerning pulmonary function and BMD.
Results
Functional vital capacity (FVC) and forced expiratory volume in 1 second ($FEV_1$) were correlated with BMD at lumbar spine, femur neck (FN), and total hip in premenopausal women ($P=0.030$, $P=0.003$, and $P=0.019$ respectively for FVC; $P=0.015$, $P=0.006$, and $P=0.059$ respectively for $FEV_1$). However, FVC and $FEV_1$ were only correlated with BMD at FN in postmenopausal women ($P=0.003$ for FVC and $P=0.006$ for $FEV_1$). BMI, FVC, and $FEV_1$ were significantly related with BMD at FN, even after adjusting for age and other confounding factors ($\beta=0.334$, $P<0.001$, $\beta=0.145$, $P=0.017$, and $\beta=0.129$, $P=0.037$ respectively) in premenopausal women. However, only age and BMI were correlated with BMD at FN ($\beta=-0.268$, $P=0.001$ and $\beta=0.384$, $P<0.001$) in postmenopausal women after adjusting for confounding factors.
Conclusions
Pulmonary function, including FVC and $FEV_1$ are associated with BMD at FN in healthy non-smoking premenopausal women but not in postmenopausal women.

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## PP262

**Bone mineral density and hip geometry in men and women with type 2 diabetes: Korea National Health and Nutrition Examination Survey 2008**

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Objective
Individuals with type 2 diabetes have increased fracture risk despite higher bone mineral density (BMD). There are few studies regarding the effect of diabetes on bone mineral density and hip geometry in Asian population. The aim of this study was to examine the gender-specific influence of diabetes on BMD and hip geometry.
Subjects and methods
A total of 1334 subjects (571 men and 763 women) $\geq 50$ years of age were analyzed using data from the 2008 Korea National Health and Nutrition Examination Survey (KNHANES IV). Femoral neck, lumbar spine BMD, and hip structural analyses were done using the dual-energy X-ray absorptiometry.
Results
Among the whole study population, 16% had type 2 diabetes (17.1% in men and 15.9% in women). Subjects with diabetes were older, and had higher BMI than those without diabetes. Lumbar spine BMD was higher in men and women with diabetes compared to non-diabetic men and women respectively, after adjusting for age, height, and weight. Femoral neck BMD was similar between those with diabetes and without diabetes. Meanwhile, after dividing by lean body mass, diabetic women had 4% lower BMD ($P=0.031$), 6% thinner cortices ($P<0.001$), and 3.4% narrower neck width ($P=0.033$) than non-diabetic women. However, the BMD, cortical thickness, and neck width in the diabetic men was similar to those in individuals without diabetes.
Conclusions
Femoral neck BMD, cortical thickness, and neck width were significantly lower after adjusting for lean body mass in women with diabetes than controls. This may partly explain the higher fracture risk in patients with type 2 diabetes.

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## PP263

**Interval changes in bone mineral density in exercising young women with apparent athlete triad syndrome**

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Objective
To evaluate the bone mineral density (BMD) of the axial and appendicular skeleton in a group of collegiate dance students undergoing intensive training between a 24 months interval and to correlate these changes to the presence or absence of apparent athlete triad syndrome (ATS).
Methods
Forty full time collegiate dance students were recruited from a tertiary Performing Arts Institute. All subjects had basic anthropometric measurements, a full hormonal profile, pelvic ultrasound, bio-impedance estimation of body fat, and dual energy X-ray absorptiometry (DXA) and quantitative peripheral CT scans (pQCT) to determine bone density. The measurements were then repeated 22–24 months after the initial assessment, Those who had persistent oligo/amenorrhoea and were underweight with a BMI $<18.5$ kg/cm$^2$ were categorized as having apparent ATS. The measurements were compared to a group of non-exercising eumenorrheic adolescents recruited from the a general gynaecology clinic undergoing similar interval measurements.
Results
A total of 40 dance students and 21 control subjects were analysed. The mean age of the exercising ($n=40$) and non-excising subjects ($n=21$) were similar (18.4 vs 18.7 years). Comparing the interval changes at the 24-month reassessment, the exercising group showed a larger interval increment in lumbar spine BMD (0.068 vs 0.016 g/cm$^2$, $P=0.001$) as well hip BMD values (neck of femur 0.0439 vs 0.008 g/cm$^2$; trochanter 0.023 vs 0.016 g/cm$^2$ and Ward's triangle 0.035 vs 0.010 g/cm$^2$; $P\leq 0.001$). Volumetric BMD also showed similar trends (distal radius core BMD 21.2 vs 13.1 g/cm$^3$ and distal tibia core 20.5 vs 14 g/cm$^3$, $P=0.001$). When dance students with ($n=13$) or without apparent ATS ($n=27$) were compared, no significant differences were seen in axial BMD values, marginally lower radial core BMD (16.3 vs 23.5 g/cm$^3$, $P=0.029$) and tibial core BMD (16.8 vs 22.2 g/cm$^3$, $P=0.048$) increments were seen in the ATS group.
Conclusion
Young women undergoing regular intensive weight-bearing exercises as in the collegiate dancers here studied have higher BMD increments as compared to non-exercising women. Even in the presence of apparent ATS, BMD increments, though somewhat attenuated, were significantly higher than non-exercising controls.

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## PP264

### Calcium and vitamin D nutritional status effect on oral health evaluated in a group of young college women

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Calcium (Ca) and vitamin D nutritional status effects on oral health were evaluated in 85 healthy women aged between 20 and 30 years (24.6±0.4). They attended the first year of the Nutrition Carrier at the Private University (ISALUD, Buenos Aires, Argentine). Usual Ca intake (CaI) was determined by a food-frequency questionnaire that included consumption of dairy products and calcium-enriched foods. Blood samples were obtained in a fasting state in winter: the end of August and the 15th September. Serum biochemical analysis of 25-hydroxyvitamin D (25OHD), Ca, phosphorus (P), bone alkaline phosphatase (BALP), and intact parathormone (iPTH) were assessed. Dental status was determined by a full odontogram including the total number of teeth and the decayed (D), missing (M), and filled (F) teeth (DMFT) index. M and D components evaluated teeth loss and the presence of caries respectively. The Plack Index Löe Silness (PI) was also evaluated.

Results

As a results of a low milk products the median CaI was lower than 600 mg/day (498 (381; 792)). Only 24% cover Ca the recommendation of 800 mg/day; 17% had a CaI $<$800 mg/day and 59% had a deficient CaI ($<$600 mg/day). Mean biochemical parameters were within normal ranges: iPTH levels: 39.2±4.9 pg/ml; Ca: 9.4±0.2 mg/dl; and P: 4.0±0.3 mg/dl. A total of 7 and 3% of women had Ca and P levels below normal values respectively. Nobody had PTH, Ca and P levels higher than the reference range. The mean 25OHD levels were 25.0±1.0 ng/ml. Only 29% had 25 OHD $\geq$30 ng/ml; a 71% had levels $<$30 ng/ml: 39% between 21 and 29 ng/ml and 32% $<$20 ng/ml and a 15% had levels $<$10 ng/ml. The 100% of the students had chronic gingivitis. The 3% had at least one missing teeth and 20% had the mouth affected by caries. The D component of DMFT increased as CaI decreased; the higher M component and PI was observed when CaI was $<$600 mg/day. The lower D component and PI was observed when 25OHD was $>$30 ng/ml.

Conclusion

The results of the present cross-sectional study in young student women evidenced a high cariogenic activity and a great severity of oral disease. This effect was related to a low CaI and vitamin D nutritional status. This finding underscores the necessity of nutrition intervention specifically designed to increase milk or milk products as a nutritional strategy to preserve the natural dentition that improves the quality of life and to prevent the development of future oral pathologies.

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## PP265

### High prevalence of vertebral fractures at time of screening for orthotopic liver transplantation

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Purpose

Osteoporosis and fractures are prevalent in orthotopic liver transplant (OLT) recipients but data on these skeletal complications are scarce in patients with end-stage liver disease awaiting liver transplantation. The aim of our study was to evaluate the prevalence of osteoporosis and vertebral fractures at screening for OLT, to determine risk factors for skeletal pathology and to evaluate the predictive value of BMD for fracture risk in these patients.

Methods

Consecutive liver transplant recipients at the Leiden University Medical Centre between 2000 and 2011 who had BMD and conventional radiographs of the spine at time of screening for OLT were studied. Clinical, laboratory and BMD data were extracted from electronic hospital records. Spinal radiographs were assessed for vertebral fractures by two independent observers using the Genant's semi-quantitative method.

Results

183 patients (median age 52 years, 74% men) were studied. Most common liver pathology was viral and alcoholic liver disease (both 28%). Osteoporosis and osteopenia were prevalent in respectively 19 and 37% at the lumbar spine and in 10 and 43% at the femoral neck. Vertebral fractures were prevalent in 56% of patients, mainly grade one fractures. Men had increased fracture risk. There was no association between any other potential risk factor studied including BMD and fracture risk.

Conclusions

Low bone mass and vertebral fractures are prevalent at time of screening for OLT but none of several potential risk factors examined, other than male gender, was predictive for increased fracture risk.

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## PP266

### Adipose tissue expression of adipocytes and osteoporosis in COPD

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Aim

Aim of our study was to investigate the serum adiponectin, leptin, and osteoprotegerin (OPG) levels and its expressions in the adipose tissue, and their relationships with bone metabolism and in patients with severe C chronic obstructive pulmonary disease (COPD).

Materials and methods

Serum leptin, adiponectin, OPG, the receptor activator of nuclear factor-kB ligand (RANKL), and bone turnover markers (osteocalcin and type 1 collagen C-telopeptide (CTx)) were determinated in 52 patients with severe COPD and 42 age- and sex-matched healthy controls. Bone mineral density (BMD) and body composition was assessed by dual energy X-ray absorptiometry at the lumbar spine (LS) and left femur neck (FN). Subcutaneous adipose tissue samples were analyzed by immunocytochemical analyses.

Results

Adipose tissue expression of leptin (LepR) was low and adipose tissue expression of adiponectin (AdipoR1) was higher in COPD group than in control. Compared to patients without osteoporosis, those with the disease had significantly lower serum leptin, OPG levels, and LepR, in association with increased serum CTx, RANKL, adiponectin, and AdipoR1 expressions ($P<0.05$). LepR was inversely related to serum CTx ($P<0.01$), and directly to serum leptin ($P<0.01$), to fat free mass (FFM) ($r=0.44$, $P<0.01$) and to BMD FN and BMD LS ($P<0.05$ for all relationships). Serum leptin was correlated positively with OPG ($P<0.05$) and negatively with RANKL ($P<0.05$); serum adiponectin was negative association with serum OPG ($P<0.05$) and positive correlation with RANKL ($P<0.05$) in severe COPD. AdipoR1 expression was negatively related to FFM ($r<-0.51$, $P=0.01$) and directly to serum adiponectin and CTx ($P<0.01$). Adipose tissue OPG expression was related to BMD FN only ($P<0.05$).

Conclusion

Our results suggest that adipose tissue leptin, adiponectin, and OPG expressions are related to development of osteoporosis in severe COPD, and appear to act as mediators between fat mass and bone density.

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## PP267

### Fracture risk and the osteoporosis treatment care gap in patients with type 1 diabetes

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Individuals with type 1 diabetes have over a sixfold increased risk of sustaining a hip fracture compared to the general population. Despite this, bone fragility is not recognized as a classic diabetes-related complication and many diabetes guidelines make no mention of fracture prevention or bone health.

We studied bone health in a population of patients with known type 1 diabetes being followed by endocrinologists at an academic centre. Patients filled out a bone health questionnaire which was used to up-date their electronic medical record (EMR). De-identified data was extracted from the EMR for all type 1 patients over age 40. Fracture risk scores were calculated using the WHO fracture risk assessment tool (FRAX). The study was approved by the Local Research Ethics Board.

There were 201 individuals identified, with mean age of 53.9 years (40–82). Of these, 7 (3.5%) were identified as 'high risk' for future fracture by their calculated FRAX score ($>$20% risk of major osteoporotic fracture over the next 10 years), and 18 (8.9%) were in the 'moderate risk' category (10–20% risk). Despite our

current national osteoporosis guidelines to treat all high risk patients, and to consider treatment of moderate risk patients, 2 of 7 (28.5%) high risk patients were not on therapy with an osteoporosis-specific medication and 14.3% were not on calcium or vitamin D. Of the moderate risk patients, 17 of 18 (94.4%) were not on bone-specific therapy and 38.9% were not taking calcium or vitamin D.
Our results identify a care gap for treatment of osteoporosis in patients with type 1 diabetes followed at an academic centre. This care gap is expected to be worse in non-specialty centres, with less time for comprehensive diabetes management, and highlights the need to up-date diabetes care guidelines to include bone fragility as a complication of type 1 diabetes.

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## PP268

### BMD and biochemical bone markers in premenopausal amenorrhea

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Objectives
This study was designed to investigate the prevalence of low bone mass, the influence of amenorrhea on bone mineral density (BMD), and the cause of amenorrhea that provoke the severe low bone mass in premenopausal women.
Materials and methods
One hundred and seventy one women diagnosed with amenorrhea in premenopausal women were included in this study. All patients underwent history taking, gynecological examination, and check serum hormone level. Dual energy X-ray absorptiometry was performed for measurement of BMD. All patients were classified into four groups; hypergonadotrophic hypogonadism, hypogonadotrophic hypogonadism, polycystic ovarian syndrome (PCOS), and control group.
Results
The mean age of all patients was $22.8 \pm 5.8$ years. There were no statistically significant differences among the groups in relation to patient's age, BMI, TSH level, prolactin level, and DHEAS level. But there were statistically significant differences in LH, FSH, estradiol, and testosterone level among the groups. *T*-score in BMD of spine was significantly lower in hypergonadotrophic hypogonadism group than others. Comparing the *T*-score in BMD according to causes of amenorrhea, premature ovarian failure group, and anorexia nervosa group had the lowest bond mass. In the multiple logistic regression model, BMI, FSH, and estradiol level were risk factors for low *T*-score in BMD. There were shown weak negative correlation between duration of amenorrhea and bone mass of spine and femur neck.
Conclusions
In this study, there was a high prevalence of low bone mass in adolescent women diagnosed with amenorrhea. BMD should be checked in all adolescent women diagnosed with amenorrhea, especially in hypergonadotrophic hypogonadism and hypogonadotrophic hypogonadism patients.

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## PP269

### Comparison of peripheral volumetric bone mineral density, quantitative ultrasound and standard radiological methods for monitoring bone mineral density changes 2 years *postpartum*

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Objective
To evaluate the BMD changes around 2 years after delivery using standard dual energy X-ray absorptiometry (DXA) of the lumbar spine and hip, peripheral quantitative computerized tomography (pQCT) of the distal tibia and radius, and quantitative ultrasound (QUS) of the oscalcis.
Methods
Consecutive patients with uncomplicated singleton pregnancies were recruited from a general obstetric clinic. Standard BMD measurements of the lumbar spine and hips were performed using DXA and volumetric BMD of the distal radius and distal tibia were also performed using pQCT techniques within 3 weeks after the index delivery, and then repeated after 21–24 months. Those that delivered preterm before 36 weeks or who had significant medical problems antenatally or postnatally were excluded.
Results
A total of 111 patients with complete data were analyzed. Uniform gains in BMD were observed between the interval assessments in the lumbar spine (L2–L4), neck of femur, as well as core and total BMD of the distal radius but not the distal tibia. The mean increase in BMD at the different sites ranged from 0.02% (distal tibia total) to 5.67% (oscalcis QUS) of the early *postpartum* value. The gain in general appeared to be higher in areal BMD values as measured by DXA compared to volumetric BMD values as measured by pQCT. QUS also showed a significant gain in the derived BMD value of 5.6% in the oscalcis. The correlation between the measured BMD gain from the three techniques were fair.
Conclusion
Significant gains in BMD at various bone sites were observable within 24 months after delivery, using both radiological methods (DXA and pQCT) for areal and volumetric BMD measurement as well as by QUS of the oscalcis. The uniform gain in BMD at all sites demonstrated clearly the recovery of BMD loss from pregnancy. There was good agreement between results from radiological assessment as compared to QUS.

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## PP270

### FRAX underestimates fracture risk in Korean postmenopausal patients with diabetes: The Korea National Health and Nutrition Examination Survey 2008–2009

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Background
Patients with type 2 diabetes (T2DM) have an increased risk of osteoporotic fracture than controls without diabetes. The aim of this study was to compare the 10-year probability of hip fracture and a major osteoporotic fracture using the FRAX algorithm between Postmenopausal Korean women with and without type 2 diabetes mellitus.
Methods
We used data from the Fourth Korea National Health and Nutrition Examination Survey 2008–2009. The measurements of anthropometric parameters and bone mass were obtained using dual energy X-ray absorptiometry (DXA) (Discovery-W, Hologic, Inc., USA). Data was available for a total of 1687 postmenopausal women (1584 without T2DM and 103 with T2DM) aged 40–90 years old. The FRAX 10-year probability was computed using the algorithm available online at http://www.shef.ac.uk/FRAX (South Korea version). To compare the 10-year probability of osteoporotic fracture between the groups with and without adjustment for age and weight, a complex-Sample general linear model was used.
Results
Mean 10-year probabilities of fracture were similar between groups for major fractures (diabetic 8.9 (8.0, 9.9, 95% CI) vs non-diabetic 8.1 (7.8, 8.4, 95% CI), $P = 0.105$) and hip fractures (diabetic 3.5 (2.7, 4.2, 95% CI) vs non-diabetic 2.9 (2.7, 3.1, 95% CI), $P = 0.161$). After adjustment for confounding factors (age and weight), there was also no significant difference between the diabetic and non-diabetic group (major osteoporotic fracture: 8.4 vs 8.1, $P = 0.485$ and hip fracture: 3.2 vs 2.9, $P = 0.346$).
Conclusion
FRAX did not reflect higher major osteoporotic and hip fracture risk in Korean T2DM patients. T2DM might be considered for inclusion in future iterations of FRAX.

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## PP271

### Secondary causes for osteoporosis significantly contribute to fracture risk in patients with osteopenia and a recent fracture

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Introduction
The reported prevalence of secondary causes for osteoporosis in men and women aged $\geq 50$ years with a fracture is 35–60%, but data on these causes are scarce in patients with osteopenia and fractures.
Objective
To investigate whether secondary causes for osteoporosis are prevalent and may contribute to fracture risk in patients aged $\geq 50$ years with osteopenia and a recent fracture.

Materials and methods
Consecutive patients of both genders aged ≥ 50 years presenting with a recent fracture over an 18-month period, and who had osteopenia, were evaluated using FRAX and laboratory investigations for screening for secondary causes for osteoporosis. Patients with a fracture and osteoporosis were used as controls.
Results
Of 553 patients presenting with a fracture, 30% had osteoporosis and 56% osteopenia. In this latter group median age was 66 years, male:female ratio was 1:3 and 76% had greater than or equal to one identifiable secondary cause for osteoporosis compared to 81% of patients with osteoporosis. Mean FRAX score was 10%/3% and captured 48% of secondary causes for osteoporosis: smoking (16%), excessive alcohol use (14%), corticosteroid use (13%), rheumatoid arthritis (2%) and other secondary causes including insulin dependent diabetes, hyperthyroidism, hypogonadism and early menopause (20%). On additional laboratory investigations 45% had serum-25OHD $<50$ nmol/l, 11% impaired renal function (eGFR $<60$ ml/min), 10% monoclonal gammopathy, and 2 and 6% primary and secondary hyperparathyroidism, respectively. There was no difference in prevalence of secondary causes for osteoporosis between patients with osteopenia and osteoporosis. There was a significant relationship between number of secondary causes for osteoporosis and mean BMD at the femoral neck. In patients with osteopenia, 54% of secondary causes were identified by laboratory investigations, 32% were amenable to lifestyle changes and 39% were treatable.
Conclusion
Our findings suggest a high prevalence of potentially reversible secondary causes for osteoporosis which may contribute to fracture risk by altering bone quality in patients with osteopenia and a recent fracture.

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## PP272

**Serum 25-hydroxyvitamin D levels of healthy adult women in Greece**

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Objective
The objective of this observational cross-sectional study is to identify the prevalence of vitamin D deficiency in healthy women in Greece, as reflected by the levels of 25-hydroxyvitamin D (25(OH)D), since recent data indicate that vitamin D deficiency can be common in countries previously considered as low risk (e.g. Mediterranean countries).
Materials and methods
A population of 840 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. Serum total calcium (Ca), phosphorus (P), creatinine, parathyroid hormone (PTH) and 25(OH)D were measured. The study was approved by the Ethics Committee of Harokopio University.
Results
The mean age of the population was 50.33 years (range, 20–86 years) while 86.9% were 20–65 years old. Mean serum 25(OH)D was 19.95 ng/ml, mean PTH was 41.16 pg/ml and mean Ca, P and creatinine were 9.50, 3.63 and 0.77 mg/dl respectively. Concerning the vitamin D levels, 55.3% of the subjects had deficient (0–19.9 ng/ml), 34.2% had insufficient (20–29.9 ng/ml) and only 10.5% had adequate (30–150 ng/ml) levels, while 8.0% had vitamin D levels $\leq 10$ ng/ml. PTH was at normal range (15–65 pg/ml) for 89.7% of the population and 8.2% had high PTH ($>65$ pg/ml). The levels of serum Ca, P, and creatinine, were within the normal range.
Conclusions
The majority of Greek women (88%) in this study had vitamin D levels below 30 ng/ml. Given that low levels of 25(OH)D are associated with increased risk for fractures and exacerbate bone loss, this study highlights the emerging issue of 25(OH)D insufficiency in Greek women and the need for targeted interventions even in age groups not previously considered as at risk.
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## PP273

**Adipokines and grelin, nutritive status and bone metabolism in chronic obstructive pulmonary disease**

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Objective
To determine associations between the adipokines tumor necrosis factor-α (TNF-α) and its receptors sTNFR I, II, leptin, adiponectin, resistin, ghrelin, pulmonary function testing, bone mineral density (BMD) and nutritive status in severe COPD.
Materials and methods
We determined BMD, serum adipokine and ghrelin in 48 patients with severe COPD and and 52 age and sex matched controls.
Bone density at lumbar spine (LS) and femoral neck (FN) and parameters of body composition were measured by dual-energy X-ray absorptiometry.
Results
The levels of adiponectin, resistin, TNF-α and its receptors sTNFR I, II were higher, but leptin and ghrelin levels were low in COPD than in controls. It was a direct correlation between leptin level ($r=0.58$, $P<0.05$ at LS; $r=0.64$; $P<0.01$ at FN) and negative relationship between adiponectin ($r=-0.54$, $P<0.05$ at L2–L4; $r=-0.62$; $P<0.01$ at FN), TNF-α ($r=-0.58$, $P<0.01$ at L2–L4; at FN $r=-0.64$, $P<0.01$) and BMD. It was negative association between serum resistin ($r=-43$, $P<0.05$), sTNFR-I ($r=-41$, $P<0.05$), sTNFR-II ($r=-0.44$; $P<0.05$) and positive correlation with ghrelin ($r=0.42$, $P<0.05$) and BMD at FN only. Leptin, ghrelin concentrations had direct relationship with BMI, fat mass. Adiponectin level significantly inversely correlated with BMI, fat mass, resistin and sTNFR-I, II concentrations. It was correlations between $SaO_2$, $pCO_2$, $pO_2$ and TNFR I, II, resistin, and the relationship between leptin, adiponectin, TNF-α levels and pCO2.
Conclusion
Results shows possibly role of adipokines and ghrelin in the increasing of bone loss in several COPD. However, larger studies are needed to further evaluate the relationship between adipokines, body weight, and BMD in patients with COPD.
Key words
bone mineral density, osteoporosis, TNF-α, leptin, adiponectin, ghrelin, resistin, chronic obstructive pulmonary disease, adipokines

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## PP274

**Hyperuricemia, bone mineral density and TBS of Ukrainian men**

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Aim of research
To determine the prevalence of hyperuricemia affecting the Ukrainian men in relation to the bone mineral density and TBS.
Object of research
The Ukrainian men ($n=132$), age of the examined patients –from 50 to 80 years. Average age of examined patients was $58.2 \pm 1.3$ years. According to the levels of uric acid in the blood serum, all patients were divided in four quartiles.
Methods of research
Uric acid level in blood plasma was determined by the uricase-peroxidase method, bone mineral density – by means of the 'Prodigy' unit (CE Medical systems, model 8743, 2005). The TBS was evaluated using the installed TBS iNsight software for an X-ray densitometer (Med-Imaps, Pessac, France).
Results
The rate of hyperuricemia affecting the Ukrainian men was 23% in the age group of 50–59 years old, 33% – in the age group of 60–69 years old, 29% – in the age group of 70–79 years old. The frequency of osteoporosis in men with hyperuricemia was lower compared with men who had a normal level of uric acid (4 and 17% at the level of the lumbar spine, and 4 and 15% at the level of femoral neck). Bone mineral density was significantly higher in case of men having the highest levels of uric acid in the lumbar spine ($F=2.78$; $P=0.04$), radius 33% ($F=3.96$; $P=0.01$) and total body ($F=2.70$; $P=0.04$). TBS was significantly higher in the patients who had the lowest levels of uric acid compared with the patients who had the highest level of uric acid ($Q1=1.17 \pm 0.02$, $Q4=1.04 \pm 0.02$; $P<0.05$).
Conclusions
We determined that men with the low levels of uric acid had the significantly lower levels of bone mineral density, but the TBS in men who have the highest levels of uric acid is higher.

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## PP275

**Influence of vitamin D deficiency to structural and functional state of bone tissue in schoolchildren**

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The aim of the work was to determine the influence of vitamin D deficiency on bone mineral density in schoolchildren.
Methods
There were examined 304 children aged 10–18 years. The boys consisted 55.0%. The average age of boys was $12.9 \pm 0.2$ and girls – $12.4 \pm 0.2$ years old. Researches included ultrasound densitometry of calcaneus by SAHARA (Hologic), blood chemistry, 25(OH)D and intact parathyroid hormone (iPTH) in plasma were determined by Elecsys 2010. Also, it was evaluated the average content of calcium and vitamin D in the diet form the products consumption frequency questionnaire.
Results
Vitamin D deficiency was founded in 92.2% of schoolchildren, and vitamin D insufficiency was diagnosed in 6.1% of cases. Secondary hyperparathyroidism was verified in 0.9% of children. The average level of consumption of calcium and vitamin D in children was below recommended data, and consisted (Me 649 (488.7; 691.86)) mg/day for calcium and (Me 68.69 (58.45; 117.3)) IU/day for vitamin D.
Children with vitamin D insufficiency had significantly higher data of structural and functional state of bone tissue in comparison with the data of pupils with severe deficiency of vitamin D: stiffness index $105.03 \pm 6.12$ vs $93.7 \pm 2.51\%$ ($P < 0.02$); BMD $0.574 \pm 0.024$ vs $0.528 \pm 0.019$ ($P < 0.02$) and speed of sound $1573.61 \pm 6.70$ vs $1557.2 \pm 5.41$ ($P < 0.01$).
Conclusion
High level of vitamin D deficiency (92.2%), secondary hyperparathyroidism (0.9%), low data of ultrasound densitometry in severe vitamin D deficient children make doctors to research the effective methods of treatment and prophylactics of revealed disorders.

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## PP276

**Is it necessary to consider fracture site in osteoporosis?**

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Objectives
To compare the incidence of osteoporosis and the related factors among fracture sites in above 50-year-old patients with fractures caused by low-energy trauma.
Materials and methods
714 patients with fracture from low energy trauma were evaluated retrospectively. By the dual-energy X-ray absorptiometry, we measured bone mineral density BMD at lumbar spine and proximal femur, and compared the incidence of osteoporosis, age, sex, BMI, previous fracture history, past osteoporosis medication history according to each fracture sites.
Results
BMD was decreased according to increasing age with statistical significance ($P < 0.001$). Sex has no significant difference according to fracture site ($P = 0.141$). Average age of patients with osteoporotic fracture was 73.8, 72.8, 66.3, 73.4, 78.3 years old according to fracture site as T-spine, L-spine, distal radius, proximal humerus and proximal femur, respectively. There was significant difference among groups ($P < 0.001$). Average BMI related with osteoporotic fracture site was 22.9, 22.7, 23.4, 23.0, 21.7 kg/m$^2$, respectively and it showed significant difference among groups ($P < 0.001$). Average BMD ($T$-score) related with osteoporotic fracture site was 0.587 g/cm$^2$ ($-3.5$), 0.614 g/cm$^2$ ($-3.1$), 0.647 g/cm$^2$ ($-2.6$), 0.597 g/cm$^2$ ($-3.1$), 0.554 g/cm$^2$ ($-3.5$), with significant difference among groups ($P < 0.001$). Previous fracture history had no significant difference among groups ($P = 0.078$). Previous osteoporosis medication history had significant difference among the groups ($P < 0.001$).
Conclusions
In conclusion, consideration of fracture site may be necessary for prevention and treatment of osteoporosis.

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## PP277

**Quantitative ultrasonometry of the phalanges in post-menopausal women with type 2 diabetes mellitus: the first results of a 3-year longitudinal study**

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Objectives
Type 2 diabetes mellitus (T2DM) is associated to an higher risk of fractures despite a normal or increased bone mineral density measured by dual-energy X-ray absorptiometry (DXA).
The purpose of this 3-years longitudinal study was to assess the changes of quantitative ultrasound (QUS) parameters in a group of postmenopausal women with T2DM and in healthy controls.
Materials and method
The analyses were performed on a group of 35 postmenopausal women attending to the OSTEOLAB within the (Prevention of Osteoporosis ad Fracture) PROF project in collaboration with the Health Local Authority of Brindisi. We selected 17 women affected by T2DM and 18 healthy controls aged 55–70 years. Subjects had baseline and 40 months follow-up measurements of phalangeal ultrasonometry, performed using DBM Sonic Bone Profiler 1200 (Igea) as well as information about medical history, current drug therapy and risk factors.
Results
General characteristics and phalangeal QUS measurements in the two groups are reported and show at baseline only ultrasound bone profile index (UBPI) was significantly lower in T2DM group ($P < 0.05$), instead at follow up were significantly lower bone transmission time (BTT) ($P < 0.01$) and amplitude dependent speed of sound (AD-SoS) ($P < 0.02$) in the same group.
During the period of the study we found an increase of BMI in the two groups of 82.3 and 55.7%, respectively in T2DM group and healthy control.
The decrease of BTT and UBPI was significantly higher in T2DM group ($P = 0.01$ and $P = 0.03$ respectively).
Conclusions
Among phalangeal QUS parameters, AD-SoS, usually associated to bone mineral density and the most used predictor parameter of fractures risk in clinical practice not showed difference between baseline and follow-up measurement in both group. Conversely BTT and UBPI, that give information about bone mineral quality, resulted decreased in T2DM subjects suggesting a future role in the clinical practice for the diagnosis of alterations of bone micro-architecture in T2DM subjects.

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## PP278

**Fluoride exposure accelerates the development of postmenopausal osteoporosis: animal model**

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Using ovariectomized rats as an animal model of postmenopausal women, we examined the causal relationship between fluoride (F) exposure and the high risk of development of osteoporosis. In order to obtain the estrogen (Es) deficient animals, 5-week-old Sprague–Dawley rats, which were overiectomized at 3- and the 5-week-old female rats without ovariectomy were purchased. The overiectomized rats were divided into two groups: the Es deficient, and the combination of Es deficiency and F exposure groups. Also rats without ovariectomy were divided into two groups: the F exposure and the control groups. Rats of both the F exposure and combined groups were given free drinking water containing 1.0 mg/l F ions (NaF), while the control and Es deficient rats were given tap water. Three months later, the samples of calvaria were subjected to soft X-ray radiography and electron microscopy. In both the Es deficient and F exposed rats, soft X-ray radiography demonstrated an increase of radiolucent areas of the calvaria at a certain degree. However, this trend was prominent in combined group rats, resulting in the appearance of a labyrinthine pattern. In the tibia, light microscopy revealed a significant decline of trabecular architecture in this same group, suggesting the onset of osteoporosis. Electron microscopy demonstrated an increase of amorphous minerals in the radiolucent areas of the calvaria. However, we could not observe a significant increase of osteoclast number. From these findings, it appears that F exposure may accelerate

the development of postmenopausal osteoporosis even at a low dose of F ions. Moreover, it is suggested that the primary cause of osteoporosis may result from a decline of the bone formation rather than excessive bone resorption. The animal protocol was approved by the Animal Care and Use Committee of Meikai University.

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## PP279

**Influence of vitamin D deficiency on bone turnover markers in men of different age**

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Introduction
Optimal 25(OH)D serum level is very important for bone health and calcium–phosphate homeostasis as well as for optimal function of many organs and tissues. The consequences of vitamin D deficiency are mineralization defects, which may lead to osteomalacia in the long-term, and muscle weakness, causing falls and fractures.
Objectives
The aim of the research is to determine the frequency of vitamin D deficiency and it's influence on bone turnover markers in men of different ages.
Methods
There were examined 215 men, aged (54.33 ± 1.74) years. The level of 25(OH)D, iPTH, bone turnover markers (osteocalcin – marker of turnover rate, β-CTx – marker of resorption, and P1NP – marker of bone formation) were evaluated by electrochemiluminescence method (Elecsys 2010, Roche). Vitamin D deficiency was defined as a 25(OH)D below 20 ng/ml (50 nmol/l), and vitamin D insufficiency as 25(OH)D of 21–29 ng/ml (50.1–74.9 nmol/l).
Results
Only 6.0% of examined men had optimal 25(OH)D level. Vitamin D insufficiency was diagnosed in 18.7%, and vitamin D deficiency was recorded in 75.3% observed patients. Severe vitamin D deficiency (25(OH)D level is below 25 nmol/l) was registered in 31.6%. All observed men were divided into four groups according 25(OH)D level: the 1st group included patients with severe vitamin D deficiency, second group – with 25(OH)D level 25–50 nmol/l, third group – with vitamin D insufficiency, and fourth group – with optimal 25(OH)D level.
iPTH level was lower in men of second group (37, 80 (25, 83; 45, 33) pg/ml, and significantly higher in observed with optimal 25(OH)D level (43.15 (26.03; 45.90) pg/ml ($P < 0.05$). It hasn't been found the significant difference in osteocalcin level and bone formation marker (P1NP) in observed men with different 25(OH)D level. The concentration of β-CTx was lower in patients with optimal 25(OH)D level (0.351 (0.251; 0.493)) ng/ml and significantly higher in group with sever vitamin D deficiency (0.545 (0.400; 0.680)) ng/ml ($P < 0.05$).
Conclusion
Only 6.0% of Ukrainian men has optimal level of 25(OH)D in blood serum. Decreasing concentration of 25(OH)D in blood serum leads to increasing the markers of bone resorption. High level of vitamin D deficiency makes doctors to search the effective treatment and prevention methods of revealed disorders.

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## PP280

**Bone health in patients with type 2 diabetes mellitus**

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Patients with type 2 diabetes (T2DM) have an increased risk of hip and vertebral fractures compared to non-T2DM subjects. The bone mineral density (BMD) does not reflect the impaired bone quality in T2DM patients. The aim of the present study was to investigate the relationship between BMD and bone formation (N-terminal propeptide of type I procollagen, PINP; osteocalcin, OC) and bone resorption (cross-linked C-telopeptides of bone type I collagen, CTX) markers, as well as changes in 25 hydroxyvitamin D (25OHD) and plasma sclerostin levels in patients with T2DM and healthy controls. In a cross sectional study, we compared 110 diabetic patients with 103 healty non-T2DM controls. The prevalance of vitamin D insuficiency in T2DM patients was higher compared to healthy controls. Our preliminary data showed that patients with T2DM without osteoporosis have lower bone remodeling markers (CTX, OC, PINP) compared to healthy controls. Patients with T2DM with a vertebral fracture have a lower CTX and OC when compared to T2DM patients with osteoporosis and/or with non-vertebral fracture and also when compared to non-diabetic controls with prevalent vertebral fracture. Serum sclerostin levels were positively correlated with bone mineral density of the lumbar spine, femoral neck and whole body bone density in patients with T2DM. Our findings suggests differences in bone remodeling in T2DM patients that can play an important role in pathogenesis of bone fragility in these patients.

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## PP281

**State of vitamin D sufficiency Ukrainian schoolchildren**

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Background
It is now known that the osteoporosis starts developing childhood. Children's age is characterized by intensive accumulation of bone mass. By the end of puberty the level of bone mass in many parts of the skeleton reaches 86% of the bone mass of an adult and in some parts it reaches 100%.
The processes of bone metabolism closely connected with the level of the vitamin D, in the body because it has a pronounced effect on calcium homeostasis.
Aim
To explore calcium and vitamin D daily intake level, determine the frequency of vitamin D sufficiency to bone mineral density (BMD) in Ukrainian school-children.
Methods
It was examined 304 Ukrainian schoolchildren aged 10–17 years. Of these 118 residents were of the west side, 91 and 95 north-eastern region of the country. Methods conducted chemiluminescent content of 25 OH vitamin D, parathyroid hormone (PTH), diet study using questionnaires, ultrasound densitometry.
Results
Only 2.7% observed had normal calcium intake level, most children (56.8%) took 500 mg of calcium per day. 60.1% children had deficiency of vitamin D in diet. Deficiency of vitamin D serum levels 25 (OH) D was 88.5%, lack of – 8.8% and only 2.6% had normal levels. Fractures were recorded in 16% of patients, 54.5% of them low energy. It was determined negative significant correlation between PTH and 25-OH vitamin D. Low mineral density was registered in 8.7% children. No significant correlation between 25-OH vitamin D and BMD.
Conclusions
High level of vitamin D deficiency, poor calcium and vitamin D level intake, frequent osteopenic syndrome in children make doctors to research the effective methods of treatment and prophylactics of revealed disorders.

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## PP282

**Vitamin D levels in Lebanese population: do we need to alter the base line for insufficiency?**

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Introduction
Vitamin D is essential for calcium metabolism as well as for fracture prevention. It plays a major role in bone health. A high prevalence of inadequacy has been reported in many studies. A serum 25-hydroxy vitamin D level of 30 ng/ml (75 nmol/l) has been proposed as the minimum for adequate vitamin D nutrition. The prevalence of inadequacy in different regions of the world has not been well characterized. The aim of this study was to evaluate and propose new base line for insufficiency.
Subjects and methods
Assessment of vitamin D was performed in 13 000 Lebanese subjects. The reference ranges used in our laboratory for 25-hydroxy vitamin D were as follows: < 10 ng/ml (deficiency), 10–30 ng/ml (insufficiency) and 30–100 ng/ml (sufficiency). The statistical soft ware package SPSS version 17.0 was used for statistical testing of the data.

Results
Age groups, gender and mean vitamin D serum levels will be presented. We consistently found low levels of vitamin D across all age groups in males and females.
Conclusion
A large group of patients fell into the category of insufficiency. We believe that it is important to redefine the vitamin D cut-off across all age groups. We require a new clarification systems to assess the vitamin D levels in our population. We need a new base line for insufficiency. Making the cut-off of vitamin D at 20 ng/ml will shift more than two-thirds of Lebanese population to the level of sufficiency.

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## PP283

**Prevalence of osteopenia using calcaneal quantitaive ultrasound among adults in Dubai**

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Introduction
Osteoporosis is a progressive bone disease characterized by low bone mineral density (BMD), decreased bone strength and increased incidence of fragility fractures. It is one of the major public health conditions, affecting millions of people worldwide. Osteopenia is a potential precursor of osteoporosis and identifying this condition is a pivotal step to institute preventive strategies at younger age, leading to delay in onset or prevention of the disease, thus related morbidity and mortality in older life can be reduced.
Objective
To determine the prevalence of osteopenia in different age groups and to identify the associated factors.
Methodology
A cross-sectional study was carried out among adults working in the governmental sector in Dubai, UAE and assessed using quantitative ultrasound device (QUS) to find the *T*-score for each participant. Results: The prevalence of osteopenia rose progressively with increasing age. It was found that, 15.3% of participants aged 20 to $<$ 30 years and 22.5% of participants aged 30 to $<$ 40 and 28.2% of participants aged 40 to $<$50 years had osteopenia. Moreover, osteopenia was more common in female participants, compared to male participants (26.5 and 17.6% respectively). Family history of osteoporosis, history of traumatic fractures and history of vitamin D deficiency were significantly associated with osteopenia ($P<0.05$).
Conclusion
The prevalence of osteopenia is high. Health-related policymakers and programs planners in the country need to address this problem and apply early intervention methods to prevent or delay a chronic condition such as osteoporosis.

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## PP284

**Low referral rate of lebanese males for vitamin D testing**

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Aim
Low serum vitamin D levels are common in sunny Lebanon. Vitamin D is essential component of osteoporosis management strategies. This study surveys the referral rate of Lebanese males for vitamin D testing.
Subjects and methods
A 13 000 Lebanese subjects included in this study were coming for routine check-ups. Patients on renal dialysis and cancer patients on chemotherapy were excluded. Serum 25-hydroxy vitamin D is considered the best indicator of vitamin D status. Insufficiency refers to those who have vitamin D blood levels lower than 30 ng/ml.
Results
Gender, age distribution and mean vitamin D levels.
Conclusions
A low referral rate of males was noticed across all age groups with significant proportion of individuals have vitamin D inadequacy as defined by a low ($<$30 ng/ml) serum concentration of 25-hydroxy vitamin D, regardless of age. Greater awareness of the importance of vitamin D among males is needed, as vitamin D adequacy is essential for optimal calcium metabolism and skeletal health.

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## PP285

**Osteoporosis risk assessment in a welsh district general hospital**

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Objective
It was to determine the prevalence of clinical risk factors for osteoporosis in medical inpatients.
Method
Data was collected on a patient questionnaire through a point prevalence study targeting patients aged 50 and above.
Results
117 patients (62 females and 55 males) met the age criteria. Non-modifiable risk factors were white/Caucasian race (100%), age 75 years and above (66%), female sex (53%), previous fragility fracture (14%) family history of osteoporosis and fragility fractures (3.4%). Potentially modifiable risk factors were restricted mobility (54%), low BMI (28.2%), frequent falls (17%), smoking (14.5%), excess alcohol use (7.7%). Secondary causes of osteoporosis (58%) were chronic kidney disease (21%), insulin dependent diabetes mellitus (6.8%), rheumatoid arthritis (5.9%), hormone therapy for breast cancer/prostate cancer (4.3%), inflammatory bowel disease (4.3%), chronic liver disease (4.3%), hyperthyroidism (1.7%), hyperparathyroidism (0.85%), premature menopause (0.85%) and Paget's disease of bone (0.85%). 11% were on long term oral steroids. 100% of study population had at least two risk factors. 71% were at high risk for osteoporosis with more than three risk factors.
Conclusion
Our study shows that clinical risk factors for osteoporosis are common. Majority of them had multiple risk factors. Screening hospitalised patients for osteoporosis can be utilised as an additional opportunity to identify and treat the disease and thereby reduce the burden of osteoporosis related falls and fractures. Ideally, all the patients at high risk clinically should be offered further assessment for osteoporosis. The literature suggests an additive effect for risk factors and guides us to prioritise patients who would need a DEXA scan thus enabling an efficient use of scarce resources.

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## PP286

**Striking osteoporosis following prolonged immobilization; a case report**

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Introduction
Bone is a dynamically changing organ. Reduction of mechanical loading on bone inhibits bone formation and accelerates bone resorption. Prolonged immobilization in casts, therapeutic bed rest and application of external fixations to treat fractures are common causes of disuse osteoporosis.
Case Report
We report a case of striking osteoporosis in a 11 years old Syrian boy with a complex open right leg wound following a blast injury (24.03.2013). He presented to ER with compound comminuted fractures of tibia and fibula with bone and soft tissue loss and vascular insult that left his ankle and foot cold and dark blue colored. He underwent immediate surgical intervention where all wounds were debrided thoroughly and a neurovascular repair was carried out after immobilization of fractures with external fixation system (orthofix). Following surgery palpable pulses were present distally but motor and sensory examination could not be obtained. Antibiotics and anti-tetanic therapies were administered. During and after surgery 12 units of PRBC's and FFP's were needed to restore normal BP. He was taken to OR four times in the 2 weeks following the initial surgery and underwent debridement of non viable tissues. On 09.04.2013 a cross leg myo-cutaneous flap was executed successfully and was divided 6 weeks later. Patient and his family fled to Lebanon in 10.09.2013 and presented to our OPD viable leg in external fixation device. Plain films and VCT study showed extensive bone tissue loss and loose four pins of the fixation device. The amount of bone lost was quite severe and patient will need aggressive and continuous treatments and his disuse osteoporosis requires a long time to recover.

Discussion
The afflicted extremity spent several months in external fixation device and this lead to striking osteoporosis distal to and including the area of injury. The period of recovery of these cases is several times longer than the period of bone loss and recovery varies widely. Hence, we have to keep in mind that there is no treatment better than prophylaxis of neglected disuse osteoporosis.

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## PP287

**Vitamin D status in sunny Lebanon**

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Introduction and aim
Vitamin D plays an important role in bone growth. In children, low levels can cause rickets. In adults, low levels can lead to osteoporosis and fragility fractures. The aim of this survey was to get information about vitamin D levels in Lebanese population.
Subjects and methods
Serum 25-hydroxy vitamin D level is considered to be the best indicator of vitamin D status. 13 000 subjects included in this study were coming for routine check-ups. Insufficiency refers to those with vitamin D levels below 30 ng/dl.
Results
Age distribution and mean of vitamin D levels will be presented.
Conclusions
We found a high prevalence of moderate to severe vitamin D levels across all age groups.
Greater awareness of the importance of vitamin D level is needed as well as more aggressive supplementation especially in age groups at high risk of inadequacy.

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## PP288

**Decrease in expression of MMP3 in osteoblast protects against bone loss**

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Osteoblastic MMPs are important actors of bone remodeling. We showed previously that osteoblast specific overexpression of TIMP1, an inhibitor of MMPs, prevents the bone loss induced by ovariectomy mainly through inhibition of bone resorption. We hypothesized that inhibition of the MMP3 could be implicated in the protective effect of TIMP1 and that MMP3 polymorphisms located in the proximal promoter of MMP3 gene could be associated to post-menopausal bone loss in women.
We evaluated the effect of MMP3 invalidation on bone mass in mice and after ovariectomy. MMP3 knockout (KO) mice exhibited significantly higher bone mineral density (BMD) compared to WT mice. MMP3 invalidation leads, alike TIMP1 overexpression, to a protective effect against bone loss induced by ovariectomy: BMD is not significantly different between ovariectomized and sham-ovariectomized MMP3 KO mice. Furthermore, plasmatic D-PYR was lower in sham-operated compared to ovariectomized MMP3 KO mice suggesting involvement of MMP3 in bone resorption.
We identified several polymorphisms within the 2 kb proximal MMP3 promoter that could result in modification of gene expression. We generated plasmids containing increasing fragments of the human MMP3 promoter containing one or more polymorphisms, cloned upstream of the luciferase gene. We used a DNA collection, established with approval of ethical committee, from a cohort of 548 post-menopausal women. Only women who had a complete bone phenotype characterization and who had no anti-osteoporotic treatment or concomitant disease were considered. Our analyses showed that three polymorphisms (rs522616, rs302590 and rs3025058) are associated to an up-regulation of MMP3 promoter activity. Moreover, women carrying the variant 5A/5A (rs3025058) showed higher *Z*-score at the femoral neck compared to the other genotypes.
In conclusion, our data indicates that invalidation of MMP3 protects against bone loss in mice. Moreover, SNPs located in its promoter affect MMP3 gene expression and are associated to bone density in post-menopausal women.

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## PP289

**The impact of common comorbidities (as measured using the Charlson index) on hip fracture risk in elderly men: a population-based cohort study**

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Purpose
To determine whether and which co-morbidities (amongst those included in the Charlson co-morbidity index) confer an increased risk of hip fracture amongst elderly men.
Methods
We conducted a population-based cohort study using a population database which contains primary care and hospital inpatient records of over >2 million people. All men aged ≥65 years registered on 1/1/2007 were followed up until 31/12/2009. Both exposure (co-morbidities in the Charlson index) and outcome (incident hip fractures) were ascertained using ICD codes. Poisson regression models were fitted to estimate the effect of each individual co-morbidity and the composite Charlson index score, on hip fracture risk, after adjustment for age, BMI, smoking, alcohol drinking, and use of oral glucocorticoids.
Results
We observed 186 171 men for a median (inter-quartile range) of 2.99 (2.37–2.99) years. In this time, 1.718 (0.92%) of them had a hip fracture. The following co-morbidities were independently associated with hip fractures: diabetes mellitus (adjusted RR 1.43 (95% CI 1.25–1.69)), chronic obstructive pulmonary disease (COPD); (adjusted RR 1.20 (95% CI 1.03–1.40)), renal failure (adjusted RR 1.32 (95% CI 1.07–1.65)), HIV infection (adjusted RR 5.03 (95% CI 1.25–20.21)), dementia (adjusted RR 1.65 (95% CI 1.30–2.09)) and cerebrovascular disease (adjusted RR 1.51 (95% CI 1.27–1.80)). A Charlson score of 3 or ≥4 conferred an increased hip fracture risk (adjusted RR 1.52 (95% CI 1.26–1.83) and 1.53 (1.24–1.88) respectively).
Conclusion
Common comorbidities including diabetes, COPD, cerebrovascular disease, renal failure, and HIV infection are independent predictors of hip fracture in elderly men. A Charlson score of 3 or more is associated with a 50% higher risk of hip fracture in this population.

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## PP290

**Novel evidence that apolipoprotein A-I deficiency is implicated in the pathogenesis of osteoporosis in mice**

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Introduction
Recent data suggest that lipid metabolism imbalances affect osteoblast and osteoclast function resulting in altered bone mass quality and quantity. Here we investigated the role of apolipoprotein A-I (apoA-I), key-element in HDL biogenesis in the pathogenesis of osteoporosis in mice.
Materials and methods
Lumbar vertebrae and femora from apoA-I deficient (*ApoA-I*$^{-/-}$) and WT (*ApoA-I*$^{+/+}$, WT) mice were used for histological, histomorphometrical, spectrometric and *in vitro* analyses. Osteoclast precursors and bone marrow mesenchymal stem cells (BMMSCs) were isolated, cultured and differentiated towards osteoclasts and osteoblasts, respectively. BMMSC were assessed for PPARγ and Runx2 expression, using western blotting, flow cytometry and qRT-PCR. Differentiated osteoblasts were stained with von Kossa and ALP and

examined for Runx2, osteopontin, osteocalcin, RankL, osteoprotegerin and Col1a1 expression. Differentiated osteoclasts were subjected to TRAP staining and qRT-PCR for Rank, Trap and cathepsin-K mRNA expression.
Results
MicroCT analysis revealed significantly reduced bone mass and Raman spectometry remarkably reduced collagen cross-linking in the *ApoA-I*$^{-/-}$ compared to the WT mice. Dynamic histomorphometry uncovered decreased osteoblast surface, bone formation and mineral apposition rate in *ApoA-I*$^{-/-}$ mice. Notably, no changes were observed in osteoclast resorption areas. BMMSCs from *ApoA-I*$^{-/-}$ mice displayed significantly decreased Runx2 but augmented PPARγ expression. Von Kossa and ALP stains were less extensive in the *ApoA-I*$^{-/-}$ compared to WT mice osteoblasts. RankL and osteoprotegerin mRNA levels were similar in the two groups, while Col1a1, osteopontin and osteocalcin mRNA expression were decreased in *ApoA-I*$^{-/-}$ mice. In accordance with TRAP staining, Rank, Trap and cathepsin-K mRNA levels showed no significant differences between osteoclasts from the two experimental groups.
Conclusions
Our findings provide novel evidence that ApoA-I deficiency diversely affects osteoblastogenesis and lipoblastogenesis resulting in impaired bone synthesis/quality, while osteoclast function remains unaffected. We hypothesize that perturbation in HDL metabolism might be implicated in the pathobiology of osteoporosis in mice.

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## PP291

**Apolipoprotein-E deficiency predisposes to the development of osteoporosis following long-term exposure to western-type diet, in mice**

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Introduction
Recent data suggest that lipid metabolism imbalances affect bone cell function and therefore may result in the development of osteoporosis. We investigated the role of apolipoprotein-E (ApoE), a plasma protein playing cardinal role in lipoprotein metabolism, in the regulation of osteoblast and osteoclast function and the pathogenesis of osteoporosis.
Material and methods
We used apoE deficient (ApoE$^{-/-}$) and C57BL/6 (control) mice fed chow (CD) or western-type diet (WTD) for 24 weeks. Calcein was injected for the determination of new bone formation rate. Following sacrifice, lumbar vertebrae and femora were removed and cortical and cancellous bone quality was evaluated using microCT. TRAP stain was used for osteoclasts detection and von-Kossa for mineralized bone visualization. Dynamic histomorphometry was employed for the assessment of bone formation–degradation rate. Bone marrow mesenchymal stem cells (BMMSC) were isolated from mice femora and then assessed for the expression of the osteoblastic and lipoblastic regulators, Runx2 and PPARγ respectively, using western blotting, flow cytometry and RT-PCR.
Results
i) ApoE$^{-/-}$ WTD did not develop obesity and their BM was devoid of adipocytes, in contrast to the control mice. ii) Osteoclast number was significantly increased, while bone synthesis was significantly reduced in ApoE$^{-/-}$ WTD mice, compared to the other groups. iii) Static and dynamic histomorphometry showed that ApoE$^{-/-}$ WTD developed osteoporosis. iv) BMMSCs from ApoE$^{-/-}$ WTD mice displayed significantly reduced Runx2 expression at both protein and mRNA levels compared to the other groups. v) PPARγ expression was significantly increased in ApoE$^{-/-}$ CD mice in contrast to the ApoE$^{-/-}$ WTD and the other animal groups.
Conclusions
i) ApoE deficiency affects osteoblast and osteoclast function and thus bone remodeling. ii) The absence of ApoE prevents obesity and BM adipocity, but predisposes to the development of osteoporosis following exposure to WTD, in mice.

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## PP292

**CXCL8 and CCL20 enhance osteoblast-mediated osteoclastogenesis**

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Osteoporosis is common in rheumatoid arthritis (RA). Since osteoblasts express receptors for CXCL8 and CCL20, which are produced by inflammatory cells around the inflamed joints in RA, we hypothesized that CXCL8 and CCL20 contribute to osteoporosis in RA by affecting osteoblast proliferation, differentiation, and osteoblast-osteoclast communication.
Primary human osteoblasts were cultured ± CXCL8 (2–200 pg/ml) and CCL20 (5–500 pg/ml) for 14 days. Osteoblast proliferation and differentiation were analyzed. IL6 production was quantified by ELISA. Human peripheral blood mononuclear cells were cultured with conditioned medium from CXCL8 and CCL20-treated osteoblasts ± IL6 inhibitor for 21 days. The number of TRACP-positive osteoclasts was counted, and osteoclast activity was determined by the resorption pit assay.
CXCL8 (200 pg/ml) enhanced mRNA expression of Ki-67 in osteoblasts by upto 2.7-fold, ALP by 1.7-fold, and IL6 concentration by 1.2-fold. CXCL8-conditioned medium enhanced osteoclast number by 1.7-fold (three to five nuclei), and 3.0-fold (greater than five nuclei). IL6 inhibition reduced these numbers by 40%.
CCL20 (500 pg/ml) enhanced mRNA expression of Ki-67 in osteoblasts up to 2.5-fold, ALP by 1.6-fold, and IL6 concentration by 1.3-fold. CCL20-conditioned medium enhanced osteoclastogenesis by 1.3-fold (three to five nuclei), and 2.8-fold (greater than five nuclei), IL6 inhibition reduced these numbers by 30%. CCL20-conditioned medium increased osteoclast activity by 2.2-fold. Neither CXCL8 nor CCL20 directly affected osteoclastogenesis.
CXCL8 and CCL20 did not negatively affect osteoblast proliferation or differentiation. However, both CXCL8 and CLC20 enhanced osteoblast-mediated osteoclastogenesis, partly via stimulation of IL6 production, suggesting that CXCL8 and CCL20 contribute to localized and generalized osteoporosis in RA.

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## PP293

**Inhibition of PDE5 decreases bone mass through inhibiting canonical Wnt signaling**

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Wnt/β-catenin signaling, also called canonical Wnt signaling, is important for regulating bone formation. Previous studies reported that deletion of secreted frizzled-related protein (sFRP1), a Wnt antagonist, enhanced trabecular bone accrual in adult mice. Tadalafil, an inhibitor of phosphodiesterase 5 (PDE5), is widely used for treating male erectile dysfunction (ED) in clinical. Inhibition of PDE5 increases cGMP levels and then activates cGMP-dependent protein kinase (PKG). However, whether PDE5 plays a role in regulating canonical Wnt signaling to affect bone formation remains unknown. Here we find that PDE5 inhibitor, Tadalafil, can decrease bone mass through inhibiting canonical Wnt signaling. First, Tadalafil treatment significantly decreased *lef1*-luciferase activity and β-catenin protein level induced by Wnt3a in 293T without affecting β-catenin mRNA expression. P33/37/41β-catenin level and GSK3β activity increased following Tadalafil treatment. Also, knockdown of PKG2 increased *lef1*-luciferase activity and inhibited GSK3β activity. Secondly, Tadalafil treatment decreased ALP activity and osteoblast marker genes expression in C310T1/2. Finally, adult C57 and sFRP1 −/− mice had a lower bone mass than control mice after Tadalafil treatment for 2 months. These studies demonstrate that Tadalafil negatively regulating canonical Wnt signaling through GSK3β to decreases bone mass.

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## Osteoporosis: treatment

### PP294

**Targeting the sealing zone, a novel strategy to prevent bone degradation while maintaining bone formation: *in vivo* proof of concept in three models of pathological bone loss**

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Molecules secreted by the osteoclast or 'clastokynes' are essential to stimulate bone formation by osteoblasts. Treatments with bisphosphates and Denosumab target osteoclast survival and differentiation. This suppresses bone turnover and is suspected to increase the risk of atypical fractures in the long term. A solution to overcome this is to develop strategies that target selectively the activity of osteoclasts without affecting their survival or differentiation. So far, this relies on the development of Cathepsin K inhibitors that do prevent osteoclast activity and preserve bone formation.

Here we propose the alternative solution to specifically target the organization of podosomes in osteoclasts. Preventing sealing zone formation by affecting the patterning of podosomes impairs the acidification of the extracellular medium by osteoclasts, which renders bone resorption ineffective. We reported earlier that Dock5 controls podosome patterning for sealing zone formation and the development of a chemical compound C21 that inhibits the activation of the GTPase Rac by Dock5. C21 prevents bone degradation by osteoclasts in culture (Vives *et al. JBMR*, 2011).

We used C21 to test if targeting podosome organization in osteoclasts could prevent osteolysis *in vivo*. We show that C21 indeed destabilizes podosomes organization rapidly and reversibly, in cultured osteoclasts. We further report that administration of C21 efficiently protects mice against bone loss, using established mouse models of post menopause osteoporosis, rheumatoid arthritis and bone metastases. C21 had no noticeable adverse effect in the mouse. Most interestingly, bone formation parameters (MS/BS, MAR and BFR/BS) are not affected by C21, while they are severely diminished in mice treated with Alendronate.

Our results provide the proof of concept that a chemical compound that destabilizes podosome organization in osteoclasts efficiently protects against pathological bone loss without affecting bone formation. Our findings open new avenues to develop innovative strategies for the treatment of osteolytic bone diseases.

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### PP295

**Bisphosphonate treatment of postmenopausal osteoporosis and circulating levels of DKK1 and sclerostin: effects of treatment regimens**

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The coupling of bone formation to bone resorption during treatment of postmenopausal osteoporosis with antiresorbers might be related to changes in Wnt/β-catenin signalling. We compared the effects of two bisphosphonate treatments on two Wnt inhibitors sclerostin (SOST) and Dickkopf-related protein 1 (DKK1). The study population included 72 women with postmenopausal osteoporosis participating simultaneously in two multicenter, placebo controlled trials. The patients were randomized to: intramuscular clodronate 100 mg/week (CLO) ($n=36$), yearly i.v. therapy with 5 mg zoledronate (ZOL) ($n=16$) and placebo ($n=20$). Bone turnover markers (intact *N*-propeptide of type I collagen (P1NP), C-terminal telopeptide of type I collagen (CTX) remained unchanged in the placebo group while they significantly decreased during treatment with the two bisphosphonates, vs both placebo and baseline. In CLO treated patients serum DKK1 remained stable over the entire period of observation while serum SOST levels increased significantly after 12 months of treatment both vs placebo group ($P<0.005$), baseline ($P<0.001$) and ZOL treated group. In the ZOL group, DKK1 levels increased significantly within one month and for the following 6 months and it fell back to baseline values at 12 months. The second ZOL infusion was again associated with an increase in DKK1 a month later, although to a lesser extent. In conclusion, in this study we have found that the treatment of postmenopausal osteoporosis with intermittent yearly ZOL is associated with transient and declining increases in DKK1 while continuous treatment with CLO, results in a progressive increase in serum SOST. These preliminary results and further *ad hoc* studies might contribute to shed light on our understanding of the bone coupling effects taking place during treatment of osteoporosis with different anti-resorbers or with different treatment regimens.

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### PP296

**Correlation between 25-OH vitamin D, γδTCR lymphocytes and acute phase reaction after the first zoledronic acid infusion for post-menopausal osteoporosis**

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Background
Zoledronic acid (ZA) is associated with a transient post-infusional acute phase reaction (APR) due to the activation of γδTCR lymphocytes (γδTcells).
Aims
To investigate if APR correlates with γδTcell percentage or cytokine polarization, or with 25-OH vitamin D levels; to identify a 25-OH vitamin D level associated with a lower risk of APR.
Methods
Sera for ELISA test (IFNγ, IL17, and IL13) and peripheral blood mononuclear cells for T lymphocyte subpopulation FACS analysis (CD3–FITC, γδTCR-PE, IL23R-Alexa Fluor700, CD27-PE-Cy7, and CD69-PERCP-Cy5.5) were drawn from 52 osteoporosis (OP) patients treated with 5 mg i.v. ZA. A second sample was drawn 1 week later from nine patients (5 APR+).
Results
26/52 (50%) patients developed APR and had higher percentages of γδTcells ($4.6\pm5.4$ vs $3.7\pm2.3$, $P=$NS) and lower 25-OH vitamin D levels ($22.1\pm8.2$ vs $33.2\pm17$ ng/ml, $P=0.005$). No correlation was found between 25-OH vitamin D and γδTcells subsets. Further, APR was associated with lower percentage of basal γδTcells Th2 ($31.6\pm27.4$ vs $33.1\pm27.9$, $P=0.022$). No differences were found in Th1, Th17, and Th2 cytokine levels before and after the infusion or in relation to APR. No significant differences were found in γδTcells subsets one week after ZA infusion. The patients with 25-OH vitamin D levels >30 ng/ml had a significantly lower risk of APR (OR 8.6, 95% CI 2.3–33.8).
Conclusion
Insufficient 25-OH vitamin D levels are associated with the risk of ZA-associated APR, suggesting that vitamin D supplementation before ZA infusion could prove protective. High percentages of γδTcells Th2 are correlated to a minor risk of APR and may provide helpful marker.

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### PP297

**Contribution of circulating sclerostin and estradiol for inadequate response to bisphosphonate therapy in women with postmenopausal osteoporosis**

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Bisphosphonate treatment reduces fracture risk in women with postmenopausal osteoporosis. However, some patients have an inadequate response to treatment. Estradiol and sclerostin play an important role in bone metabolism. Sclerostin is an endogenous inhibitor of osteoblastic activity and estrogen deficiency increases osteoclast activity and bone resorption.

We examined the influence of both measures on fracture incidence in postmenopausal osteoporosis in 120 women on bisphosphonate therapy. Patients were classified in adequate responders (AR, $n=66$) without incident fractures during 5 years of treatment and inadequate responders (IR, $n=54$), with incident fractures between 1 and 5 years of treatment. Several variables were measured; anthropometric, biochemical, bone mineral density (DXA), structural analysis of the proximal femur and structural/fractal analysis of the distal radius. Circulating

sclerostin concentrations were measured by ELISA and 17β-estradiol levels by RIA based on ultrasensitive methods.
In AR group, sclerostin serum levels were significantly lower ($P=0.02$) and estradiol concentrations significantly higher ($P=0.023$) than IR group. In a logistic regression model the independent predictors of inadequate response were: previous history of fragility fracture (OR 11.27, 95% CI 1.88–67.34, $P=0.008$) and sclerostin levels (OR 1.11, 95% CI 1.02–1.20, $P=0.011$). Estradiol levels were protective (OR 0.93, 95% CI 0.86–1.01, $P=.09$). We observed a nonlinear, inverted U-shaped relation between estradiol and sclerostin levels. Lower sclerostin levels were found at both low and high estradiol levels.
In conclusion, sclerostin and estradiol circulating levels are related to inadequate response to treatment with bisphosphonates in women with postmenopausal osteoporosis.

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## PP298

### Investigating effects of novel conjugate drugs for the treatment of osteoporosis

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Introduction
Prostaglandin $E_2$ has bone-anabolic effects through EP4 receptor but its clinical utility is hindered by gastrointestinal side effects. To avoid these side effects, EP4 agonists (EP4a) were covalently linked to the bisphosphonate alendronate (ALN) to create two ALN-EP4a conjugate drugs, C1 and C2. When administered systemically, C1 and C2 will be target delivered to bone through ALN, where local hydrolytic enzymes liberate EP4a from ALN to exert bone anabolic effects. Although C1 and C2 both have the same ALN and EP4a components, C1 has a short linker between the two components while C2 does not, making C1 more labile *in vitro*. Here we seek to characterize and compare effects of C1/C2 in a curative *in vivo* study. We hypothesize that C1 and C2 show differential levels of bone anabolic effects due to presence or absence of the linker.
Methods
Three-month-old female Sprague–Dawley rats were ovariectomized (OVX) or sham operated, and allowed to lose bone for 3 months. Animals were then treated via tail–vein injections for 3 months and sacrificed at 9 months. Seven treatment groups were: C1-L (5 mg/kg biweekly), C1-H (5 mg/kg weekly), C2-L (15 mg/kg monthly), C2-H (15 mg/kg biweekly), vehicle for OVX and sham control (PBS biweekly), and ALN/EP4a mixture without conjugation (0.75 mg/kg each, biweekly).
Results
MicroCT showed C1 significantly increased vertebral vBMD and trabecular bone volume compared to OVX controls but not in C2 treated animals. Mechanical testing of C1 vertebrates and femurs revealed significant improvement in load bearing abilities compared to OVX but not in C2 treated animals. Undecalcified histomorphometry of proximal tibial metaphysis showed C2 treatment had no effect while C1 treatment significantly increased bone formation compared to sham.
Conclusions
C1 form of ALN-EP4a conjugate drug showed significant bone anabolic effects while C2 conjugate did not, possibly due to slow cleavage of EP4a from ALN of the C2 conjugate in the local bone environment.

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## PP299

### Association between allopurinol use and hip fracture in older patients discharged from rehabilitation

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Background
Allopurinol reduces oxidative stress and interacts with purinergic signalling systems important in bone metabolism and muscle function. We assessed whether allopurinol use was associated with a reduced incidence of hip fracture in older people who had undergone rehabilitation.
Methods
Analysis of prospective, routinely-collected rehabilitation and hospitalisation data. Data on patients discharged from a single inpatient geriatric rehabilitation centre over a 12-year period were linked to community prescribing data and ICD-10 coded hospitalisation data. Exposure to allopurinol was derived from prescribing data, and hip fracture was derived from hospitalisation data. Time-dependent covariate analysis was used to model time to hip fracture, incorporating ever-use of allopurinol, cumulative exposure to allopurinol, and covariates (age, sex, Barthel Index, comorbid disease, concomitant medication and biochemistry indices)
Results
3517 patients were alive at discharge from rehabilitation without a previous diagnosis of hip fracture; mean age 84 years. 1474 (39%) were males, and 253 (7%) had at least one exposure to allopurinol. A total of 313 (9%) sustained a hip fracture, and 2628 (75%) died during a mean follow-up of 3.1 years. In fully adjusted analyses, each year of allopurinol exposure showed a hazard ratio of 0.17 (95% CI 0.01–2.70) for hip fracture, 1.22 (0.87–1.70) for death, and 1.14 (0.81–1.61) for time to death or hip fracture. Ever-use of allopurinol was associated with a hazard ratio of 1.48 (0.75–2.91) for hip fracture, 1.48 (1.16–1.90) for death and 1.49 (1.16–1.91) for death or hip fracture.
Conclusion
Allopurinol use may be a marker of increased risk of death and hip fracture, but greater cumulative exposure to allopurinol may be associated with a reduced risk of hip fracture. Studies with more events are required to confirm or refute these initial non-significant findings

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## PP300

### Resveratrol supplementation increases vBMD at the spine in obese men: a randomized controlled trial

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Background
Resveratrol (RSV) is a natural polyphenolic compound, found in fx grapes, which possesses anti-inflammatory properties. RSV inhibits osteoclast activity and promotes osteoblastogenesis *in vitro*. Furthermore, RSV protects against bone loss after ovarectomy and immobilization in animal models. RSV treatment of obese men for 4 weeks resulted in an increase in bone specific alkaline phosphatase (BAP).
Hypothesis
Low-grade inflammation is associated with poor bone quality and/or quantity, and if RSV can reduce inflammation, it could result in improved bone quality.
Methods
A placebo-controlled double-blind trial with 76 obese men with metabolic syndrome, randomized to either placebo, low-dose RSV (75 mg×2 daily), or high-dose RSV (500 mg×2 daily) supplementation for 16 weeks. Ethical approval was granted.
Results
Markers of inflammation; hs-CRP and IL6 were not affected by RSV. In contrast, vBMD at the spine (QCT) increased 2.59±1.26% in the high-dose RSV group after 16 weeks of treatment ($P=0.04$ compared with placebo and $P=0.009$ compared with baseline vBMD), while the low-dose RSV group increased 1.0±1.10% compared with placebo ($P=0.39$). A linear regression analysis indicated a dose-dependent increase in vBMD at the spine with increasing RSV dose ($R=0.268$, $P=0.036$). Additionally, aBMD at the spine (DXA) increased significantly with high-dose RSV treatment ($0.011\pm0.004$ g/cm$^2$, $P=0.02$, corresponding to 1.03%), although the change was not different from the change seen in the placebo group ($P=0.88$). vBMD and aBMD at the hip did not differ between groups. BAP increased dose-dependently ($R=0.471$, $P<0.001$), and the increase in the high-dose RSV group was significantly different from the placebo group (15.20±3.71%, $P<0.001$). Osteocalcin and P1NP increased around 5–8% duing RSV treatment, but not statistically significant, and CTx was unaffected.
Conclusion
RSV supplementation increases vBMD at the spine dose-dependently in obese men with metabolic syndrome, despite no effect of RSV on inflammation. Bone markers indicate improved mineralization/bone formation, while resorption is unaltered.

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## PP301

**Effect of gastrointestinal events on treatment patterns, discontinuation, resource utilization, and cost in osteoporosis: an analysis using danish health registries**

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Objectives
To investigate the burden and impact of gastrointestinal (GI) events in osteoporosis (OP) patients by i) examining the association of GI events with the likelihood of OP treatment initiation; ii) measuring the effect of GI events on treatment discontinuation; and iii) determining the impact of GI events post-OP treatment on health care resource utilization and cost. All subjects are identified utilizing national health registries which cover the whole Danish population.
Methods
In women aged $\geq 55$ with an OP diagnosis, we assessed the effect of post-diagnosis GI events on treatment initiation ($n=34\,614$). In women aged $\geq 55$ who initiated an oral bisphosphonate, we determined the likelihood of discontinuation after 12 months in patients with and without GI events ($n=60\,291$). In a matched subset of post-OP treated patients with ($n=1373$) and without ($n=43\,680$) GI events (cases and controls respectively), health care resource use and cost were tallied during the year after the first GI event. Subjects in the three analyses were identified within years 2002–2009.
Results
Treatment patterns differed between patients with and without post-diagnosis GI events as patients with post-diagnosis GI events had significantly lower odds of treatment initiation (OR 0.43, 95% CI 0.39–0.47). Post-diagnosis GI events had no significant effects on the choice of treatment (OR 0.89, 95% CI 0.68–1.16). Treatment discontinuation was 36% more likely at 12 months in patients with post-initiation GI events (OR 1.36, 95% CI 1.29–1.43). All-cause cost accrued by cases (€9363) was more than double that of controls (€ 3996; $P<0.001$).
Conclusions
In Danish women aged $\geq 55$, GI events decreased the likelihood of OP treatment, but not the choice of treatment. In OP patients GI events increase the odds of discontinuation, and GI events post-OP treatment initiation are associated with more resource use and higher cost.

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## PP302

**Comparison of osteosarcoma incidence between abaloparatide (BA058) and PTH (1–34) in long term rat studies**

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It has been previously reported that prolonged treatment with rhPTH(1–34) or rhPTH(1–84) in rats is associated with the development of bone neoplasms including osteosarcomas. However, to date there has been no evidence of an increased osteosarcoma risk of in patients treated with rhPTH(1–34) or rhPTH(1–84), or in diseases associated with chronic PTH elevation. Abaloparatide (ABL) is a novel analog of PTHrP(1–34) currently being developed as a treatment for osteoporosis. In completed clinical studies, earlier and greater BMD gains were seen with ABL compared to rhPTH(1–34). This study was conducted to evaluate the potential for ABL to induce osteosarcomas in rats following long-term treatment. Six-week-old F344 rats (60/sex per dose) were treated with 0, 10, 25, or 50 μg/kg ABL or 30 μg/kg hPTH(1–34) daily for 2 years. Human PTH(1–34) was included as a positive control, at a dose known to result in osteosarcomas in rats. Hyperostosis, increased bone radiopacity and BMD were noted in rats treated with ABL at $\geq 10$ μg/kg or hPTH(1–34), consistent with expected pharmacodynamics. Osteosarcomas were observed in both ABL and hPTH(1–34) treated rats. Comparison of these findings for ABL and hPTH(1–34), at similar exposure multiples to the human therapeutic dose (25 μg/kg ABL and 30 μg/kg hPTH), showed comparable incidence of osteosarcomas. In female rats, there was one primary osteosarcoma in vehicle controls, 11, 22 and 37 with ABL at 10, 25 and 50 μg/kg respectively, and 24 with hPTH(1–34). There were no metastatic osteosarcomas observed in vehicle controls, two, three and 16 with ABL 10, 25 and 50 μg/kg respectively, and nine with hPTH(1–34). In conclusion, close to whole life treatment with ABL in rats resulted in the dose and time dependent formation of osteosarcomas, with a comparable incidence to hPTH(1–34) at similar exposure. These results suggest no increased risk of osteosarcoma would be expected in patients treated with ABL compared to rhPTH(1–34).

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## PP303

**Gastrointestinal issues and osteoporosis treatment: the medication use patterns, treatment satisfaction, and inadequate control of osteoporosis study (MUSIC-OS)**

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Objective
This multi-objective prospective observational study is intended to examine treatment patterns, occurrence of gastrointestinal (GI) complications and clinical and health outcomes in post-menopausal women with osteoporosis (OP) in Europe and Canada. This abstract presents the baseline results of the treated population.
Materials and methods
Post-menopausal osteoporotic women currently on treatment completed surveys intended to measure the incidence and prevalence of GI problems; the associations between GI problems and medication adherence, discontinuation or switching; the impact of GI problems on quality of life, treatment satisfaction and healthcare resource utilization; and estimate the proportion of participants with inadequately controlled osteoporosis. This survey was administered at study enrolment (baseline), and 3, 6 and 12 months after enrolment. The results of the baseline data are presented here.
Results
A total of 2959 patients were enrolled in the treated participant cohort. GI problems were prevalent in 69.4% of patients at baseline. 68.1% of participants reported any GI problem in the past 6 months. Patients with GI problems reported lower medication adherence than those without GI problems, with ADEOS scores of 18.6 and 19.1 respectively ($\geq 20$ indicates adherence). Patients with GI problems also reported lower quality of life than those without GI problems, with EQ-5D summary scores of 71 and 77.3 respectively (score of 100 indicating the highest quality of life). Additionally, patients with GI problems reported lower treatment satisfaction than those without GI problems, with OPSAT-Q scores of 78.6 and 84.6 respectively (score of 100 indicating highest treatment satisfaction). Finally, two-thirds of patients with fractures in the past three months utilized the emergency room, indicating high healthcare resource use.
Conclusion
Participants with GI problems have poorer treatment adherence to osteoporosis medications, lower treatment satisfaction and quality of life than those without GI problems. Fractures correlate with higher resource utilization.

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## PP304

**Activin decoy receptor (IIA) ameliorates immobilisation induced loss of bone and muscle mass in mice**

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Activin is a known physiologic regulator of bone metabolism. Ovariectomy-induced osteopenia has been shown to be attenuated by injection of the activin type IIA decoy receptor (ActRIIA-Fc). However, immobilization-induced osteopenia is driven by different pathways than ovariectomy-induced osteopenia, and the role of activin in immobilization-induced osteopenia has not yet been elucidated.
The purpose of the study was to investigate the possible attenuation of the muscle and bone deterioration following botulinum toxin A (BTX) induced immobilization in mice.

Sixteen-week-old C57BL/6 mice were divided into five groups: baseline ($n=10$), control ($n=12$), ActRIIA-Fc ($n=12$), BTX ($n=12$), and BTX + ActRIIA-Fc ($n=12$). Immobilization was induced by injecting 2 IU/100 g BTX in the right hind limb musculature. The mice were euthanized after 21 days. Rectus femoris muscle mass and BMD of the whole femur were determined and μCT analyses of the mid-diaphyseal femur were performed. All experimental procedures were approved by the Danish Animal Experiments Inspectorate.
The ActRIIA-Fc treatment resulted in greater muscle weight (+8%, $P<0.05$) in the left (non-injected) limb compared with the control group. In the right limb (BTX-injected) of the BTX + ActRIIA-Fc group, treatment resulted in a greater muscle weight (+11%, borderline significant, $P=0.05$) than in the BTX group. ActRIIA-Fc mice had an 8% ($P<0.05$) higher femoral BMD than control mice and BTX + ActRIIA-Fc mice had a 14% ($P<0.05$) higher femoral BMD than BTX mice. At the mid-diaphyseal femur ActRIIA-Fc mice had significantly (+9%, $P<0.05$) higher bone area with no difference observed in tissue area or marrow area compared with control mice. BTX + ActRIIA-Fc mice had a significantly greater tissue area (+5%, $P<0.05$) and bone area (+13%, $P<0.05$) than BTX mice.
Conclusion
In conclusion The immobilization-induced loss of muscle mass, bone density, and bone area may be ameliorated by treatment with ActRIIA-Fc.

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## PP305

**Treatment with PTH 1–84 influences glucose metabolism trough undercarboxylated osteocalcin**

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In the recent years the role of the skeleton in glucose and energy homeostasis has been studied. In particular the osteoblast-specific protein osteocalcin (OC), in its undercarboxylated form (uOC) has been shown to influence glucose homeostasis in animal models.
The aim of our study is to evaluate if the intermittent administration of 1–84 PTH could influence glucose metabolism through its anabolic action on the skeleton.
We enrolled in the study 43 women affected by postmenopausal osteoporosis; the patients were randomly assigned to treatment with:
i) 1-84 PTH 100 μg plus calcium 1200 mg and vitamin D 800 UI daily (21).
ii) calcium 1200 mg and vitamin D 800 UI daily (22).
Glucose and bone metabolism were evaluated at basal and after 3, 6, 12 and 18 months of treatment. Glucose metabolism was evaluated trough an oral glucose tolerance test with 75 g of glucose and blood sampling for glucose and insulin at 0′, 30′, 60′, 90′, and 120′. OC, uOC, adiponectin, and leptin were measured at each visit by ELISA technique. Body fat was measured by plicometry at each visit.
In PTH treated patients fasting plasma glucose significantly decreases during therapy, without increase in insulin secretion. The treatment with PTH increases the production of uOC, and this increase is inversely correlated with fasting plasma glucose, whereas there is no effect of treatment on fat distribution or adipokines secretion.
Our data demonstrated that treatment with intermittent PTH lowers plasma glucose metabolism and suggest that this effect is mediated by the increase in uOC.

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## PP306

**The effect of bisphosphonate treatment on sclerostin levels in postmenopausal osteoporosis: the TRIO study**

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Treatment of postmenopausal osteoporosis with bisphosphonates reduces bone resorption and formation. Sclerostin, an osteocyte regulator of bone formation may be involved in these changes. Some studies have reported an increase in sclerostin associated with bisphosphonate treatment while others have reported a decrease. The aims were to determine the effect of bisphosphonates on i) circulating sclerostin and ii) PINP in postmenopausal women with osteoporosis.
We studied 92 postmenopausal women with osteoporosis (<85 years) from a parallel group trial of bisphosphonates. They were randomised to receive ibandronate 150 mg/month ($n=28$), alendronate 70 mg/week ($n=33$) or risedronate 35 mg/week ($n=31$). They all received 1200 mg of calcium and 800 IU of vitamin D3/day. Fasting blood samples were collected at baseline (weeks −1 and 0) then at 1, 2, 4, 12, 13, 48 and 96 weeks and from 57 healthy premenopausal women (mean age 37 years) on one occasion. Serum sclerostin was measured by immunoassay (TECO Medical Group, Switzerland). The intra- and inter-assay coefficients of variation were 2.3 and 4.2% respectively. PINP was measured using an automated immunoassay analyser (the ISYS-IDS, UK). Median levels of sclerostin were significantly higher in postmenopausal than premenopausal women, 0.49 and 0.38 ng/ml respectively ($P<0.0001$), (Table 1). There was no significant change in sclerostin over the 96 weeks in any of the three treatment groups. There was a significant decrease in PINP by 67% ($P<0.0001$).

**Table 1** Serum sclerostin (ng/ml) (mean ± S.D.) in the three treatment groups over 96 weeks.

| | Baseline | 1 week | 4 weeks | 12 weeks | 48 weeks | 96 weeks |
|---|---|---|---|---|---|---|
| Ibandronate | 0.51 + 0.13 | 0.50 + 0.11 | 0.52 + 0.12 | 0.53 + 0.13 | 0.51 + 0.14 | 0.51 + 0.13 |
| Alendronate | 0.48 + 0.13 | 0.48 + 0.13 | 0.47 + 0.12 | 0.48 + 0.13 | 0.47 + 0.12 | 0.50 + 0.13 |
| Risedronate | 0.51 + 0.10 | 0.51 + 0.11 | 0.52 + 0.10 | 0.51 + 0.11 | 0.51 + 0.11 | 0.52 + 0.14 |

Friedman test (ibandronate) $P=0.08$, (alendronate) $P=0.11$, and (risedronate) $P=0.88$. Dunns *post hoc* test for comparison to baseline not significant. Changes in sclerostin are unlikely to explain the decrease in bone formation with bisphosphonate therapy for osteoporosis.

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## PP307

**Effect of daily intake of milk enriched with a high dose of vitamin D in healthy postmenopausal women: preliminary results from a randomized, controlled and double-blind nutritional trial (The EFICALCIO study)**

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Background
Vitamin D deficiency is highly prevalent and can be associated with adverse health outcomes. Few studies have evaluated the effects of daily consumption of milk fortified with a high dose of vitamin D in a large cohort of healthy postmenopausal women.
Objectives
To determine the effect of daily intake of milk enriched with vitamin D (with or without fructooligosaccharides (FOS)) on vitamin D status, bone mass and cardiovascular risk factors.
Subjects and methods
This was a 2-year randomized controlled study in which 500 healthy postmenopausal women (mean age 58.1 ± 4.8 years) were assigned to receive 500 ml/day of a dairy product to one of three groups: control group (C) with skimmed milk (120 mg/100 ml calcium and vitamin D 0.75 μg/100 ml), group A with skimmed milk enriched with calcium and vitamin D (180 mg/100 ml and 3 μg/100 ml) and group B with skimmed milk enriched with calcium and vitamin D (180 mg/100 ml and 3 μg/100 ml) and FOS (5 g/l). We evaluated serum levels of 25-OH-vitamin D. We also measure anthropometric parameters, biochemical data of glucose metabolism and lipid prolife, and body composition by electrical impedance. Preliminary results in 292 posmenopausal healthy women after 12 months of the nutritional intervention are presented.
Results
After 12 months, changes in vitamin D in the control group ($n=105$) were non-significant (22.21 ± 7.5 vs 24.16 ± 7.8 ng/ml, $P=0.067$). In group A ($n=108$) and group B ($n=79$) we observed a significant increase in vitamin D (group A 21.5 ± 6.5 vs 27.35 ± 8.8 ng/ml and group B 23.7 ± 10.7 vs 29.0 ± 13.6 ng/ml, $P<0.001$ for both). In groups A (84%) and group B (72.6%) a high percentage of women reached vitamin D levels >20 ng/ml, compared with the control group (65.5%), $P<0.01$ for the comparison with control group.

Conclusions
Preliminary data confirms that daily intake of milk highly enriched with vitamin D, with or without FOS, in postmenopausal healthy women induces a significant improvement in vitamin D status.

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## PP308

**Association of gastrointestinal events and osteoporosis treatment initiation in newly diagnosed osteoporotic Israeli women**

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Objectives
To examine the association of gastrointestinal (GI) events and osteoporosis (OP) therapy initiation patterns among postmenopausal women following a diagnosis of OP from a large health plan in Israel.
Methods
Women aged $\geq$55 years were included in the analysis if they had $\geq$1 OP diagnosis (ICD-9 733.0X) (date for first OP diagnosis was index date), no estrogen use, no diagnosis of Paget's disease or malignant neoplasm. OP treatment initiation was defined as use of OP therapy: bisphosphonates (BIS) (alendronate, ibandronate, risedronate, and zolendronic acid) and non-BIS (raloxefene, calcitonin, and teriparatide), during 12 months post-index. GI events (diagnosis of GI conditions) were reported for 12-month pre-index and post-index (from index to treatment initiation or end of 1-year post-index whichever occurred first). The association of post-index GI events (yes/no) and initiation of OP treatment (yes/no), and the type of therapy initiated (i.e. BIS vs non-BIS) were examined with logistic regression and also Cox proportional hazard regression as sensitivity analysis.
Results
Among 30 788 eligible patients, aged 65.0$\pm$7.6 (mean$\pm$s.d.) years, 17.5% had pre-index GI event, and 13.0% had post-index GI event. 70.6% of patients didn't receive OP therapy in the year following OP diagnosis, 25.1% received BIS and 4.2% received non-BIS. The logistic regression showed that post-index GI events were associated with a lower odds of OP medication initiation by $\sim$85% ($P<0.001$), and upon treatment initiation, post-index GI was not significantly associated with type of therapy initiated (BIS vs. non-BIS), controlling for baseline GI and patient characteristics.
Conclusions
Among newly diagnosed osteoporotic women from a large health plan in Israel, 70.6% did not receive pharmacological OP treatment within 1 year of OP diagnosis. Patients with post-index GI events were about 85% less likely to initiate OP treatment.

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## PP309

**Treatment of severe vertebral osteoporosis with teriparatide or 1–84-PTH: results from a Danish Database Initiative**

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A Danish database initiative was established in 2003 with the purpose of evaluating patients treated with teriparatide. After the introduction in 2006 of 1–84-PTH, these patients were also included. A total of 1494 patients from 19 centers were reported to the database, until closure in 2011. The reported patients correspond to a third of all patients in Denmark receiving anabolic treatment during this time period. With regard to age and sex the reported patients do not differ from the entire group of patients treated with teriparatide or 1–84-PTH. Almost 80 patients. of patients treated were having at least two vertebral fractures, while the remaining group had a single vertebral fracture in combination with a $T$-score $<-3.0$. Thirty-five patients. of the patients were reported to be treatment naïve, while 38% had suffered a vertebral fracture despite antiresorptive treatment. This pattern did not change over time.
Eighty-three patients. were treated with teriparatide, while 17 patients. received 1–84-PTH. The vast majority was treated for 18 months, since this was the maximum reimbursement period for the main part of the study. Comparing teriparatide and 1–84-PTH parallel patterns in changes in BMD of the spine and total hip were seen, with $\sim$10 patients. increase at the lumbar spine and one patient. increase at the total hip. Identical responses were found in females and males, as well as in patients previously treated with bisphosphonates compared to treatment naïve. Side effects reported were few and equally distributed between the two treatments, except for hypercalcaemia that was more frequently reported in patients treated with 1–84-PTH. Only very few patients stopped treatment prematurely due to side effects.
With the use of voluntary clinical database additional information on how a new treatment is adopted by clinicians and how patients respond to treatment.

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## PP310

**Risk factors for the development of chronic back pain after percutaneous vertebroplasty vs conventional treatment**

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In a recent randomized controlled trial comparing vertebroplasty (VP) vs conservative treatment (CT) in patients with symptomatic vertebral fractures (VF) we observed the development of severe chronic back pain (CBP) in nearly one quarter of patients. The aim of this study was to evaluate the risk factors related to the development of CBP in these subjects.
Methods
We evaluated risk factors including: visual analog scale (VAS) at baseline and during the 1-year follow-up, age, gender, symptom-onset time, number, type and severity of VF at baseline, number of vertebral bodies treated, incident VF, and antiosteoporotic treatment, among others. CBP was considered in patients with VAS $\geq$7 at 12 months.
Results
91/125 patients completed the 12-month follow-up. CBP was observed in 23% of VP-treated patients vs. 23% receiving CT. Patients developing CBP after VP showed a longer symptom-onset time (82%$\geq$4 months in VP vs 40% in CT, $P=0.03$). On univariate analysis, female gender (OR 1.52; 95% CI 1.47–1.57, $P<0.0001$), multiple acute VF (OR 1.79; 95% CI 1.71–1.87, $P<0.0001$), VAS $\geq$7 2 months after treatment (OR 11.04;95% CI 6.71–18.17, $P<0.0001$) and type of antiosteoporotic drug (teriparatide) (OR 0.12; 95% CI 0.03–0.60, $P=0.0236$) were risk factors of CBP development in both groups. In the multivariate analysis the main risk factors were baseline and post-treatment VAS $\geq$7, longer symptom-onset time and type of antiosteoporotic treatment.
Conclusions
23% of patients with symptomatic osteoporotic VF developed severe CBP independently of the type of treatment. Symptom-onset time before VP and persistence of severe CBP after treatment were the main factors related to CBP with teriparatide treatment decreasing the risk of this complication.

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## PP311

**Rates and reasons for lack of persistence with anti-osteoporotic drugs: analysis of the Campania Region Database**

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Background
Osteoporosis treatment involves several therapeutic tools, including long-term drug therapy. Subjects with chronic disorders are more likely to be non-adherent and/or non-persistent to treatment than those with other diseases. Adherence is the extent to which patients take medication as prescribed by their physicians, whereas persistence is the time from treatment initiation to discontinuation. Lack of persistence is common among subjects using oral anti-osteoporotic drugs, and leads to increased risk of fragility fracture. The aim of our study is to analyze the rates and reasons for discontinuation of anti-osteoporotic drugs in the Campania Region.
Methods
The study was designed as a retrospective cohort study. Subjects aged over 40 years were included if they receive at least one prescription for any anti-osteoporotic drugs in 2009. Data were obtained from an administrative database of regional data on outpatient drug prescriptions reimbursed by the National Health Service (NHS). Patients were followed until the discontinuation of anti-osteoporotic therapy or until the end of the observation period (31st December 2010).
Results
A total of 30 048 were incident users of anti-osteoporotic drugs: 1731 (5.8%) males and 28–317 (94.2%) females. The mean age (s.d.) of the cohort was 69.0 (10.0) years. Weekly bisphosphonate (BP) (51.1%), was the most commonly prescribed drug. In the overall population, persistence rates were 34.8% after 6 months, 13.4% at 1 year. A multivariate Cox proportional hazard analysis showed that daily regimen (HR 1.9) treatments remained at a higher risk of early discontinuation compared to weekly regimen therapies. Patients who started treatment with a co-prescription with calcium and vitamin D had a lower risk of early discontinuation (HR 0.7).
Conclusions
Our data showed that the persistence to osteoporosis therapy is significantly worse than reported in literature. A better osteoporosis management should include drugs with less frequent dosing, to obtain both an increase in rate of persistence and a reduction in side-effect.

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## PP312

**Farnesylpyrophosphate synthase rs2297480 polymorphism and the response to the zoledronic acid in the treatment of postmenopausal osteoporosis**

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Research design and methods
A characterisation of 225 European (Russian) osteoporotic postmenopausal women, treated for 2 years with amino-bisphosphonate zoledronic acid (zol), with respect to the adenosine/cytosine (A/C) rs2297480 farnesyl pyrophosphate synthase (FDPS) gene polymorphism, was carried out by PCR-based enzymatic digestion and quantitative PCR allelic discrimination on genomic DNA extracted from blood leukocytes. The association between these polymorphism genotypes and the response of spine and femur bone mineral density (BMD) and of bone turnover markers (BTM): b-crosslaps (CTX) and osteocalcin (OC) to treatment with zol was statistically examined.
Results
225 postmenopausal Russian women with mean age of 59 years old (between 54 and 66 years old, mean length of menopause of 7 years (2–13), and mean BMI of 27.2 (23.6–29.05) kg/m$^2$. were characterized for (AA:AC:CC) allele distribution in FDPS gene. The ratio of alleles was AA 55.1 ($n=144$): AC 39.5 ($n=103$), and CC 5.4 ($n=14$). There was no statistically significant correlation between the FDPS genotype and baseline levels of BTM. The average BMD in the spine/femur indicated presence of osteoporosis. All the patients were treated i.v. with 5 mg of zol once in 12 months for 2 years. Additionally all the patients were taking Ca at 1000 mg/day and vitamin D at 800 ME/day. Difference in BMKP after zol infusion was FDPS genotype dependent.
There was a statistically significant decrease in CTX 92% (91–93%) in patients with C/C genotype ($P=0.0056$) in comparison to 82% in A allele carriers 6-month post first infusion ($P=0.07$). Nine months after infusion CTX were up to 75% in A allele carriers with osteocalcine being 44.4% of the baseline. In the meatime, C/C genotype carriers still had low CTX (87%) and OC (53.6%) levels ($P=0.056$). By the 12th month of treatment – CTX and OC levels were increasing in A allele carriers but in the C/C allele genotype stayed within the limits of the lower levels of normal for pre-menoupause. Patients carrying C/C genotype showed significant decrease in BMD (5.6%) and bone turnover markers after the second infusion of zol (not sure about abbreviations and my translation)
Conclusions
The difference in reaction to zol treatment in postmenopausal women correlated with FDPS gene polymorphism. Zol therapy in C/C genotype was characterized by hypersuppression of bone turnover and possibly is a reason for the negative dynamics of MPK in the spine after 2-year therapy.

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## PP313

**Preclinical evaluation of the link module from human TSG–6 as a novel anti-resorptive agent for postmenopausal osteoporosis**

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We have shown previously that TSG–6 acts as an autocrine regulator of osteoclast activity *in vitro*, capable of inhibiting RANKL-mediated osteoclastic bone resorption with a similar potency to OPG[1,2]. Thus, the TSG–6 protein has the potential to be developed as a novel treatment for osteoporosis, which is associated with excessive bone loss[3].
The aim of this study was to determine the therapeutic potential of the isolated link module domain from human TSG–6 (Link_TSG6; ~11 kDa), which, like the full-length protein, binds to RANKL[1]. Here we have demonstrated that Link_TSG–6 inhibits lacunar resorption when osteoclast precursors are cultured on dentine slices in the presence of M-CSF/RANKL, with similar effects seen for both human and mouse cells (IC50 = ~1 nM). The finding that Link_TSG6 impairs F-actin ring formation (85% reduction at 0.85 nM) provides a likely mechanism for its anti-resorptive activity. We have also tested the efficacy of Link_TSG–6 *in vivo* using the ovariectomised mouse model of post-menopausal osteoporosis; all work was carried out in accordance with UK Home Office regulations. Mice (ten per group) treated with Link_TSG–6 (over a period of 4 weeks) showed a statistically significant reduction in serum levels of CTX-1 (a marker of bone breakdown) compared to vehicle controls. Furthermore, unlike zoledronate, Link_TSG–6 did not reduce the levels of P1NP, a marker of bone formation. Importantly, analysis of femurs by micro-CT revealed a significant reduction in trabecular bone loss in Link_TSG–6-treated animals.
Inhibition of bone resorption by Link_TSG–6, in the absence of effects on bone formation, might represent an advantage compared to existing anti-resorptive treatments for osteoporosis, which significantly impair the bone-remodelling unit.
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## PP314

**The effect of risedronate on hypogonadal osteoporotic HIV males treated with highly active anti-retroviral therapy: a pilot study**

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Objective
We evaluated the effect of risedronate on BMD and bone turnover markers in HIV infected osteoporotic males, according to their gonadal status.

Methods
41 HIV patients treated with highly active anti-retroviral therapy (HAART) were followed for 24 months and divided into two groups: patients with osteoporosis or osteopenia with fractures (Group A, $n=20$) and those without (Group B, $n=21$). Group A and B were further divided according to the presence of a reduced calculated free testosterone ($\leq$225 pmol/l), a better marker of androgenization in HIV given the raised SHBG level, and/or severe symptoms of hypogonadism defined as $>37$ score at the aging male symptoms scale (AMS). Both groups were treated with cholecalciferol 800 IU and calcium 1000 mg orally every day for the first 12 months. Risedronate 75 mg for 2 consecutive days a month orally was then added in Group A, for another 12 months. Group B continued treatment with calcium and vitamin D for the entire study length (24 months). Every 6 months each patient underwent biochemical evaluation, AMS questionnaire, lumbar and femoral BMD measurement.
Results
During the study period, the HAART therapy was unchanged. A significant increase in lumbar BMD was observed in HIV males with adequate androgenization after 12 months of risedronate treatment in Group A compared to hypogonadal subjects ($5.2\pm1.0$ vs $3.1\pm0.8\%$, S.E.M., $P<0.05$); a significant greater reduction of βCTX ($-19.8\pm0.8$ vs $-16.1\pm0.9\%$, S.E.M., $P<0.05$) was also observed. BMD remained stable with a concomitant significant modest reduction ($P<$ 0.05 vs basal values) of bone turnover markers in Group B.
Conclusions
Risedronate increased BMD and reduced bone turnover markers at a greater extent in eugonadal compared to hypogonadal osteoporotic HIV males, emphasizing the role of gonadal status in determining response to treatment in young HIV males.

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## PP315

**Combination therapy with ibandronate and eldecalcitol enhances bone strength without severe suppression of bone formation in aged ovariectomized rats**

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Ibandronate (IBN), a nitrogen-containing bisphosphonate, and eldecalcitol (ELD), an active vitamin $D_3$ derivative, are used for osteoporosis treatment. Vitamin $D_3$ often used in combination therapies, however, there are no reports on the effects of combined IBN and ELD treatment for osteoporosis.
In this study, we examined the effects of combination treatment with IBN and ELD in aged ovariectomized rats. Eight-month-old Wistar-Imamichi rats were ovariectomized and treated for 12 weeks with either vehicle, IBN (3 μg/kg per month, s.c., monthly), ELD (15 ng/kg per day, p.o., daily), or a combination of IBN and ELD.
Urinary DPD was reduced in each monotherapy group. The combination treatment showed further decreases in DPD compared to each monotherapy group. Osteocalcin in all therapy groups was not lower than the level in the sham group.
Lumbar BMD was higher in the monotherapy groups than in the vehicle control group, and was higher in the combination group than in both monotherapy groups. A mechanical strength of lumbar was improved in monotherapy groups and in the combination group. The maximum load was higher in the combination group than in the IBN monotherapy group.
In femurs, BMD was increased in both monotherapy groups, and was higher in the combination therapy group than in both monotherapy groups. The maximum load of femurs was higher in the combination group than in the vehicle control group. Bone histomorphometry analysis revealed that combination therapy result in additive effects on reduction of ES/BS in cancellous and endosteal cortical bone in femurs, but bone formation parameters such as MS/BS or BFR/BS were not less than Sham level.
This study suggests the additive effects of IBN and ELD combination therapy on the mechanical strengths of the lumbar vertebrae and femur. These effects may be induced by increased BMD via reduction of bone resorption without severe suppression of bone formation.

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## PP316

**Odanacatib treatment improves lumbar vertebral bone mineral density and strength in orchiectomized rabbits**

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The selective cathepsin K inhibitor odanacatib (ODN) is currently in development for the treatment of postmenopausal osteoporosis. Our goal was to evaluate the effects of ODN vs alendronate (ALN) on bone mass and strength of lumbar vertebrae (LV) in orchiectomized (ORX) rabbits, a model of male osteoporosis. Adult male rabbits (11 months old) were subjected to sham- ($n=20$) or ORX-surgery ($n=24$/group) for 7.5 months before dosing initiated. ORX animals were randomized by LV BMD and dosed with either vehicle (Veh), ODN (1.5 and 6 mg/kg per day, providing approximately one to seven times the clinical exposure respectively), or alendronate (ALN; 300 μg/kg per week, s.c.) for 14 months. Endpoints included *in vivo* spine DXA, urine, serum collected at baseline and at every 3 months; and *ex vivo* pQCT and strength testing. Compared to Veh, ODN significantly increased LV BMD ($P<0.01$) by 9% at 3-months and 19% at 14-months with the 1.5 mg/kg dose, and by 6% at 3-months and 27% at 14-months with the 6mg/kg dose, vs ALN by 9% at 14-months. ODN at both doses and ALN reduced the bone resorption marker helical peptide by 50–60% vs Veh. While ALN reduced BSAP, ODN maintained this bone formation marker at comparable levels as in Veh. ODN increased pQCT-based trabecular vBMC by 28 and 38% respectively vs Veh ($P<0.001$), compared to ALN increased this parameter by 11% ($P<0.05$). From LV compression testing, significant treatment related increases in multiple strength parameters (peak load, apparent strength, yield load, and yield stress, stiffness) compared to Veh were demonstrated. Peak load was positively correlated with vBMC ($R=0.9383$, $P<0.001$). Taken together, this study demonstrated ODN dosed in treatment mode was highly efficacious in restoring lumbar spine bone mass and increasing bone strength of ORX rabbits. Our results support the clinical evaluation of ODN for the treatment of osteoporosis in men.

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## PP317

**Eldecalcitol improves endothelial function in the femoral artery and prevents bone loss in ovariectomized rats**

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Eldecalcitol (ED-71; ELD), a 2β-hydroxypropyloxy derivative of 1α,25(OH)2D3, was approved to treat osteoporosis in Japan in 2011. The endothelial protective effect of vitamin D3 in osteoporosis is not clear. This study evaluated the endothelial protective effect of ELD in ovariectomized (OVX) rats. ELD (20 ng/kg) was orally administered five times a week for 4 weeks from 1 day after OVX surgery. Four weeks after surgery, flow-mediated dilation (FMD) as an indicator of endothelial function was measured by ultrasound in the femoral artery (FA) of living rats and the BMD of the L2–L4 vertebrae was measured by DXA. FMD was significantly reduced in FA. Nox4 expression and nitrotyrosine content (NT) were increased, indicating oxidative stress, and the eNOS dimer:monomer ratio was decreased, indicating eNOS dysfunction; moreover, PPARγ expression was decreased and NF-κB p65 expression was increased in FA. ELD ameliorated the reduction of FMD. ELD reduced Nox4 and NT and improved the eNOS coupling state and also increased PPARγ expression and decreased NF-κB p65 expression in FA. On the other hand, PPARγ in bone marrow (BM) is a known risk for bone loss, and PPARγ expression was increased in BM. BMD was significantly lower in OVX rats than in sham-operated rats. ELD prevented the reduction of BMD. ELD tended to suppress PPARγ expression in BM. These results suggest that ELD ameliorated endothelial dysfunction in OVX rats. An antioxidative effect via increased PPARγ expression by ELD is thought to improve the eNOS coupling state in FA in this osteoporosis model. Change in PPARγ expression differed between FA and BM in OVX rats, indicating that the effect of ELD on PPARγ signaling might vary according to organ or to disease condition. ELD is expected to act as an anti-osteoporotic agent with the additional value of improving endothelial function in osteoporosis patients.

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## PP318

### Preferences of patients and health care professionals for osteoporosis drug treatment: a discrete choice experiment

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Objectives
This study aims to evaluate and compare the preferences of patients and health care professionals for osteoporotic drug treatment.
Materials and methods
A discrete choice experiment was conducted among patients and health care professionals (general physicians, rheumatologists, and geriatricians) in Belgium. Participants were asked to choose between two hypothetical unlabelled drug treatments (and an opt-out option) that vary in several attributes: efficacy in reducing the risk of fracture, type of potential common side-effects, mode and frequency of administration and out-of-pocket costs. Patients completed the questionnaire from their own perspective and professionals were presented with a specific patient profile corresponding to the average patient that completed the experiment. An efficient design was used to construct the treatment option choice sets and a mixed logit model was used to estimate patients' preferences.
Results
A total of 257 patients and 59 professionals completed the experiment. Both patients and professionals preferred a drug treatment with a higher risk reduction and a lower cost. They disliked more being at risk of gastro-intestinal disorders than at risk of skin reactions and preferred 6-month s.c. injection and monthly oral tablets compared with weekly oral tablets. Some differences between patients and professionals were significant. Health care professionals attached higher value for treatment efficacy and treatment costs, and the preference for yearly i.v. was higher (and significant compared with weekly oral tablet) for professionals. Patients also disliked more a treatment with a risk of having skin reactions. The constant (representing the preferences for drug treatment compared with no treatment) was also higher for the patients.
Conclusions
Although there were similarities in the preferences of patients and health care professionals for osteoporosis drug medications, differences for levels of some attributes were observed that should not be ignored when aiming to adjust treatment to the preferences of patients.

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## PP319

### Perceived vs objective knowledge in patients with osteoporosis

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Previous studies have demonstrated that patients with osteoporosis (OP) have a poor understanding of their bone disease and that this negatively influences treatment decisions and medication compliance. The aim of this study is to determine if there is a discrepancy between perceived and objective disease knowledge (PK and OK, respectively) in patients with OP and whether this correlates with certain patient characteristics.
Design and methods
After ethics approval, 121 patients of any age/gender referred to the Osteoporosis Clinic were provided questionnaires to assess their PK and OK overall and in each of four domains: general OP, prevention and treatment, risk factors, and consequences of OP. Overall correlation between PK and OK was assessed using the Spearman's correlation coefficient. Multivariate analysis was performed to look at association of demographic variables with PK and OK. Internal consistency for PK surveys overall and in each of the four domains was established using Cronbach's alpha. A Spearman's correlation coefficient was calculated between our PK and OK surveys and results obtained using previously validated surveys addressing the same.
Results
There is a low-moderate correlation (Spearman's coefficient 0.542) between PK and OK, with PK generally greater than OK (67.1 vs 51.7%). PK is significantly increased by attendance at an osteoporosis educational session ($P$ 0.0021) and by a prior visit at the Osteoporosis Clinic ($P$ 0.0021).Conversely, OK correlates positively with increasing education level ($P$ 0.0002). Internal consistency for overall PK was reasonable with a Cronbach's alpha of 0.836, however was poor in the consequences of OP domain (Cronbach's alpha of 0.547). Spearman's correlation coefficient showed a high-moderate correlation between survey results and those achieved with previously validated surveys.
In conclusion, patients require further education regarding OP to minimize the disparity between PK and OK. This may be best achieved through additional education sessions. Questionnaires have been modified to improve poor internal consistency in the consequences of OP domain and this study is ongoing using the modified questionnaires.

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## PP320

### Effects of preventive long-term treatment with strontium ranelate and zoledronic acid to ovariectomized rats on bone microstructure

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Many pharmacological treatments have been developed in order to prevent or treat postmenopausal osteoporosis. There is not any study in the literature comparing long-term treatments of osteoporosis. The aim of this work was to studythe effects of long-term prevention treatment with zoledronic acid (ZA) and strontium ranelate (SrR) on bone microstructure in ovariectomized rats.
Sixty 6-month-old female Wistar rats were used in this study and divided into four groups: SHAM ($n=15$), simulated intervention; OVX ($n=15$), ovariectomized; OVX+ZA ($n=15$), ovariectomized and treated with ZA (0.08 mg/kg i.v. at the begining of the study); OVX+SrR ($n=15$) ovariectomized and treated with SrR (0.033 g/kg per day by oral gavage. Treatments started one day after ovariectomy. Eight months later all rats were sacrificed and bone microstructure by microCT scan analysis was performed.
Orchidectomy produced a significant reduction of bone quality, as all the microestructural parameters of the trabecular region showed (BV/TV, BS/TV, Tb.N, Tb.Sp, Tb.Th, Tb.Pf, SMI and Conn.Dn). ZA treatment avoided this bone deterioration, even enhancing microCT parameters with respect to the SHAM group. OVX+SrR group did not show any change in these parameters with respect to the OVX group. Microstructural parameters of the cortical region were not modified in any of the studied groups with respect to the SHAM group.
SrR long-term preventive treatment is no capable of avoid the effects of ovariectomy. Long-term treatment of ovariectomized rats with ZA prevented effects of ovariectomy on bone microstructure, even improving bone quality of healthy group.

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## PP321

### On-treatment safety and efficacy and short-term efficacy of 1–34 parathyroid hormone treatment in severe osteoporosis

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Objectives
To assess safety and clinical efficacy during 1–34 parathyroid hormone treatment (1–34-PTH) in real clinical practice; to describe fracture outcome after 1–34-PTH discontinuation in real clinical practice.
Methods
We performed an observational study in real clinical practice of all consecutive severe osteoporosis (sOP) patients referred to our Rheumatology Department from Feb'10 until Jan'14. All patients were referred both by general practitioners and geriatrists from a 150 000 population community (Hospitalet Llobregat). Patients were classified as sOP if they accomplished one of the following: incidental osteoporotic fractures during previous anti-OP treatment, need of chronic systemic steroid treatment and presence of previous osteoporotic fracture, several previous osteoporotic fractures, and serious risk of fall with previous osteoporotic fracture. 1–34-PTH was given to all of them. The incidence of clinical vertebral and nonvertebral fragility fractures were assessed, and fractures outcome were asked in 6-month-follow-up visits. All safety issues were registered

in a questionnaire-sheet. A 12-month vertebral X-ray was performed during treatment, and blood tests (assessing bone metabolism and renal function after 1–34-PTH treatment) and previous-BMD were collected. All data concerning to age, 1–34-PTH onset/discontinuation, previous treatment, number of fractures, side-effects, incidental fractures and fractures outcome were registered in a database.
Results
A total of 111 patients fulfilled our sOP criteria. All patients showed OP-BMD level. 83% retained an 18-month course of 1–34-PTH. 17 patients discontinued treatment due to a mild side-effects (12 by GI intolerance, four by headache, and one site-injection pain), all recovered. No serious side-effects were found. A total of 79.8% were women, mean age 71.3 (s.d. ±6.7), 38% had one previous fracture (63% vertebral, 19% hip, and 18% others), and 62% greater than or equal to one previous fracture; mean number of fractures was 1.94 (s.d. ±1.49). The 38% had suffered fracture during previous treatment (95% on byphosphonates). No new fractures were observed during treatment follow-up. Fifty-four (57%) had finished the 18-month course 1–34-PTH (mean follow-up 9.2 months; s.d. ±2.4). Of these, three patients showed one each new vertebral fractures (5.2%) after 1–34-PTH discontinuation.
Conclusions
1–34 PTH showed benefit during treatment and a very low rate of fracture after its discontinuation in sOP in real clinical practice. Safety issues were mild and fully recovered, and observed in only 15% of patients. Longer follow-up time is needed to elucidate post-1–34 PTH fracture outcome. These results should be interpreted in the context of the design of an observational study.

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## PP322

**Non attendance at a Bone Health Clinic following hip fracture**

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Hip fractures are a major cause of burden in terms of mortality, disability, and costs. In Ireland, 3000 hip fracture occur annually and is expected to increase over the coming years[1]. Estimated cost of hip fractures is €14 300/admission[2]. Outpatient non-attendance is a source of inefficiency, wasting time, resources and lengthens waiting lists. Non attendees have a significant negative impact on productivity, their own care and resources. In 2008, an estimated 25 000 outpatient appointments were broken in our institution, at an estimated cost of €3 800 000[3].
Aim
To identify the reasons for non attendance at an osteoporosis clinic following hip fracture.
Methodology
Hip fracture patients admitted to study site from June 2008 to June 2010 assessed by an orthogeriatric team and offered an appointment in a Bone Health Clinic.
Results
394 hip fracture patients were admitted to study site. 57 (14%) got no appointment as 26 (7%) did not want one, 13 (3%) were too frail, 8 (2%) were non-residents in country/county and 10 (2%) had metastatic disease. 197 (50%) attended for a clinic appointment while 140 (36%) did not. Mean age of non attendees 77.5 years. Reasons for non attendance are tabulated below.

| | |
|---|---|
| Longterm care (cognitive impairment ± reduced mobility) | 21 (15%) |
| Cancer | 4 (3%) |
| Alcohol excess | 5 (4%) |
| Cognitive impairment | 25 (18%) |
| Reduced mobility | 24 (17%) |
| RIP | 33 (23%) |
| Cancelled | 28 (20%) |

Conclusion
Non-attendance at outpatient appointment is considered an indicator of poorer access to health care services and may lead to worse health outcomes, increasing costs and waiting times. In order for health service providers to be able to allocate adequate resources for the management of hip fractures, accurate figures for fracture rates and outcomes should be measured. Given the current economic climate, methods need to be employed to reduce non-attendance.

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## PP323

**To decline screening for osteoporosis: a study on individual decision making, meaning and reasons for non-participation**

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Purpose
This combined qualitative and quantitative study aimed to investigate women's personal reasons for and choices to decline the screening for osteoporosis. The ROSE study is a randomized prospective population-based trial investigating the efficacy of a screening program to prevent fractures in women aged 65–80 years.
Methods
A triangulated approach combining data from interviews and questionnaire. 7020 questionnaires from the screening group in the ROSE-study were included. In total 205 telephone interviews and five in-depth interviews were carried out. Principles from critical psychology guided the analysis of the qualitative data.
Results
Women reasoned their choice on declining screening by three themes: Not at risk – embodied knowledge, osteoporosis – a minor health concern, and consequences and harms of screening. Age, bodily comprehension of risk, experience of osteoporosis, health-seeking behavior in daily life was important to the women's decision on declining screening. Data from the questionnaire showed that a majority of women who declined screening significantly perceived themselves at low risk for fracture, were older and had less risk factors compared to participating women. Perceived low risk was found to be the main reason for cancelling DXA appointment. Furthermore, issues on co-morbidity distance to the DXA-scan, immobility, concerns about side-effect to treatment and to be labeled were important to the women.
Conclusion
Women, who declined screening, were older, perceived own fracture risk lower than their peers, and reported a lower proportion of risk factors. Furthermore, the interviews unfolded that some women decline screening due to co-morbidity and immobility or long distance to DXA. Women's choices in relation to participating in screening or not, are interpreted by perceived susceptibility and severity of osteoporosis, and benefits or harms of participating in the screening program. Knowledge on these issues is important to the effectiveness of the screening programs, the management of osteoporosis and informed decisions making.

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## PP324

**The effects of risk factors on raloxifene, alendronate, and vitamin D treatment in postmenopausal osteoporosis**

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Objective
The purpose of this study was to compare the efficacy of raloxifene (RLX) 60 mg daily, vitamin D (VD) 200 IU daily, and alendronate (ALN) 35 mg once weekly on bone mineral density (BMD) of lumbar spine (L) and total femur (F) in postmenopausal women with osteoporosis, and the effects of the osteoporosis risk factors on BMDs and these treatments.
Design and methods
The subjects were 242 postmenopausal Japanese women aged 48–87 years (mean 65.4 ± 7.8 years). BMDs of L (L2–L4) and F were measured by the DXA. Those who were diagnosed with osteoporosis were allocated in three groups and treated with RLX 60 mg daily, VD 200 IU daily, and ALN 35 mg once weekly for 24 months. L and F BMDs were measured at baseline and after 24 months treatment. Factors those are thought to be affecting BMD such as eating habits, the differences in lifestyles, and the physical differences were investigated. The relationships among these risk factors and the effects of each treatment on L and F BMDs were analyzed by correlation analyze test
Results
L and F BMDs were increased from baseline at 24 months in ALN and RLX groups but not in VD group ($P < 0.01$), with greater increases in the ALN group (each $P < 0.05$).
Significant correlations were found between baseline L BMD:F BMD ratio and the daily number of walks, family history of bone fracture, and alcohol intake.

However, significant correlations were not found among these risk factors and the effects of each treatment on L BMDs or F BMDs.
Conclusion
The differences between lumbar and femoral BMDs were associated with walking habit, family history of bone fracture, and habits of alcohol intake in these postmenopausal women. But these risk factors were not associated with the effects of ALN, RLX, and VD treatment.

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## PP325

**Effect of implant to radiologic result in treating osteoporotic intertrochanteric fracture**

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Objectives
To determine whether kinds of implants would influence on the radiologic outcomes in the treatment of osteoporotic intertrochanteric fractures.
Materials and methods
In this retrospective study, radiologic outcomes of 151 patients with unstable osteoporotic intertrochanteric fractures undergoing surgical treatments were compared based on the types of fixation implants as follows: PFNA (53 cases, group 1), gamma nail 3 (31 cases, group 2), CHS with TSP (43 cases, group 3), and helical blade type LCP–DHS with TSP (24 cases, group 4). On the follow-up radiographs after operations, we assessed differences of bone union durations, neck-shaft ankle changes, lag screw or helical blade slippages, and varus $\alpha$ angle changes among the study groups.
Results
All the radiologic outcomes evaluated in this study were not significantly different among the study groups. The average bone union durations of the groups 1, 2, 3 and 4 were 17.7, 18.0, 18.2, and 17.8 weeks respectively ($P=0.429$). The average variation of neck-shaft angle of the groups 1, 2, 3 and 4 were 3.6°, 3.1°, 3.7° and 2.9° respectively ($P=0.273$). The average lag screw or blade slippage of the groups 1, 2, 3 and 4 were 5.1, 3.3, 3.6 and 2.7 mm respectively ($P=0.154$). The average variation of varus $\alpha$ of the groups 1, 2, 3 and 4 were 5.3°, 4.7°, 4.1° and 4.6° respectively ($P=0.894$).
Conclusions
This study indicates that four typical types of fixation implants for treating osteoporotic intertrochanteric fractures would not lead to differences in postoperative radiological outcomes.

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## PP326

**Undertreatment of osteoporosis: the Medication Use Patterns, Treatment Satisfaction, and Inadequate Control of Osteoporosis Study (MUSIC-OS)**

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Objective
This multi-objective prospective study (MUSIC-OS) is intended to examine treatment patterns, occurrence of gastrointestinal (GI) complications and clinical and health outcomes in post-menopausal osteoporotic (OP) women in Europe and Canada. Surveys were administered to both physicians and patients as part of the MUSIC-OS study to document reasons for undertreatment in OP from both physician and patient perspectives.
Materials and methods
A physician questionnaire was employed to document physician practice patterns regarding the diagnosis and management of OP, and to understand the role of gastrointestinal complications in the management and treatment of OP. Additionally, a survey was administered to untreated patients who were diagnosed with OP, to document their reasons for not receiving a medication for their condition.
Results
Ninety-seven physicians participated in the physician survey and 292 patients participated in the patient survey. Seventy-five percent of patients reported having a pre-existing GI problem. 18.6% of physicians were unwilling to prescribe pharmacological OP treatment for their osteoporotic patients with a pre-existing GI problem and of all patients with pre-existing GI problems, 15% reported GI problems as the main reason their doctor did not prescribe OP treatment. Most patients who had made the decision to not take OP treatment cited 'fear of side effects' as the most common reason they are untreated (58.3%). Approximately 62% of patients reported that they were not treated for OP due to physician concern. Conversely, only 17% of physicians reported they were unwilling to prescribe treatment because of a patient's medical condition.
Conclusion
There is a disparity in perception between patients and physicians regarding decision making for treatment of OP. Sixty-two percent of patients believe non-treatment is physician driven, compared to 17% of physicians. Physicians (18.6%) will not prescribe pharmacological OP treatment due to patient GI sensitivity.

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## PP327

**Baseline data of the DEVIDE-study: DEnosumab vs Intravenous Ibandronate: a 2-year retrospective head to head real life study**

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Introduction
Effective treatment of postmenopausal osteoporosis (PMO) is frequently compromised by poor adherence to short-term ($\leq$1-monthly) medications. Aim of this study is to investigate the effect of parenteral ibandronate (IBN) compared to denosumab in a cohort of IBN pretreated PMO patients.
Methods
In a retrospective analysis, a total of 808 women were treated with quarterly 3 mg IBN injections for 27+3 months and were regularly monitored at the osteoporosis outpatient clinic at two large academic centers. After denosumab became available, 366 women were switched to receive this therapy due to their own and their physician's decision, whereas the remaining 442 preferred to stay on IBN. Fracture incidence, BMD, BTM, and adherence/safety of these two parenteral therapies will be analyzed.
Results
Important baseline characteristics are given in the Table 1**.** There were no differences in any of the parameters investigated including renal and liver function, vitamin D, PTH and bone turnover markers. Laboratory testing, recording of adverse events was done every 6 months, BMD readings, spinal X-ray and fracture assessment were performed at baseline and after 12 and 24 months of ongoing IBN or denosumab therapy.

**Table 1** Baseline characteristics

| | **Denosumab ($n=366$)** | **Ibandronate ($n=442$)** |
|---|---|---|
| Age (years) | 67±7 | 68±6 |
| Femoral neck BMD (*T*-score) | −2.1+0.9 | −2.0+1 |
| Lumbar spine BMD (*T*-score) | −2.8+1.2 | 2.7+1.4 |
| Non-vertebral fracture, *n* (%) | 26 (7.1%) | 34 (7.7%) |
| Vertebral fracture, *n* (%) | 69 (18.9%) | 84 (19%) |

Conclusion
The results of this study not only will provide clinicians with insight into persistence with denosumab in comparison to quarterly IBN therapy, but will also compare the effect of denosumab for the first time with a parenteral administered bisphosphonate in respect to safety, changes in BMD as well as fracture incidence and changes in SDI over a time period of 2 years.

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## PP328

**Effects of a preventive long duration treatment of ovariectomized rats with strontium ranelate and zoledronic acid on bone mineral density and bone remodeling**

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Long-term use of pharmacological treatments of osteoporosis is currently a controversial subject within the scientific community.
The aim of this work was to study the effects produced by a preventive long duration treatment of strontium ranelate and zoledronic acid on bone mass and remodelling when they are administered to ovariectomized rats.
Sixty female albino Wistar rats were divided into four groups ($n=15$): SHAM group (simulated surgery), OVX group (ovariectomized rats), OVX+ZA (ovariectomized rats treated with Zoledronic acid, 0.08 mg/kg, i.v., at the start of the study), OVX+SR (OVX rats treated with 0.033 g/kg per day of strontium ranelate, oral gavage). Treatments started the day after ovariectomy, and were administered during 8 months. After sacrifice, bone mineral density (BMD) in lumbar spine and femur by DXA and osteocalcin (BGP), aminoterminal propeptide of procollagen I (PINP), 5b isoenzyme of serum tartrate resistant acid phosphatase (TRAP) and carboxiterminal telopeptide of collagen I (CTX) by ELISA were performed.
Femoral and lumbar BMD were significantly decreased in OVX rats without significant differences with those of rats treated with SR. Femoral and lumbar BMD of (OVX+ZA) group was significantly increased with respect to those of OVX group and even with respect to SHAM group. OVX produced a significant increase in BGP, PINP and CTX/TRAP rate. Levels of BGP of (OVX+SR) and (OVX+ZA) groups were similar to those of SHAM group. PINP levels of (OVX+SR) group were similar to those of SHAM group, being levels of (OVX+ZA) group lower than those of SHAM rats. Levels of CTX/TRAP of both treated groups were increased with respect to SHAM group and similar to those of OVX rats, being levels of group treated with ZA significantly lower than those of rats treated with SR.
Conclusions
A long duration preventive treatment with SR was not able to avoid the negative effects of ovariectomy on bone mass. Treatment with ZA avoided these effects and improve bone mass with respect to that of control group.

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## PP329

**Analysis of adequacy in prescribing calcium supplements based on the daily dairy intake in patients at risk of osteoporosis**

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Introduction
Clinical practice guidelines recommend a daily intake of calcium of 1-1, 5 g for posmenopausical osteoporosis. However, giving the possible adverse effects of calcium supplements, their use remains controversial.
Aims
Validate the dietary assessment of calcium. Evaluate the degree of compliance of the medical advice and discuss the adequacy of prescribing calcium supplements.
Patients and methods
Sample cohort comprised 337 patients (292 women; and 45 men) recruited from consecutive patients submitted to perform a bone densitometry. Anthropometric measurements, osteoporosis risk factors, dairy intake of calcium, antecedents of bone fractures, co-morbidity, and treatments were recorded in all participants. Also, a sample of 79 female subjects was re-evaluated across the time to evaluate the adherence to the recommendation of increase the intake of Ca. Data were analyzed with the statistical package IBM-SPSS.
Results and discussion
Daily intake of calcium showed a good correlation ($r=0.42$; $P<0.001$) with information obtained by means of a 24 h dietary record administered by trained personal. Patients increased the intake of Ca from $663\pm321$ to $762\pm359$ mg/day, ($P=0.015$) after medical advice. A 24.0% of the patients were treated with anti-osteoporotic drugs and a 31.0% of them takes Ca supplements but their medical prescription has not been associated with the intake of calcium of the sample (no prescription in 86.8% of patients with dairy Ca intake $<700$ mg/day and 18.2% with Ca intake $\geq 1200$).
Conclusions
As patient's follows medical recommendation, it would be useful for the future, the evaluation of the real requirement of calcium supplements before supplements prescription. The questionnaire previously used for calcium assessment seems to be valid for this purpose.

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## PP330

**Determinants of bone turnover marker response to three oral bisphosphonate therapies in postmenopausal osteoporosis: the TRIO study**

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Three oral bisphosphonates ibandronate, alendronate, and risedronate are commonly used for the treatment of osteoporosis but they have not previously been compared in the same study. Our aim was to identify determinants of change in bone turnover markers (BTM) in response to these bisphosphonates in a 2-year randomised parallel group trial. We recruited 171 postmenopausal osteoporotic women ($<85$ years) who were randomised to receive ibandronate (150 mg monthly), alendronate (70 mg weekly), or risedronate (35 mg weekly) plus daily calcium (1200 mg) and colecalciferol (800 IU). Ninety women returned at 2 years. The study had Local Research Ethics Committee approval. Fasting blood and urine samples were collected at baseline, 1, 2, 4, 12, 48, and 96 weeks. Biochemical measurements included: serum bone ALP, osteocalcin, PINP, CTX, PTH, and 25(OH)D (IDS-iSYS, Immunodiagnostic Systems, UK), urine NTX (Vitros ECi, OrthoClinical Diagnostics, UK). Mixed model statistical analysis was used to account for repeated measures and identify the determinants of percentage change in BTM. We evaluated the influence of baseline BTM, age, BMI, and BMD T-score stratum.
There was a significant difference between treatment groups for bone resorption markers (Table 1) with greater responses for alendronate and ibandronate than for risedronate.

**Table 1** *P* values for mixed model analyses.

| | Bone ALP | OC | PINP | CTX | NTX |
|---|---|---|---|---|---|
| Treatment | 0.6 | 0.9 | 0.2 | $<0.0001$ | $<0.0001$ |
| Visit | $<0.0001$ | $<0.0001$ | $<0.0001$ | $<0.0001$ | $<0.0001$ |
| Baseline | $<0.001$ | 0.007 | $<0.001$ | $<0.001$ | $<0.001$ |
| BMI | 0.006 | 0.4 | 0.07 | 0.5 | 0.05 |

Bone resorption markers were significantly decreased by week 1 ($P<0.001$), bone formation markers by week 4 (bone ALP, $P=0.02$, OC and PINP $P<0.001$). High baseline bone turnover was related to a greater decrease in BTM. BMI was associated with change in bone ALP and NTX (Table 1). Age and BMD stratum were not significant. Response to oral bisphosphonate is determined by the type of bisphosphonate, the baseline BTM and the BMI.

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## PP331

**Prevalence of osteoporosis treatment depending on the risk evaluation of the osteoporotic fractures in patients with rheumatoid arthritis in Russia**

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Background
It is known that one of the features of rheumatoid arthritis (RA) is a generalized bone loss, therefore important tasks for the physician are timely evaluating of the risk of fractures and approving osteoporosis treatment in patients with RA.
Objectives
Through the Programme of the Russian Association of Rheumatology for the diagnosis, prevention and treatment osteoporosis (OP) in patients with RA we evaluated the 10 year probability of major osteoporotic fracture by FRAX for Russia in patients with RA, and we identified prevalence osteoporosis treatment in patients with high or low risk of osteoporotic fractures (OF).
Material and methods
Through the Programme of a large amount of patients with RA from 12 clinical centers in Russia 534 patients aged 41– 89 years were selected and have been evaluated the risk of OF by FRAX.
Results
459 (86%) women and 75 (14%) men were involved in our study. Female average age was 60.85 years, male one was 60.1 years. OP was diagnosed in 88 (16%) patients, 61 (11%) had a history of OF, however, of these people OP treatment was approved only 80 (91%) and 31 (51%) respectively. 168 (31%) patients had indications to approve OP treatment, defined using FRAX, only 51 (30%) of these people treatment was imposed. Assignment of treatment didn't need for 366 (69%) patients, however treatment was approved 45 (12%) from them. 65 (68%) patients were taking prescribed medications, 42 (65%) of them – bisphosphonates plus calcium and/or vitamin D supplementation, 14 (21%) – calcium and vitamin D, 9 (14%) – calcitonin.
Conclusion
Osteoporosis management is not quite enough in patients with RA in Russia. There is substantial variation in the quality of risk evaluation of the osteoporotic fractures and treatment. Introduction of FRAX for allocation of group of patients with RA with a high risk of fracture will contribute to a personalized approach to approving treatment.

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## PP332

**Denosumab therapy of osteoporosis in patients after solid organ transplantation and impaired renal function**

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The therapy of osteoporosis in patients with end organ failure and after the transplantation (Tx) becomes more frequent problem with the better patients and graft survival rates. Moreover some of these patients have impaired renal function and therefore cannot use bisphosphonates. The supplementation therapy with calcium and/or vitamin D is frequently not sufficient to improve bone mass. The possible innovative therapy is Denosumab (Prolia inj.) - MAB to RANK, which is not contraindicated in patients with elevated creatinine level.
We have treated osteoporosis in 21 patients (14 females, seven males, age 39–68 years) after solid organ Tx performed in years 1996–2013 with impaired renal function-the mean creatinine level 171.9 μmol/l (110–306 μmol/l). Kidney Tx was performed in 11 patients, liver Tx in eight patients, and two patients underwent heart Tx. The osteoporosis was diagnosed with standard DEXA examination on GE instrument Lunar Prodigy. We have used Denosumab 60 mg inj. s.c. every 2 months in years 2011–2013. All these patients were also treated with calcium and D vitamin and the plasma Ca level was within normal limits during the treatment (mean 2.44 mmol/l). The mean osteocalcin level was 22.6 μg/l (6.5–54.1 μg/l) parathormone (PTH) 7.12 pmol/l (2.48–15.8), (beta-cross laps 0.389 μg/l (0.040–1.020), $25OHD_3$ 33.4 μg/l (14.5–67.6 μg/l), $1.25(OH)_2D_3$ ng/l (16.4–95.1 ng/l). All patients were treated with Denosumab minimally for 1 year and 13 patients had repeated DEXA examination after therapy. The BMD of lumbar spine has increased in ten patients, in two patients did not change and slightly decreased in one patient. The BMD of total hip has improved in eight patients, unchanged in four patients and decreased in one patient. The BMD of distal radius has improved in six patients, unchanged in five patients and decreased in two patients.
Summary
The Denosumab therapy increased bone density in most patients after Tx. We did not observe any complications after this therapy. The administration of Denosumab seems to be useful in osteoporotic patients after organ transplantation in which bisphosphonate therapy is contraindicated because of impaired renal function.

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## PP333

**Patients with atypical femoral fractures: characteristics, anabolic treatment and long term follow-up**

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Background
Atypical fractures (AF) of femoral shaft have distinct radiologic features and are probably related to prolonged bisphosphonate (BP) exposure. They are currently considered to be insufficiency fractures occurring in osteoporotic bone. After initial damage, the repair process calls for accelerated remodeling, but the later is prevented by BP accumulation on the remodeling site. Thus, the fracture expands instead of healing. The management is not postulated, but anabolic treatment makes sense and a few case reports regarding teriparatide use have been published. Long-term follow-up data is lacking. The aim of this report was to summarize our experience with AF patients – clinical characteristics, treatment and outcomes.
Patients
Seven female patients were followed prospectively since 2008. The median age was 66. All of them were BP exposed, and mean treatment duration was 8 years. Two patients were steroid treated. The majority (57%) sustained osteoporotic fractures prior to the index event. None smoked. Their vitamin D levels were $>20$ ng/ml, in all. Bone density prior to AF was in the osteoporotic range: lumbar spine *T*-score $-3.48+1.1$ and femoral neck $-2.9\pm1$.
Results
Three patients presented with complete shaft fracture, four had lateral cortex incomplete fractures (one of those bilateral). Bisphosphonate was discontinued in all patients and six (85%) were offered teriparatide (TPT) treatment. Two patients with lateral cortex incomplete fractures progressed to a complete fracture while on TPT. Median follow-up was 48 months. Hip bone density was significantly improved. None sustained another fracture.
Conclusions
AF occurred in high risk patients. In our small cohort, TPT failed to prevent incomplete fracture progression in half of the participants. Anabolic treatment was effective in terms of bone density improvement.

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## PP334

**Comparison of efficacy teriparatide between denosumab and on hip BMD in women with severe post-menopausal osteoporosis**

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Introduction
Teriparatide is a potent anabolic drug that has demonstrated efficacy on fracture risk reduction in women with severe postmenopausal osteoporosis. Denosumab, designed to inhibit RANKL (RANK ligand), is a fully human MAB for the treatment of osteoporosis. BMD changes, measured by DXA, is an established tool for monitoring the effects of anti-osteoporotic therapy. Our purpose is to compare teriparatide vs denosumab efficacy on hip BMD variations, in postmenopausal women with primary osteoporosis.
Methods
68 female patients (mean age $75.5\pm7.0$ years), with severe postmenopausal osteoporosis, characterized by multiple vertebral fractures, were treated either with Denosumab (*n*: 34, group D) or with Teriparatide (*n*: 34, group T), for 12 months.

Results
Since the treatments were not randomly administered, baseline unbalancing between two groups should be taken into account, even more than in case of randomized trial. We found that the denosumab group was slightly lower in terms of BMD (0.59 vs 0.63, $P=0.138$), clearly lower in terms of $T$-score ($-3.3$ vs $-2.4$, $P<0.001$) and older (78.4 vs 72.6, $P<0.001$). Therefore, baseline measures were entered as covariate to statistically control their effect on treatment effects. ANCOVA indicated that the increase of BMD was larger ($P=0.028$) after teriparatide ($+0.06$, 95% CI: $+0.04$, $+0.08$) than after denosumab ($+0.02$, 95% CI: $+0.01$, $+0.04$). The increase of $T$-score was significant in the whole sample ($P<0.01$), but without difference between the two treatments ($P=0.132$). In particular, after teriparatide $t$-score increased of 0.44 (95% CI: $+0.28$, $+0.61$), while after denosumab of 0.25 (95% CI: $+0.08$, $+0.41$).
Conclusions
Our preliminary observation suggests a possible greater efficacy of teriparatide, when compared to denosumab. This finding could be related to the faster anabolic effect of Teriparatide, in comparison to denosumab, which displays a slower and more gradual efficacy. However, prolongation of follow-up is needed to confirm our data.

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## PP335

### A survey of oral health attitudes and practices amongst bisphosphonate users in UK

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Introduction and aim
Many patients with osteoporosis are elderly and oral health is an important aspect of patient care especially in patients on bisphosphonates. Our aim was to determine oral health attitudes and practices in patients with osteoporosis receiving bisphosphonates.
Methods
A questionnaire study was conducted after informed consent on cognitively intact patients diagnosed with osteoporosis who were attending hospital Osteoporosis clinic and received bisphosphonate treatments.
Results
200 patients age range 50–95 years (average 70 years) 75% females. Patients receiving treatments were alendronate (80%) risedronate (10%) ibandronate (4%) zoledronate (6%). 100% considered care of teeth is important. 92% considered cleaning of teeth daily is important. 20% considered they do not need regular specialist dental care. 90% thought tooth loss is normal consequence of ageing. 90% considered tooth loss has an association with osteoporosis. 32% felt access to dental services was a barrier towards improving oral health. 20% used dentures. 65% had visited dentist in last 1 year. 50% regularly visit dentist twice a year. 10% were not registered with a dentist. 59% clean their teeth twice daily. 80% had informed their dentist about osteoporosis medications. 10% were aware of rare side effect of jaw osteonecrosis.
Conclusions
This large study shows that most elderly have favourable attitudes towards improving their oral health. But there are gaps in patients knowledge and there is suboptimal dental access, behaviour and practice amongst a large number of patients. Further education and improved provision of facilities for dental care is needed.

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## PP336

### The effect of alendronate oral jelly in the treatment of osteoporosis in clinical settings

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Alendronate is one of the most popular anti-resorptive drugs and widely used to treat osteoporotic patients in the world. In Japan, alendronate oral jelly (ALN-J) as a new formulation was launched in 2013. Dissolution test and bioequivalence study were performed between ALN-J and alendronate oral weekly tablet (ALN-T), and bioequivalence of both drugs was approved. Dosage and administration of ALN-J is the same as ALN-T. Moreover, ALN-J has special features of easy swallowing and obvious distinguishability compared with ALN-T. However, little is known about clinical evidence such as BMD, bone turnover marker, safety and adherence in the treatment with ALN-J. We conducted the present study to verify the efficacy and safety of ALN-J in the treatment of osteoporosis. A number of 32 patients were enrolled in the present study, and age, history of prevalent fractures, BMD, bone turnover marker and others were investigated. Baseline characteristics of the patients were as follows; mean (S.D.) of age (years) was 78.25 (6.58); radius BMD (g/cm$^2$), 0.45 (0.07); TRACP5b (mU/dl), 497.42 (160.92); P1NP (μg/l), 64.90 (25.66) respectively. After treatment, mean change in TRACP5b decreased by 36.7% and P1NP decreased by 43.7% for 3 months. The effect of ALN-J for change in bone turnover marker was confirmed in the treatment of osteoporosis in brief duration.

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## PP337

### Monitoring of calcium intake and vitamin D saturation in Slovak postmenopausal women

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According various guidelines the basic recommendation in prevention and treatment of postmenopausal osteoporosis (PMO) is adequate intake of calcium (1000– 1300 mg/day) and vitamin D (800–1000 IU/day).
Aim
To determine the intake dose of calcium and also the vitamin D saturation in women treated for PMO.
Patients and methods
465 women with PMO, divided into two groups: patients with newly diagnosed osteoporosis ($n=203$) and treated patients with PMO ($n=262$). We used a questionnaire to evaluate demographic dates, eating habits and risk factors for PMO. We measured serum calcium level and 25-OH-vitamin $D_3$.
Results
Milk consumes only 70% of newly diagnosed patients and 78% of treated patients with PMO. About 18% (group 1) and 13% (group 2) of the patients declared lactose intolerance. Low-fat milk (with the highest content of calcium) prefers 8–11% of the patients. Calcium supplements were used in 41% of group 1 and 87% of group 2. The daily calcium intake in group 1 was 452, and 848 mmol/l in treated group of patients. The serum calcium was the same in both groups (2.35 mmol/l). In both groups was very low saturation of vitamin D – in newly-diagnosed patients, 63% of women had insufficiency of vitamin D, with the mean level of 25-OH-$D_3$ 17 nmol/l. But also in treated group of patients with PMO was the mean 25-OH-$D_3$ level was only 35.3 nmol/l, and 41% of these women had vitamin D insufficiency.
Discussion
Also according international surveys is the daily calcium intake very low. Bruyera *et al.* (2009) find out, that the mean calcium intake in European patients with PMO is 930 mg/day. Our study demonstrated that the calcium intake in our country was lower than Hungary, the country with the lowest calcium intake.
Conclusion
According the result of our study the calcium intake in Slovak women patients with PMO is insufficient and also the saturation of vitamin D is inadequate. Unpleasant is that it is also in treated group of patients. We must in the future clarify causes of this result, if is it noncompliance of the patient, or other reasons.

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## PP338

### Patients at high risk of fragility fracture and teriparatide: report from a third level osteoporosis clinic

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Background
Osteoporotic fractures are a major cause of disability and results in extensive utilization of health care resources. The history of previous fractures increases the risk for future fragility fractures.
Subject
To describe the diagnostic therapeutic protocol of our tertiary care clinic dedicated to the osteoporosis (OP) treatment. To estimate the use of effective therapies for OP in those patients at highest risk for future fracture.

Methods
Patients with a history of OP or with high risk of fracture (calculated using DEFRA or frax) were preliminarily assessed by general practitioners and/or by rheumatologist at a primary level clinic or at bone densitometry service. In presence of a suspected fracture patients were referred to our tertiary center.
If not available second level laboratory evaluation, bone densitometry and morphometry X-ray of the spine had been performed. We adopted SIOMMMS guidelines for treatment of osteoporosis.
Results
We analyzed data from 300 consecutive patients who received OP consultations for fragility fractures at our referral centre from December 2011 to December 2013. The consultation included an extensive risk factor analysis for OP.
In this time lapse overall 49 patients were suitable to start teriparatide (16.3%). Of these, primary osteoporosis was diagnosed in 24 patients and secondary osteoporosis in 25. Median age was of 75 years (range 53–84) and ratio of women to men was 7/1 (43 females, and 6 males). 46 patients have started treatment with teriparatide: among them, three discontinued treatment for adverse events. A follow-up of 12 months was available for 18 patients: no patient had new fractures during this period.
Conclusion
Sharing of diagnostic and therapeutic pathways allows to identify patients at increased risk for fragility fractures in order to offer them the best available treatment and to provide monitoring throughout the follow-up.

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## PP339

**Recommendations for osteoporosis management and fracture prevention for the frail elderly in long-term care**

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Objectives
Clinicians practicing in long-term care (LTC) face unique challenges caring for frail elderly individuals including multiple co-morbidities, polypharmacy, and end of life care, and practice guidelines typically do not address this population. Guidance regarding the management of osteoporosis and fracture prevention in LTC, a high-risk population, is needed.
Materials and methods
A survey of LTC physicians informed key questions and outcomes, and interviews with resident representatives and a literature review informed values and preferences. The GRADE approach was used.
Results
For residents at high risk for fracture, we suggest multifaceted interventions that are individually tailored to reduce the risk of falls and fractures, and suggest balance, strength and functional training exercises only when part of a multifaceted intervention. There may be an increase risk of falls in this high-risk population if risk factors for falls are not addressed in addition to exercise interventions. We recommend hip protectors. We recommend vitamin $D_3$ supplements daily (800–2000 IU) and calcium supplementation up to 500 mg daily if unable to meet recommended dietary allowances through food. We recommend the following therapeutic agents: alendronate, risedronate, zoledronic acid, or denosumab. Teraparatide is a suggested option. We suggest raloxifene and etidronate not be used. For every 1000 persons who are treated for 1 year with the recommended pharmacological therapies there would be 22–24 fewer hip fractures, 89–124 fewer vertebral fractures and 13–18 fewer non vertebral fractures. The evidence which informed these recommendations included adverse effects, patient values and preferences, lifespan and costs.
Conclusions
These are the first guidelines developed for the care of osteoporosis and fracture prevention in LTC using GRADE. In LTC, strategies to prevent fractures and falls must consider resident values and preferences, co-morbidities, life expectancy, and quality of life.
They emailed a full list of disclosures to be included as follows
Dr Alexandra Papaioannou has received grants/research support from Amgen, Eli Lilly, Merck and Warner Chilcott. Dr Papaioannou has been on a speakers bureau and received honoraria from Amgen, Eli Lilly, and Merck. Dr Papaioannou has received consulting fees from Amgen, Eli Lilly and Merck and is an employee of McMaster University. Nancy Santesso, no conflicts. Dr Suzanne Morin, has received grants/research support from Amgen. Dr Morin has received consulting fees for Amgen, Merck and Eli LIlly. Dr Morin has been on speakers bureau and received honoraria from Amgen and Eli Lilly. Dr Angela Cheung has received grants and honorarium from Amgen, Eli Lilly, Merck. Dr Richard Crilly, no conflicts. Dr Lora Giangregorio has received grants/research support from Merck. Kerry Grady, no conflicts. Dr Robert Josse has been an advisory board member, received speaker honoraria and/or research grants from Lilly, Amgen, Novartis, Warner Chilcott, Merck. Dr Susan Jaglal, no conflicts. Ravi Jain, no conflicts. Dr Sharon Kaasalainen, no conflicts. Dr Andrea Moser, no conflicts. Laura, Pickard, no conflicts. Carly Skidmore, no conflicts. Dr Hope Weiler, no conflicts. Dr Susan Whiting, no conflicts. Dr Jonathan Adachi, has participated in clinical trials for Amgen, Eli Lilly, Merck, Novartis. Dr Adachi has been on a speakers bureau, received honoraria and consulting fees from Amgen, Eli Lilly, Merck, Novartis, Warner Chilcott. Dr Adachi is an employee of McMaster University.

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## PP340

**A clinical case of pregnancy, delivery and lactation in a patient treated with ibandronic acid 3.0 intravenously**

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There are few cases of pregnancy described in which the fetus was exposed to some bisphosphonates, but not ibandronate to our knowledge. Meanwhile, ibandronate has a high protein binding rate (87%) and short tissue's half-life (24 days) and might be a less potentially harmful drug in the event of pregnancy. A 23-year-old woman was admitted to our hospital with evident ACTH-dependent Cushing's syndrome CS (24 h urinary free cortisol – 2413 nmol/24 h (reference range 59.2–413)). The etiological diagnosis was unclear. At the time of admission the patient had vertebral compressions of ThX, VII, V with a BMD loss −2.4 (*Z*-score) at the femur neck. After informed written consent was signed, the patient started treatment with ibandronic acid 3.0 intravenously every 3 months and a daily dose of ketokonasol 400–600 mg. The first injection was done on the 30th October 2009. The full remission of CS was achieved on 10th June 2010. Nevertheless, because of following severe adrenal insufficiency the patient received prednisolone 7.5–10 mg daily and continued the treatment with ibandronic acid with a total +11.9% L1–L4 and +6.8% proximal femur BMD increase. The final injection of ibandronic acid was performed on 28th April 2011. The pregnancy was diagnosed on 14th June 2011 at gestation age of 2–2.5 weeks. The pregnancy was normal with a caesarian section at the gestation age of 38 weeks. The child suffered from inspiratory insufficiency because of cord entanglement. A boy was born with a length of 49 cm and weight of 3120 g and without any signs of birth defects. The child was breastfed until 11 months of age. At the age of 1.5 years his height was 89 cm and his weight was 11 400 g. The child was healthy.
Conclusion
Treatment with ibandronate did not have any deleterious effects on the above mentioned pregnancy and fetus.

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## PP341

**Changes on bone turnover markers and bone mineral density in women with postmenopausical osteoporosis treated with denosumab**

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Denosumab (DNMB) is an effective treatment for osteoporosis with antiresorptive effect that has demonstrated to increase the bone mass and reduces the incidence of both vertebral and nonvertebral fractures in randomized controlled trials (RCTs).

Objectives
To confirm that this drug is actually effective in our experience into daily clinical practice as well.
Material and methods
30 postmenopausal women, mean age 71 years ($r=41–89$), were treated with DNMB (60 mg Q6M) during 1 year. Calcium, phosphorus, and BTM of formation (P1NP) and resorption (CTX) were measured in serum at baseline, 6 months and 12 months by the following techniques: CTX by electrochemisluminescence and PINP by RIA. BMD were measured by DXA (Hologic *c.v. in vivo* 1.2%) at the lumbar spine (LS), femoral neck (FN), total hip (TH), and forearm at baseline and 12 months.
Results
DNMB did not produce significant variations in serum calcium and phosphorus. BTM decreased very significantly ($P<0.001$): CTX decreased 66% at 6 months and 65.9% at 12 months. P1NP decreased 55.11% at 6 and 44.9% at 12 months. BMD increased in LS 5% ($P<0.001$), TH 2.36% ($P<0.001$) and forearm 2.18% ($P<0.01$). BMD in FN also increased (1.57%) but was not statistically significant. There were not differences in BMD responses related with the use of previous antiosteoporotic treatments although the BTM were a different magnitudes in their decrease along the time. We found a correlation between changes in CTX and the gain in BMD at the level of CL, we did not found any other correlation between changes in BTM and the BMD in other localizations.
Conclusion
One year of treatment with DNMB causes a very significant decrease in the bone resorption and bone formation markers. DNMB produced a significant increase of the bone mass at LS, TH and forearm. The changes in BTM occurred just after 6 months of treatment with DNMB and changes in CTX are associated to progressive increasing of BMD in LS. Thus, we suggest that changes in CTX can be used as precocious indicators for the BMD later response after DNMB treatment

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## PP342

**Assessment treatment with denosumab in clinical practice**

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Introduction
Denosumab monoclonal antibody approved for Osteoporosis's treatment in Europe union and U.S.A. Dose of 60 mg every 6 months reduces the risk of vertebral, non vertebral and hip fractures. What is more increases BMD
Material and methods
Descriptive observational study with densitometric characterisques and risk factors of 64 patients in Osteoporosis unit of HGUG Marañón from November 2011 to December 2013. Analyzing occurrence of new fractures and densitometric change in lumbar spine (LS) and femoral neck (FN) in patients with 3 doses.
Results
Median age: 70.7 years old. 50% had previous fractures, 48% of them with more than 1 fracture. 69.7%vertebral fracture, 24.2% wrist fracture, 9% hip, peroneal, and humerus respectively Basal analytic characteristics: Ca 9.53, P 3.45, PTH 61.13, VitD 30.3 (media de BMD) LS FN.
35.9% received previus treatment with biphophonates, 12.5% with PTH, 4.7% with SERMS, 1.56% renelate of estroncium, 7.8% BF+PTH, 6.25% BF+SERMS and a 31.2% none.
90% with FRAX hip greater than 3%, with an average of 9.4%.
In patients with 3 doses an improvement in DXA at LS was observed in the 100%, with an average of the 11.24%. In FN an improvement in the 75%, with an average of a 4.03%.
The amelioration was higher in patients with previous fractures, but without relation with DXA value.
The tolerance to the treatment was good in the 100%. In none of the patients ocurred new fractures, neither jaw osteonecrosis.
Conclusion
Denosumab antiresortive drug presents an excellent clinical tolerance, as well as a great therapeutic answer in our population.These answer was higher al lumbar spine, which is coincident with previous studies.its a good therapeutic target for osteoporosis.

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## PP343

**Clinical experience with Denosumab in the treatment of osteoporosis**

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Background
Denosumab is licensed for the treatment of osteoporosis in the UK. Guidance issued by NICE and SMC in indicated that Denosumab may be used in the treatment of patients with postmenopausal osteoporosis who are unsuitable for other treatments. Here we have reviewed the clinical characteristics of patients treated within a secondary care referral centre adverse events encountered during treatment.
Methods
We reviewed data from 75 consecutive patients treated with Denosumab between 2010 and 2012.
Results
Most patients (88%) had postmenopausal osteoporosis and 80% had previously been on other osteoporosis treatments. Seven male osteoporotic patients (9%) were treated. All patients were given vitamin D supplements prior to commencing Denosumab and combined calcium and vitamin D supplements during treatment. The mean duration of Denosumab therapy was $21.7\pm5.4$ months, with the most common indications being GI intolerance with oral bisphosphonates ($n=24$), poor renal function ($n=17$) and poor compliance with oral bisphosphonates ($n=10$). Overall compliance with NICE guidelines was 63% and SMC guidance was 65%. Treatment was generally well tolerated and mild hypocalcaemia was observed in only one patient, who in error had also been treated with alendronic acid.
Conclusion
Denosumab is a useful and safe treatment option for many patients in whom oral bisphosphonates are poorly tolerated or where compliance is an issue. Although most patients being treated with Denosumab fulfilled the guidance issued by NICE and SMC a substantial proportion did not.

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## PP344

**Appropriateness of osteoporosis treatment within a medical in-patient population in a welsh district general hospital**

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Objectives
To assess the pharmacological management of osteoporosis in a cohort of hospitalized medical in-patients within a Welsh district general hospital.
Materials and methods
We collected data using medical records on a structured proforma as a point prevalence study targeting medical inpatients aged fifty and above in an admitted hospital cohort.
Results
Out of the 117 patients, 88 (75.21%) of them had greater than three risk factors. Out of the 117 patients only 28 (24%) of the in-patients were on any form of treatment for osteoporosis. And an equal number (i.e. 24%) had undergone a fracture risk assessment. Only 16 (13.6%) of them were on both calcium-vitamin D3 and a bisphosphonate. Out of 117, 25 of them were receiving calcium and vitamin D. Of them were on Bisphosphonates (17 on alendronic Acid, one on denosumab, and one on risedronate)
Conclusion
A significant percentage of hospitalized inpatients have risk factors for osteoporosis. Several of them have multiple risk factors but are inadequately treated for osteoporosis. Creating an awareness in this aspect among health professionals for identifying and adequately treating patients with osteoporosis would be a step towards reducing the burden of this chronic and treatable condition.

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## PP345

**Standardized diagnostic and therapeutic pathway for management of patients with skeletal fragility fractures in the Orthopedic and Physiatrist Unit in an Italian hospital: impact on patient outcome**

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It is well known that osteoporotic fragility fractures are an important risk factor for subsequent new vertebral and non-vertebral fractures. Many countries published recommendations to prevent fractures. Also in Italy the Minister of the Health highlighted the importance to prevent osteoporosis and the subsequent fractures, without practical instructions on the management of patients with fragility fractures in the daily clinical practice. Up to date there is no standardized diagnostic and therapeutic pathway (DTP), with a non-homogeneous patient

management, different costs and patient outcomes. With the aim to improve the outcome of patients with femoral, vertebral and wrist fragility fractures, a standardized DTP has been created by a multidisciplinary working group (Physiatrist, Orthopedic and quality management control representative) in an Italian hospital. Patients admitted in the Orthopedics Unit with a fragility fracture are identified by a specific 'patient record', with prescription of first level blood tests for osteoporosis diagnosis according to the national guidelines. Then a discharge letter is specifically created with the diagnosis of osteoporotic fragility fracture. Patients discharged from orthopedics have a priority access to the rehabilitation unit, where other diagnostic tests are performed (i.e. second level blood tests and others such as X-rays, MRI, etc.). Upon discharge from the rehabilitation unit, a letter is drawn up with the fragility fracture diagnosis, drug prescriptions, according to the National reimbursability criteria. Follow-up visits are required at 6 and 12 months to measure activity, process and outcome indicators, with the objective to verify: i) percentage of DTP implementation. ii) percentage of patients with new fragility fracture occurred within one year from hospital discharge. In conclusion, the working group expectation is the 100% of the DTP application, with consequent patient re-fractured reduction in the 1-year period, compared to the patient fracture incidence without DTP implementation.

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## PP346

**Efficacy of Denosumab on bone metabolism after a 12-month treatment, in women with severe post-menopausal osteoporosis**

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Introduction
Denosumab, a fully human monoclonal antibody to RANK ligand, effectively reduces bone resorption by inhibiting RANK-L binding to RANK. We aimed to evaluate the efficacy of denosumab in post-menopausal women, by monitoring the evolution of different parameters of bone health: *T*-score, morphometry, osteocalcin and β-Ctx.
Methods
A 34 women with severe postmenopausal osteoporosis, characterized by multiple vertebral fractures were studied. At baseline, hip and spine *T*-score were measured, by dual energy X-ray absorptiometry (DXA, Hologic) and fulfilled WHO criteria for osteoporosis. At baseline and following a 12-month treatment with Denosumab, all patients underwent morphometry, in order to investigate the prevalence of vertebral fractures during treatment, and measurement of bone metabolism parameters (serum calcium, osteocalcin, and β-Ctx).
Results
At the end of the 12 months, *T*-score raised at the lumbar level ($-2.0\pm0.5$ at baseline and $-1.7\pm0.8$ after 12 months, $P=0.05$, Wilcoxon's test) and significantly increased at the hip level ($-3.4\pm0.8$ at the baseline and $-2.7\pm1.2$ after 12 months, $P=0.02$, Wilcoxon's test).
Serum calcium, osteocalcin, and β-Ctx were within the normal range and no new vertebral fractures were observed, after 12 months of therapy with Denosumab.
Conclusion
Our preliminary results confirm literature data, showing a significant efficacy of Denosumab in the treatment of post-menopausal osteoporosis. The observation of the statistically significant increase of *T*-score at the hip level, and the absence of incident vertebral fractures, although very promising, requires further data from long term follow-up.

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## PP347

**The efficacy of the treatment with intravenous ibandronate in common population.**

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Objectives
To confirm effect of intravenous treatment with ibandronate every three months in common population of postmenopausal women.
Methods
Patients with postmenopausal osteoporosis, who fulfil ACR criteria for postmenopausal osteoporosis and were treated with intravenous ibandronate for two years, underwent DEXA examination.
Results
A 50 women suffering from postmenopausal osteoporosis were treated with ibandronate i.v. every three during 2 years. (It means 8 doses of i.v. ibandronate.) They were examined between May 2010 and April 2013. Average age was 71.2 years, range 47–86 years.The previous antiosteoporotic treatment had 44/50 = 88%.
The previous osteoporotic fracture had 28/50 = 56%. Average time from menopause was 21.3 − 9.2 years, range 0–35 years. Average time of previous antiosteoporotic treatment was 3.3 − 0.5 years, range 0–16 years.

| BMD (g/cm$^2$)Trend | New fractures 0 |
|---|---|
| L spine +3.3% after 1 year, +1.9% after 2 years | |
| hip +0.5..+0.9%, | |
| neck +2.7..+3.3% | |

| Difference (*T*-score) after 1 year | Difference (*T*-score) after 2 years compare to baseline |
|---|---|
| L-spine: $P=0.23$ | L-spine: $P=0.23$ |
| total hip: $P=0.22$ | total hip: $P=0.95$ |
| neck: $P=0.001$ | neck: $P < 0.0001$ |

There was no difference in all regions between the first and secon years.

| Difference (g/cm$^2$) after 1 year | Difference (g/cm$^2$) after 2 years |
|---|---|
| L-spine: $P=0.004$ | L-spine: $P=0.009$ |
| hip: $P=0.933$ | hip: $P=0.96$ |
| neck: $P=0.004$ | neck: $P=0.18$ |

There was no difference in all regions between the 1 and 2 years.
Conclusion
These results of common population of postmenopausal women confirm BMD gain in all regions especially after the 1 year of treatment, but significant gain in *T*-score and g/cm$^2$ was only in femur neck and significant gain in g/cm$^2$ was also in L spine. After the first year there was stagnation.
Postmenopausal osteoporosis, BMD, *T*-score, ibandronate

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## PP348

**Physical performance and risk of fall in elderly people with severe osteoporosis**

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Objective
This study evaluated the differences in physical performance and risk of fall in old oldest people affected by severe osteoporosis.
Methods
The subjects were all ≥85:45 women (mean age $88\pm3$) and six men (mean age $88\pm2$) affected by severe osteoporosis. In seven women and one man we discovered a new spinal fracture after treatment. Thirty-eight women and three men had multiple spinal fractures (>3). The subjects were prescribed teriparatide treatment (PTH 1–34).The design of the study included at T0–T24: i) spine and hip DEXA densitometry; ii) spine X-ray with morphometry; and iii) blood tests. The physical performance was assessed through the Short Physical Performance Battery (SPPB) which results in a combination of a balance test according to three increasingly difficult positions, a walking test on a 4-m-course and a standing-up test from a chair, and whose final SPPB score was comprised between 0 and 12. The Tinetti balance and gait scale inspects the balance and the gait and shows a variability in score: score <1 indicates non walking; 2<score<19 walking but with a high risk of fall; and score >20 walking with a low risk of fall.
Results
At T0 we considered: i) SPPB Geriatric: mean score seven in 78.9% subjects ($P<0.05$); ii) Tinetti balance and gait scale: mean score 8 (high risk of fall) 83.8% subjects ($P<0.5$); mean score 1 (non walking) 16.2% subjects ($P<0.5$). At T24 we evaluated: i) SPPB: mean score nine in 61.3% subjects ($P<0.05$); ii) Tinetti balance and gait scale: mean score 14 (high risk of fall) 93.1% subjects ($P<0.5$), mean score 1 (non walking) 6.9% ($P<0.5$). At T24 in all subjects we detected no new spine fractures through spine X-rays and morphometry.
Conclusion
Since a reduced physical performance and an increase in the risk of fall indicate frailty in the elderly affected by severe osteoporosis, we inspected, after teliparatide treatment (PTH 1–34), the markers' severity variations.

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## PP349

**Bisphosphonate-associated atypical femoral fracture, effect of teriparatide**

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Long-term bisphosphonate (BP) therapy can be complicated by atypical femoral fracture (AFF). A positive effect of teriparatide (TPTD) has been suggested.
A woman (A, 81) with densitometric OP was put on alendronate (ALN) from 1997 until 2006, substituted by ibandronate (IBA), with an increase in FN-BMD of 6.6%. In August 2010, a fall from her standing height provoked a right transverse femoral fracture, which was operated. She was weaned from BP therapy. After operation, she complained of pain of the left thigh, and was seen in our hospital. sCTX and BSAP were not suppressed. Scalcium, GFR and 25OHD were normal. Bone scan and X-ray showed a fissure fracture of the left lateral femoral cortex. She received TPTD 20 μg/day with rapid pain alleviation. After 1 year, she could walk unaided without pain and healing of the fissure-fracture. She developed hypercalcemia (10.8 mg/dl) and TPTD was stopped, without pain recurrence.
If AFF does not heal after stopping BP, TPTD can be considered as a rescue therapy.

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## PP350

**Bilateral transient osteoporosis of hip: a case report**

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Transient osteoporosis of hip (TOH) is a spontaneous resolving skeletal disorder characterized by sudden onset of severe pain which resolves within 6–12 months. It is seen more commonly in middle aged men, though also seen in third trimester of pregnancy. MRI is the main diagnostic tool. It is idiopathic in nature.
We present a case report of a young adult male who presented with migratory transient osteoporosis of both hip joints separated by a period of around 2 years. He was managed conservatively and recovered completely both times.

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## PP351

**A transdermal patch delivering the $PTHrP_{1–34}$ analog, abaloparatide (BA058), dose-dependently increases spine and hip bmd compared to placebo**

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Abaloparatide (BA058) is a synthetic analog of $PTHrP_{1–34}$ which greatly increases bone mass and bone strength with preservation of normal bone quality in animal models of osteoporosis. Daily s.c. abaloparatide ($ABL_{SC}$) at doses of up to 80 μg daily in postmenopausal women with osteoporosis for up to 48 weeks were associated with increases in spine and femoral neck BMD of up to 12.9 and 4.1% respectively and good safety and tolerability. The increases in BMD at the 80 μg dose exceeded those seen with teriparatide (Forteo) 20 μg s.c. daily (TER) in a head-to-head study. $ABL_{SC}$ is in a large phase three fracture study due to complete this year. Many, but not all, patients with osteoporosis can self-administer injections of TER or $ABL_{SC}$, but a dosage form that increases BMD and avoids the need for injections would clearly be a valuable alternative for some patients.
Using 3M's solid Microstructured Transdermal System (sMTS), which consists of an array of 316 microprojections that penetrate the skin to about 250 μm, through the stratum corneum into the upper dermis, we developed a short-wear-time abaloparatide transdermal ($ABL_{TD}$) patch coated with doses of 50, 100 and 150 μg, and a placebo patch. 199 postmenopausal osteoporotic patients applied a patch containing either one of these three doses or placebo daily to their peri-umbilical skin for 5 min once daily for up to 24 weeks. At the end of treatment spine and hip BMD increased dose dependently, with 150 μg increasing these by 2.9% ($P<0.001$) and 1.5% ($P=0.002$) relative to placebo, respectively. Therefore, this study provides strong proof of concept that a transdermal patch delivering abaloparatide produces meaningful increases in spine and hip BMD. $ABL_{TD}$ was generally well tolerated. With further optimization, this approach holds substantial promise for a future alternative to existing and investigational injectable treatments for osteoporosis.

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## PP352

**Abaloparatide (BA058), a novel human PTHrP analog, restores bone mass and strength in the aged osteopenic ovariectomized cynomolgus monkey**

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Abaloparatide (ABL) is a novel analog of hPTHrP (1–34) in clinical development for treatment of osteoporosis. This study evaluated the long-term effects of ABL on BMD and bone strength in aged osteopenic, ovariectomized (OVX) monkeys. Four groups of ≥9-year-old female cynomolgus monkeys underwent OVX and one group underwent Sham surgery. After a 9-month bone depletion period, increases in bone markers and decreases in BMD by DXA and pQCT were observed for OVX groups, consistent with estrogen deprivation. Treatment was then initiated with either vehicle (Sham and OVX controls) or ABL at 0.2, 1 or 5 μg/kg for 16 months. Daily administration of ABL resulted in marked bone anabolic effects. Spine and femoral neck BMD was comparable to, or above, pre-surgery levels after 4 months of treatment, with further increases at 12 months, which were sustained at 16 months. Total tibia metaphysis BMD was also increased at all doses, with gains maintained at 16 months. These BMD gains were associated with increases in bone formation markers. As expected for osteopenic animals, decreases in bone mass were associated with decreased bone strength parameters (yield load) for lumbar vertebral cores (−27%) and vertebral body (−20%) in compression. ABL treatment resulted in complete reversal of OVX-induced strength loss at the spine with increased yield load for the vertebral core by +42 and +47% with 1 and 5 μg/kg ABL, and by +21 and +19% with 1 and 5 μg/kg ABL at the vertebral body. At the femoral neck, peak load shear was decreased in OVX controls (−4%), compared to sham, while this loss was reversed with 1 and 5 μg/kg ABL (+6%), compared to OVX controls. In summary, abaloparatide demonstrated the ability to rapidly build high-quality new bone in osteopenic monkeys, with sustained gains over time, and improved bone strength at clinically relevant sites.

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## PP353

**PTH treatment induces WNT10b expression in humans lymphoid cells**

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Intermittent PTH reduces vertebral fractures risk in osteoporotic patients. The mechanisms trough which PTH acts are not completely understood, it has been observed to activate Wnt pathways in osteoblasts (OBs). Activation of this pathway induces OB proliferation, differentiation and prevents apoptosis. Recently increased expression of Wnt10b by T cells during intermittent PTH, and no increase during continuous PTH has been demonstrated in mice.In order to evaluate if PTH increases Wnt10b expression lymphoid humans cells, we measured this molecule at baseline and during treatment in osteoporotic women and in patients with primary hyperparatiroidism before and after surgery.
Forty postmenopausal osteoporotic women were randomly assigned to therapy with: 1–84 PTH 100 μg plus calcium 1200 mg and vitamin D 800 IU daily, or with calcium and vitamin D alone and return for control visit and exams at 3, 6, 12 and 18 months of treatment.
Twenty patients affected by primary hyperparathyroidism and subjected to surgical parathyroidectomy were enrolled and evaluated at baseline and 1 month after surgery.
Real time PCR for WNT10b was performed on peripheral blood lymphoid cells, osteocalcin and bone alkaline phosphatase were measured by ELISA.
Our data show an increase in WNT10b expression by lymphoid cells that was maximum (more than 20-fold) after 6 months of treatment, after 18 months WNT10b returned to basal expression. The WNT10b curve acts similarly to the bone formation markers curve. In patients treated with calcium and vitamin D alone no increase in WNT10b was observed. Also in patients affected by hyperparathyroidism there was no difference in WNT10b before and after surgery.
Our data suggest an effect of intermittent, but not continuous PTH on the expression of WNT10b by lymphoid cells, this could be one of the mechanisms trough which PTH treatment increases OB formation and function in humans.

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## PP354

**Denosumab treatment in women with osteoporosis reduces hip cortical porosity**

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Bone strength is influenced by cortical thickness, area, mass and porosity, all of which contribute to nonvertebral fracture risk. Cortical porosity is one parameter of structural decay associated with bone fragility. This is caused by unbalanced and accelerated remodelling of Haversian units which enlarge, coalesce and fragment the cortex. Antiresorptive therapies will limit progression of cortical porosity; reducing existing porosity would be a goal for those already at increased risk of fracture. Using multi-detector computed tomography (MDCT) hip images from FREEDOM, a 3-year, randomized, double-blind trial in which postmenopausal women with osteoporosis received placebo (Pbo) or 60 mg denosumab (DMAb) every 6 months, we previously reported that hip cortical mass and thickness improved over 3 years of DMAb administration. We postulated that this could be explained by infilling of porosity in the inner cortical region adjacent to the medullary canal. For this analysis, percentage porosity in both the compact and the trabecularized (outer and inner transitional zones) cortical volumes of the subtrochanter region were measured at baseline and year 3 based on a subset of the same MDCT hip images (Pbo, $n=22$ and DMAb, $n=28$) using StrAx1.0 Software (Zebaze *et al*., *Bone* 2013). At baseline, 72% volume was occupied by porosity in the inner transitional zone adjacent to the medullary compartment, 37% in the outer transitional zone, and 29% in the compact-appearing cortex. Cortical porosity was positively correlated with serum CTX ($P=0.017$) and negatively correlated with hip strength estimated using finite element analysis ($P=0.027$). At year 3, DMAb reduced cortical porosity compared with baseline and Pbo across the entire cortex and in each compartment, reaching treatment effect improvements (DMAb–Pbo) of −1.8% (inner transitional zone), −5.6% (outer transitional zone) and −7.9% (compact-appearing cortex; all $P<0.001$). This is the first report of the response of hip porosity *in vivo* to pharmacological therapy. Reductions in cortical porosity equate to increased mineralized bone matrix and both are relevant to strength. This improvement in cortical bone parameters is likely to contribute to the observed significant reductions in hip and nonvertebral fractures associated with DMAb administration.

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## PP355

**Continuous modelling-based bone formation could explain sustained increases in hip bone mineral density with denosumab treatment**

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In clinical studies, denosumab (DMAb) administration up to 8 years is associated with continued increases in bone mineral density (BMD) and low fracture incidence despite persistently low bone turnover markers and limited iliac crest tetracycline labelling (Papapoulos 2013). We tested the hypothesis that, with persistently low bone remodelling, BMD increases may result from a non-remodelling dependent mechanism to accrue bone matrix. We examined the fluorochrome labelling pattern in proximal femur sections from ovariectomized (OVX) cynomolgus monkeys (cynos) treated with DMAb. Following OVX, mature 9+ years old cynos were treated with vehicle ($n=20$) or 25 mg/kg DMAb ($n=14$) monthly for 16 months. Fluorochrome labels were administered at months 6, 12 and 16. As expected from the potent anti-remodelling effect of this regimen (25× clinical DMAb dose), bone resorption and formation indices histologically and by serum markers were very low (Kostenuik 2011). However, DXA femoral neck BMD continued to rise from baseline in the DMAb group: 5.9 and 11.3% at months 6 and 16 respectively. There was little surface label within the trabecular compartment in proximal femur sections in the DMAb group. In contrast, consistent and prominent labelling was observed in the cortex, primarily on both the superior endocortex (12/14 cynos) and the inferior periosteal surface (11/14 cynos). These regions typically contained all three superimposed labels over smooth cement lines, spanning months 6–16, suggesting that modelling-based bone formation was continuous during administration of DMAb. Persistent cortical bone modelling on a background of maximal suppression of remodelling provide a possible explanation for the progressive increases in BMD and mass observed with DMAb treatment at the hip. Importantly, augmentation of bone mass occurred at biomechanically relevant sites of the femur neck, corresponding to significant increases in bone strength (Ominsky 2011). Thus, there is evidence in cynos that continual modelling-based bone formation occurs during DMAb therapy. If applicable to human studies, this could provide an explanation for the increase in cortical thickness and mass observed in clinical trials. This study may provide the first histological evidence of a potential mechanism responsible for clinical observations of continued BMD increases and low fracture rates with long-term DMAb treatment in the FREEDOM Extension.

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## PP356

**Changes in lumbar spine QCT, DXA and TBS with denosumab, alendronate or placebo in postmenopausal women with low bone mass**

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Patients with osteoporosis require treatment with therapies that reduce the risk of fracture which at the spine is significantly influenced by bone microarchitecture. Quantitative computed tomography (QCT) and dual X-ray absorptiometry (DXA) allow measurement of bone mineral density (BMD), a known indicator of fracture risk. Trabecular bone score (TBS) is a novel gray-level measurement derived from spine DXA image texture that is related to microarchitecture and associates with fracture risk independent of BMD. Denosumab (DMAb) results in greater gains in BMD than oral bisphosphonates and in a recent study, this greater improvement in BMD was associated with a larger reduction in vertebral fracture with DMAb compared with alendronate (ALN; Nakamura *ASBMR* 2012). To further characterize the bone response with DMAb and ALN, we compared QCT vBMD, DXA aBMD *T*-score and TBS from spine scans obtained in postmenopausal women with low BMD. In a randomized, double-blind, double-dummy study, postmenopausal women aged 50–70 years with low BMD at the spine or total hip received DMAb (60 mg s.c. every 6 months), branded ALN (70 mg orally every week) or placebo (Pbo) for 12 months (Seeman *JBMR* 2010). Lumbar spine (LS) QCT vBMD, DXA aBMD and TBS were measured from scans obtained at baseline and month 12. TBS values at baseline and month 12 were available for 215 women (73 DMAb, 68 ALN, and 74 pbo). At baseline, mean age was 60 and, at the LS, mean vBMD was 90.4 mg/cm$^3$, mean aBMD *T*-score was −2.4 and mean TBS was 1.234. Overall, vBMD, aBMD and TBS decreased with Pbo; increased or were maintained with ALN; and improved with DMAb compared with both Pbo and ALN. At baseline, TBS was better correlated with vBMD ($r=0.42$, $P<0.001$) than with aBMD *T*-score ($r=0.13$, $P=0.051$). TBS% changes did not positively correlate with those in QCT vBMD or DXA aBMD in any of the treatment groups. In postmenopausal women with low BMD, DMAb increased vBMD, aBMD and TBS vs both ALN and Pbo over 12 months. Altogether these results suggest that changes in TBS are not biased by BMD changes. These data support the concept that TBS acquires information not captured by standard bone density and warrant further investigation of the clinical significance of the positive changes in TBS in response to denosumab.

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## PP357

**In postmenopausal women previously treated with an oral bisphosphonate and at higher risk of fracture, denosumab significantly increases bone mineral density compared with ibandronate and risedronate**

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Low bone mineral density (BMD) is an important and modifiable risk factor for fracture in postmenopausal women with osteoporosis. Denosumab (DMAb) shows a stronger relationship between BMD increases and antifracture efficacy than oral bisphosphonate (BP) therapies. Subjects who remain at higher risk of fracture despite current BP therapy need treatment. In two studies, DMAb significantly increased BMD and decreased bone turnover markers vs a BP (ibandronate (IBN) or risedronate (RIS)) in subjects previously treated with, but suboptimally adherent to, a BP. We evaluated the effects of DMAb vs a BP (IBN and RIS) to increase BMD in a subset of subjects at higher risk of fracture. Both studies had multicentre, randomized, open-label, parallel-group designs in which postmenopausal women $\geq$55 years were randomized 1:1 to DMAb 60 mg s.c. Q6M or a BP 150 mg p.o. QM for 12 months. In this combined *post-hoc* analysis, higher-risk subjects were identified by meeting $\geq$1 risk criterion (baseline age $\geq$75 years, total hip (TH) or femoral neck (FN) BMD *T*-score $\leq -2.5$, TH or FN BMD *T*-score $\leq -1.0+$ prior osteoporotic fracture, sCTX-1 $>$0.9 ng/ml and TH or FN BMD *T*-score $\leq -2.0$) and % BMD change from baseline at TH, FN and lumbar spine (LS) at month 12 was calculated. Subjects (852 DMAb and 851 BP) had a mean (s.d.) age 67 (7.4) years and mean (s.d.) TH, FN and LS *T*-score of $-1.7$ (0.8), $-2.0$ (0.7), and $-2.4$ (1.0) respectively. For subjects at higher risk of fracture (475 DMAb and 469 BP), compared with BP (IBN and RIS), at 12 months DMAb significantly increased TH (2.2 vs 0.8%), FN (1.8 vs 0.3%) and LS BMD (3.7 vs 1.4%; $P<0.0001$ for all). These results are consistent with the overall study population and lower risk subgroup (treatment-by-risk subgroup interaction, $P>0.05$). In general, adverse events and serious adverse events were similar between DMAb and the comparator BP groups. In conclusion, for subjects who were previously suboptimally treated with a BP and remained at higher risk of fracture, transitioning to DMAb led to significantly greater increases in BMD at 12 months than cycling to another BP. These results in higher-risk subjects are consistent with those obtained in the overall population and support DMAb as an alternative therapeutic option for women at higher risk of fracture.

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## PP358

**Zoledronate reverses bone marrow adiposity in disuse osteopenic rats**

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Objective
I.m. injections with Botox (BTX) leads to a paralysis of the muscles, resulting in a rapid loss of muscle and bone mass (disuse osteoporosis). Adipocytes and osteoblasts derive from the same mesenchymal stem cell, and commitment to one linage represses commitment to the other. Furthermore, the existence of a close communication between bone cells and endothelial cells is widely accepted.
The purpose of the study was to quantify the changes in adiposity and vasculature in disuse osteopenic rats with and without treatment with the bisphosphonate zoledronate (Zln).
Materials and methods
Sixty female Wistar rats were randomized into five groups. One group served as baseline, while two groups were injected with BTX in the right hind-limb and two groups with saline. Zln was given to one BTX and one saline group. After 6 weeks the animals were sacrificed. Subsequently, the distal femora were μCT scanned, and the proximal femoral bone strength was determined with mechanical testing. Finally, a histological quantification of the bone marrow adiposity and vascularity was performed on the proximal tibial and distal femoral metaphyses. All procedures were approved by the Danish Animal Experiments Inspectorate.
Results
BTX resulted in lower BV/TV ($-32.2\%$, $P<0.01$) and bone strength ($-17.1\%$, $P<0.05$), and higher bone marrow adiposity in the tibia (247%, $P<0.05$) and femur (1511%, $P<0.05$). Zln prevented loss of BV/TV (67.2%, $P<0.001$) and bone strength (20.5%, $P<0.05$) and significantly attenuated ($-64.4\%$, $P<0.05$) the BTX-induced increase in adiposity. Furthermore, the vascularity of the tibia was significantly higher (43.5%, $P<0.01$) in the BTX-Zln group compared to the BTX group.
Conclusion
BTX-induced disuse osteopenia resulted in a significant increase in bone marrow adiposity, whereas it did not affect the vasculature. The BTX-induced increase in adiposity was significantly reduced by treatment with Zln. No antiangiogenic effect of Zln on the bone marrow was found.

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## PP359

**Ten year alendronate use does not adversely affect bone quality compared to 5 years use: a human iliac crest biopsy study**

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Bisphosphonates (BPs) including alendronate (ALN) are the most widely prescribed therapy for post-menopausal osteoporosis. Despite their overall excellent safety record and efficacy in reducing fractures, concerns have been expressed lately regarding potential detrimental effects due to prolonged bone turnover reduction, although no definite cause–effect relationship has been established to date. The purpose of the present study was to determine bone material quality by Raman microspectroscopic analysis of iliac crest biopsies from postmenopausal osteoporosis patients that were either on 5-year ALN therapy followed by another 5 years of placebo (PLC) ($n=14$), or on 5 mg/day ALN for 10 years ($n=10$), or 10 : mg/day ALN for 10 years ($n=6$). The parameters monitored and expressed as a function of tissue age defined on basis of double tetracycline labels and geometrical centra of trabeculae were: i) the mineral : matrix ratio (MM), ii) the relative proteoglycan content (PG), iii) the relative lipids content (LPD), iv) the mineral maturity/crystallinity (MMC), and vi) the relative pyridinoline content (PYD). The results indicate that 10-year ALN use (both 5 and 10 mg/day) does not result in any detrimental changes compared to use for 5 years. The only differences were transient ones at bone forming surfaces, implying potential differences in bone matrix maturation that nevertheless did not result in differences of these values in mature tissue. These results indicate that as far as the parameters monitored are concerned, 10 years ALN use does not change the bone material quality at the examined tissue ages compared to 5-year use. Additionally, it is suggested that prolonged bisphosphonate use when accompanied with residual bone turnover may not be responsible by itself for an impairment in bone material quality contributing to adverse conditions such as atypical femoral fractures.

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## PP360

**Predictors of second fracture while on treatment with oral bisphosphonates: a multinational retrospective cohort study**

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Objective
To identify predictors of inadequate response to oral bisphosphonate therapy, defined as the incidence of $\geqq$2 fractures while on treatment among incident oral bisphosphonate users with high refill compliance ($\geqq$80%).

Material and methods
Data from computerized records and pharmacy invoices were obtained from SIDIAP (Catalonia, E) and Danish Health Registries (DK) for all incident users of oral bisphosphonates in 2006–2007 and 2000–2001 respectively. Exclusion criteria: Paget disease, age $<40$, anti-osteoporosis treatment in previous year, and suboptimal refill compliance ($<80\%$). Fine and Gray survival models accounting for the competing risk of therapy cessation were used to identify predictors of $\geq 2$ fractures while on treatment after 6 months of treatment initiation.
Results
7449/21 385 (34.8%) and 7885/13 949 (56.5%) were compliant oral bisphosphonate users in Catalonia and Denmark respectively. Significant predictors of $\geq 2$ fractures while on treatment were older age, and history of recent fracture. Sub-hazard ratios (SHRs) for each of the predictors in each of the datasets are reported in Figure 1.

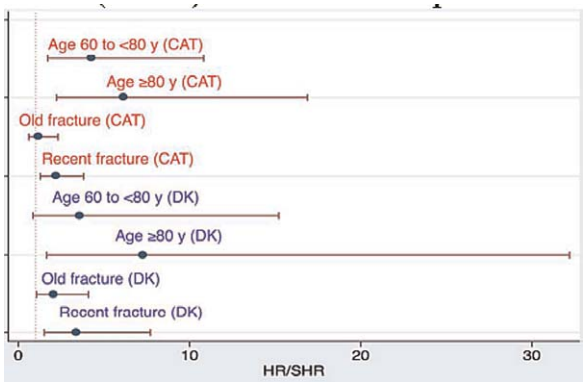


**Figure 1** Sub-hazard ratio for each of the predictors in each of the datasets.

Conclusion
Older age and recent fracture history are predictors of inadequate response as confirmed in two separate cohorts. Monitoring strategies and/or alternative therapies should be considered for these patients.

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## PP361

**Protein malnutrition attenuates bone anabolic response to PTH in female rats**

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We previously demonstrated that an isocaloric low protein diet attenuates the bone anabolic response to GH. Whether such a dietary manipulation could affect the bone anabolic effects of PTH was assessed. We studied 6-month-old female rats pair fed with isocaloric diets with a low protein (2.5% casein, LP) or normal protein content (15% casein, NP) for 2 weeks. Then, the animals were treated with 5 or 40 µg/kg recombinant human amino-terminal fragment PTH(1–34) (PTH-5 and PTH-40), or with vehicle for 4 weeks. Before starting PTH treatment, the 2-week LP diet was associated with reduced plasma IGF1, but no change in bone strength nor microstructure. After 4 weeks of PTH, proximal tibia trabecular BMD, microarchitecture and compressive bone strength were increased in a dose dependent manner in NP. These changes were attenuated in rats fed the LP diet. In the cortical compartment, PTH was anabolic in NP but not in LP group. Bone cellular mechanisms underlying the attenuated bone anabolic response to PTH under LP were analyzed by histomorphometry. At trabecular level, osteoid surfaces were similarly dose-dependently increased by PTH treatment compared to vehicle treated and diet matched groups, in both in NP and LP, surprisingly osteoclastic surfaces were lower in PTH-treated NP. Trabecular bone formation rate was increased in PTH treated NP group (PTH-5: +271 and PTH-40: +443%), with a lower response in LP (PTH-5 +171 and +343%). At the cortical level, periosteal and endosteal bone formation rate were increased by PTH-40 in both NP and LP, with a 30% greater periosteal formation in NP compared to LP. In conclusion, protein malnutrition attenuates anabolic PTH response through lower bone formation cellular activities.

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# Other diseases of bone and mineral metabolism

## PP362

**Osteogenesis imperfecta in adults: a cross sectional trial**

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Osteogenesis imperfecta (OI) is a hereditary disease with a generalized involvement of the connective tissue caused by collagen type 1 mutations. The clinical appearance is broad with fractures as the key symptom. Only few genotype–phenotype correlations have been established. We aim to characterize the Danish OI population thoroughly including DXA and HRpQCT, anthropometry, patient history, and genetic background.
This cross-sectional study includes 85 Danish adult OI patients, aged 19–72 (mean 44.9 ± 15.5 years; 38 men, 47 women). All patients are classified according to the Sillence classification: 58 type I, 12 type III and 15 type IV. In a subset of patients collagen sequencing ($n=53$) and structural collagen analyses ($n=60$) were performed. We found OI causing mutations in 44 patients (27 in COL1A1, 17 in Col1A2), however, no obvious mutations was found in nine patients. A quantitative collagen defect was found in 35 patients, and a qualitative defect in 25 patients.
The most severely affected patients (type III) had the highest fracture rate; 1.16 (0.43–3.96) fractures/year compared to 0.27 (0–2.26) and 0.32 (0–0.55) fractures/year in patients with type I and type IV, respectively, $P=0.001$. Lumbar aBMD correlates with fracture rate, $R^2=0.126$, $p=0.003$. This is not the case for hip aBMD, forearm vBMD(HRpQCT) and tibial vBMD(HRpQCT). The patients with a qualitative collagen defect had lower lumbar aBMD, $P=0.004$ and a higher fracture rate, $P=0.002$ than patients with a quantitative collagen defect. They also had reduced height (147 ± 25 vs 161 ± 10 cm, $P=0.02$), sitting height (72 ± 12 vs 81 ± 8 cm, $P=0.003$) and armspan (151 ± 24 vs 169 ± 13 m, $P=0.002$).
We found poor correlation between OI disease severity and aBMD and vBMD. Since the most severe cases of OI have qualitative collagen defects, we suggest extending routine patient evaluation with mutation screening and collagen structural analyses. Our data suggest that a classification of autosomal dominant OI based on biochemical and genetic findings may be developed.

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## PP363

**Sustained efficacy and tolerability in infants and young children with life-threatening hypophosphatasia treated with asfotase alfa**

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Background
Hypophosphatasia (HPP) results from inactivating mutation(s) in the gene for tissue non-specific alkaline phosphatase (TNSALP). Substantial improvement has been reported in skeletal mineralization and physical function in patients (pts) with life-threatening perinatal and infantile HPP treated for 48 weaks with asfotase alfa, a bone-targeted recombinant human TNSALP.
Objective
To evaluate long-term efficacy and tolerability of asfotase alfa in these pts.
Design/methods
Continued assessment using a Radiographic Global Impression of Change (RGI-C) scale ($-3$=severe worsening; $+3$=complete healing), Rickets Severity Scale (RSS; 0–10, 10=severe) and respiratory status during asfotase alfa treatment (Rx; s.c. injection, 3x/week, 1–3 mg/kg) for $\geq 3$ years.
Results
The study enrolled 11 points (median age 6.8 months; range 2.9 weeks-3 years). One point withdrew after first Rx and one point died at Rx 7.9 mo (sepsis

unrelated to Rx). Median Rx duration was 35 months ($n=11$, range 1 day-49 months). Asfotase alfa was well tolerated; the most common adverse events (AEs) were mild/moderate injection-site reactions and upper respiratory tract infection (six points each). Three serious AEs were reported as possibly Rx-related; craniosynostosis (a known complication of HPP), conductive deafness and chronic hepatitis. Median RGI-C ($+2.50$, range: 1.67–3.0; $P=0.008$) and median change from baseline in RSS ($-6.25$, range $-9.5$ to 0.0; $P=0.016$) showed sustained improvement from baseline at 3 years of Rx ($n=8$, one pt missing data at 3 years). Ten pts (including the two who died or withdrew) required respiratory support at some time during the first 48 weeks, after which three points remained on respiratory support. Subsequently two discontinued support and one improved to supplemental oxygen at last assessment ($\geq 3$ years). Probability of survival at 3 years of Rx was 90%.

Conclusion

Infants and young children with life-threatening HPP treated with asfotase alfa show sustained improvement in skeletal mineralization and respiratory status for $\geq 3$ years.

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## PP364

### Hypophosphatasia: a retrospective natural history study of the severe perinatal and infantile forms

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Background

Hypophosphatasia (HPP) is caused by inactivating mutation(s) in the gene for tissue non-specific alkaline phosphatase. Extracellular accumulation of inorganic pyrophosphate can lead to profound hypomineralization resulting in limb and chest deformity, respiratory complications and vitamin B6-dependent seizures in the severe forms of HPP. The natural history of HPP is poorly understood, but the perinatal and infantile forms are often considered lethal.

Objective

To clarify the natural history of perinatal and infantile HPP.

Methods

This was a multicentre, multinational, retrospective chart review study of patients (pts) with perinatal or infantile HPP (onset $<6$ months of age) with at least one of the following features: respiratory complications, rachitic chest deformity or seizures. Data collection spanned the first 5 years of age. The primary and secondary outcome evaluations were survival and invasive ventilator-free survival, respectively.

Results

48 pts were eligible, 14 of whom had signs of HPP *in utero*. At the time of diagnosis (median age 8.6 weeks), 32/48 patients (67%) had radiographic evidence of rachitic chest deformity. At the time of our study, 13 (27%) patients were alive (median age: 7.7 years (range: 1.6–19.7 year)); 35 (73%) patients had died (median time to death 8.9 months (95% CI: 5.1–14.1)), with a 31 and 58% probability of death by 3 and 12 months respectively. All ten patients with seizures died. Respiratory support was required for 29 patients; of these, 19 required invasive ventilation, with a median time to invasive ventilation or death of 7.8 months (95% CI: 2.6–9.9). Nearly 50% of the infants requiring respiratory support received maximal respiratory support within the first 6 days of life. Among the 13 patients alive at the time of chart review, three had received respiratory support and one of these had required invasive ventilation.

Conclusion

Perinatal or infantile HPP complicated by respiratory compromise, seizures or chest deformity is associated with high mortality.

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## PP365

### Alkaline phosphatase bone isoform B1x: a marker of impaired osteoblastic function in patients with renal osteodystrophy

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Renal osteodystrophy encompasses the bone histologic abnormalities seen in patients with chronic kidney disease (CKD). The bone-specific alkaline phosphatase (BALP) isoform B1x is exclusively found in serum of some CKD patients. The aim of this study was to examine the relationship between serum BALP isoforms and histologic abnormalities of bone in patients with CKD on chronic dialysis (CKD-5D).

Anterior iliac crest bone samples from 40 CKD-5D patients were selected from the IRB approved Kentucky Bone Registry based on the level of bone turnover. There were samples from 40 patients with low and non-low bone turnover. Bone histomorphometry was performed using the Osteoplan system. BALP levels were measured by an ELISA assay. BALP isoforms (B/I, B1x, B1 and B2) were determined by HPLC. Parathyroid hormone (PTH) levels were measured using an electrochemiluminescence assay.

B1x was found in 21 patients (53%). BALP, other BALP isoforms, PTH, osteoblast number (NOb/BPm), and activity (Obv) were lower in these patients compared to patients without B1x (Table). B1x correlated inversely with osteoblast number and activity ($r=-0.30$ and $-0.26$ respectively, $P<0.05$).

| | BALP (U/l) | B/I (μkat/l) | B1 (μkat/l) | B2 (μkat/l) | PTH (pg/ml) | NOb/BPm (#/100 mm) | Obv (%/day) |
|---|---|---|---|---|---|---|---|
| B1x+ | 24.3±3.24† | 0.13±0.02† | 0.53±0.09† | 1.57±0.24† | 141±38.3* | 77.1±19.4† | 0.27±0.07† |
| B1x− | 69.6±14.0 | 0.24±0.03 | 1.44±0.30 | 5.72±1.81 | 484±106 | 333±94.3 | 0.50±0.09 |

Mean±S.E.M.; *$P<0.05$; †$P<0.01$.

This study shows that the release of B1x from bone into serum is a sign of perturbed osteoblast activity. Measurement of B1x may provide a valuable tool for the assessment of patients with renal osteodystrophy.

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## PP366

### Gene expression in vascular calcification: are there differences between atherosclerotic changes and media sclerosis?

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Background

Pathophysiological calcification in the vasculature is a risk factor for cardiovascular disease (CVD). CVD are among the most common causes of death in patients with chronic kidney disease and crucial for kidney transplantation (RTX) outcomes.

Aim

The aim of this study is to identify differences in the pattern and the onset of expression of regulators of calcification (RC) in atherosclerosis (AS) and media sclerosis (MS).

Methods

We investigated the expression of RCin the arteria iliaca externa in 26 donors (D) and 25 recipients (R) of RTX.

Gene expression of RC was performed using TaqMan gene expression assays with a LC480 system. Determination of calcification type in donors (AS) was done histologically and in recipients (MS) via computed tomography (CT) by applying a score ranging from 0 to 3 in 0.5 intervals. Classification of stages in

donors and recipients: 0 (unaffected vessels), 1 (AS: intima thickening, MS: CT score 0.5), 2 (AS: intima calcification, MS: CT scores 1–3).
Results
Gene expression of OPG, OPN, RANKL, SMAD6, RunX2 and BSP was significantly higher in donors than in recipients ($P=0.004$, $P=0.001$, $P=0.004$, $P=0.026$, $P=0.027$ and $P=0.068$ respectively).
Gene expression did not significantly differ in vessels of D and R at stage 0. In stage one of AS, OPG expression increased, whereas expression in stage one of MS was unchanged. In progressive calcification (stage 2), expression of OPG, OPN and SMAD6 was significantly higher ($P=0.048$, $P=0.024$, $P=0.048$ respectively) in D (AS) than in R (MS); RANKL and RunX2 ($P=0.089$, $P=0.085$ respectively) were only slightly higher. AS and MS were compared separately.
Conclusion
We demonstrate a different gene expression pattern in a clinical model of AS and MS and a different onset of calcification. These data might lead to a more comprehensive insight in the mechanisms of calcification.

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## PP367

**Blockade of Wnt inhibitor Dickkopf-1 improves bone mass and microstructure of osteogensis imperfecta**

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Osteogenesis imperfect (OI) is an inheritable bone disease with low bone mass, fragility, deformity and multiple fractures in skeletal tissues. Modulation of Wnt signalling components reportedly alleviates excessive bone and joint remodelling in various skeletal disorders. This study is undertaken to explore whether loss of Wnt inhibitor Dickkopf-1 (Dkk1) action affects bone formation or skeletal homeostasis in OI. In clinical vignettes, OI patients had severe osteoporosis (*T*-score) in spines and hips in association with high serum Dkk1 levels compared to normal subjects. Treatment with Dkk1 antibodies or recombinant Wnt3a increased the expression of Runx2, collagen 1$\alpha$2 and osteocalcin and mineralized nodule formation in primary bone-marrow mesenchymal stem cells from OI patients underwent osteotomy. In experimental OI models, oim mice had lower bone mass and higher serum Dkk1 levels than WT mice. Administration of nanoparticles with phosphorothioate antisense oligonucleotide for Dkk1 decreased serum and bone tissue Dkk1 expression in OI mice. Loss of Dkk1 function increased bone mineral density and trabecular bone volume and reduced cortical bone porosity in OI mice. Dkk1 antisense oligonucleotide treatment increased mineral acquisition in skeletal tissue and promoted osteogenic gene expression and *ex vivo* osteoblastogenesis in primary bone-marrow mesenchymal cells. Knockdown of Dkk1 reduced the OI promotion of osteoclast surface and *ex vivo* osteoclast differentiation and resorption capacity of primary bone-marrow macrophage precursor cells in OI mice. Taken together, high Dkk1 level impedes bone formation activities and bone homeostasis in OI patients. Dkk1 interference has therapeutic potential for ameliorating the OI-mediated excessive bone remodelling and skeletal deterioration.

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## PP368

**Vitamin D supplementation decreases the occurrence of acute phase response following i.v. bisphosphonate treatment in Paget's disease of bone**

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Acute phase reaction (APR) is the most frequent side effect following i.v. nitrogen-containing bisphosphonates (N-BPs) infusion. A recent observation in osteoporotic women treated with N-BPs evidenced a negative association between 25(OH)D levels and APR, likely due to the immuno-modulatory effects of vitamin D on $\gamma\delta$Tcells. However, this association remains to be demonstrated in patients with Paget's disease of bone (PDB). Moreover whether vitamin D supplementation is able to prevent the occurrence of APR has not yet been demonstrated. We initially performed a retrospective analysis of 205 patients treated with intravenous N-BPs for PDB. Overall APR occurred in 31% of cases, more frequently in previously untreated patients (43%). Neither gender nor disease severity nor use of other drugs apart previous N-BP treatment was associated with APR. 25(OH)D levels before treatment were lower in PDB cases with APR than in patients who did not experience APR. Following this observation we performed a prospective study in 30 naïve PDB patients. Hypovitaminosis D was common in this cohort (mean $18.0\pm5$ ng/ml; 63% $<20$ ng/ml). All patients received oral vitamin D3 (7000 IU/week) for 60 days before zoledronate 5 mg (16 patients) or neridronate 200 mg (14 patients) infusion. Moreover a single baseline oral dose of 25 000 IU was given to patients with 25(OH)D levels $<20$ ng/ml. All adverse events occurring after N-BPs infusion were listed. Interestingly, APR occurred in 12.5% patients treated with zoledronate and 7% patients treated with neridronate (overall incidence rate 10%). In two of these cases 25(OH)D levels at the time of infusion were within the normal range while in the third case remained below the range. These results suggest that APR following N-BPs infusion for PDB is a common event particularly in patients with hypovitaminosis D and that vitamin D supplementation prior infusion is able to reduce the occurrence of this complication.

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## PP369

**A homozygous 20 bp intronic deletion in front of exon 8 of the *ALPL*-gene causes infantile hypophosphatasia: a functional characterization**

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Mutations of the *ALPL*-gene are closely related to hypophosphatasia (HPP), an inherited disorder of bone and mineral metabolism with clinically heterogeneous symptoms. To date 278 different mutations have been described, leading to reduction or completely loss of enzymatic activity of the tissue nonspecific alkaline phosphatase (TNAP).
We present the case of a 6-year-old boy with clinical features and laboratory results consistent with infantile HPP, but without any mutation in the coding region of the *ALPL*-gene. Intensified genomic DNA analyses, performed with informed consent provided by the parents, revealed a homozygous 20 bp-deletion in front of exon 8: c.793del-14_33. Both parents, who are consanguine, are heterozygous. Examination of mRNA transcripts resulted in three different splice variants; the main transcript shows a deletion of exon 8, a second, weaker transcript has a deletion of exons 7 and 8, and only a very weak signal referred to the full length transcript. Deletion of exon 8 or both exons leads to a shift of the reading frame resulting in a stop codon in exon 9. Since the main transcript misses exon 8 and we detected a protein of $\sim$ 40 kDa in PBMC and serum from the patient by western- blotting with a TNAP-specific antibody, we generated an expression construct for the deduced C-terminally truncated TNAP protein of 275 aa and investigated intracellular localization by immunocytochemistry and enzymatic activity in transfection/co-transfection studies with not mutated TNAP expression plasmid.
The protein cannot be localized in the cell-membrane due to the loss of its C-terminal membrane-anchor. Furthermore it has no basal enzymatic activity and it does not seem to affect an unimpaired dimerization partner.
This intronic deletion can explain the reduced alkaline phosphatase activity and clinical symptoms of HPP, which cannot be compensated by an unaffected allele like in the case of the healthy parents.

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## PP370

**Isoform-specific effects of Sequestosome-1 UBA domain mutations on NF-κB signalling**

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Paget's disease of Bone (PDB) is caused by mutations in the gene encoding Sequestosome-1 (Q17STM1 or p62) that affect the C-terminal Ubiquitin-Associated (UBA) domain. A second isoform of Q17STM1 exists (referred to hereafter as 55kDa-Q17STM1), which lacks the N-terminal Phox and Bem1 (PB1) domain and has previously been reported to be ∼45x more abundant than Q17STM1/p62 in osteoclasts. Mutations in the UBA domain will also occur in this isoform. Several of the UBA mutations in 62kDa-Q17STM1 have been reported to increase the activation of NF-κB in overexpression systems. No study has yet examined the effect of mutations in 55kD-Q17STM1 on NF-κB signalling.

Human Embryonic Kidney (HEK293)-derived cell lines were stably transfected with wild-type (WT) or mutated (392L, 396X or 425R) 55kDa- or 62kDa-Q17STM1. Expression of endogenous Q17STM1 renders these cell lines heterozygous for each mutation. NF-κB activation for all cell lines was assessed using dual luciferase reporter assays.

55kDa-WT and 62kDa-WT cell lines similarly activated NF-κB. Marked differences were observed in NF-κB activation between mutations in the two isoforms: 62kDa-P392L exhibited 80.1% NF-κB activity of 62kDa-WT whereas 55kDa-P392L increased activation by 17.3% compared to 55kDa-WT. For the E396X mutation the results were opposite (62kDa-E396X increased activation by 57.5% and 55kDa-E396X decreased activation by 26.1%). No differences between isoforms were seen for the G425R substitution, where NF-κB activation was reduced by 35% compared to 62kDa-WT for both isoforms.

In our physiologically-relevant cell model, we found that the effect of UBA domain mutations on NF-κB signalling is context-dependent, with opposing effects occurring depending on which isoform the mutation is present within. *In vivo*, this may regulate NF-κB signalling in a manner related to isoform abundance, and this warrants further investigation. The 55kDa isoform could, therefore, play an important role in the development and/or limitation of PDB exclusively in osteoclasts.

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## PP371

**Effect of complete spinal cord injury on bone turnover and bone mineral density evolution: a 1 year follow-up study**

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Spinal cord injury (SCI) has been associated with marked bone loss and an increased risk of fractures under the SCI level. The aim of the study was to analyze the effect of recent complete SCI on bone turnover and bone mineral density (BMD) evolution and factors related to bone loss.

Methods

Prospective study including patients with complete motor SCI (ASIA A or B) (<6 months). Bone turnover markers (bone formation: P1NP, bone ALP and resorption: sCTx) and BMD (lumbar spine and femur (DXA)) were assessed in all patients at baseline and at 6 and 12 months. Risk factors for osteoporosis, SCI level (paraplegia/tetraplegia), lesion type (spastic/flaccid), SCI severity (ASIA score) and fractures were evaluated, comparing results with a control group.

Results

42 patients (40M:2F) (mean age 35±14 years) were included 100±33 days after SCI (ASIA A 39:B 3). 55% had paraplegia and 78% spasticity. 24 were followed-up at 6 months and 22 at 12 months. Compared to controls, bone turnover markers were markedly increased just after SCI (P1NP: 194±124 vs 50±19 ng/ml, $P<0.001$; bone ALP: 15±7 vs 12±4 ng/ml, $P=0.04$; and sCTx 1.48±0.52 vs 0.49±0.23 ng/ml, $P<0.001$), with maintained, although with less increased, values at 6 and 12 months. BMD decreased progressively at proximal femur (−13±5% at 6 months, $P<0.001$, −20±8% at 12 months, $P<0.001$), with no significant changes at lumbar BMD. At 12 months 59% of the SCI patients presented densitometric osteoporosis. Patients with higher sCTX values after SCI (third tertile) had the highest femur BMD loss at 12 months (−23%). No relationship was observed between BMD and bone marker changes, or SCI level or lesion type.

Conclusions

Patients with complete SCI have increased bone turnover after injury with marked bone loss under the injury level of ∼−20% of BMD at the proximal femur at 1 year, being more marked in individuals with the highest sCTX levels. Awareness of this complication and its therapeutic approach are mandatory.

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## PP372

**Comparative effect of Denosumab vs Teriparatide on bone and energy metabolism**

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Recent studies have demonstrated the role of osteocalcin in energy metabolism regulation having a connection between this and bone metabolism. According to this, anti-osteoporotic drugs may exert different effects on energy metabolism. Thereby, our aim is to evaluate the effects of antiresorptive (Denosumab) and osteoanabolic (Teriparatide) drugs that reduce or increase respectively osteocalcin levels, on energy and bone metabolism by assessing of under-carboxilated osteocalcin (ucOC), myostatin and sclerostin levels.

We performed a prospective study of 3 months duration in patients with postmenopausal osteoporosis who are treated with two different antiosteoporotic drugs: i) dose of 60 mg Denosumab semiannually ($n=22$) compared to ii) 20 μg Teriparatide s.c. daily ($n=16$).

We measured the percent change from baseline in serum ucOC, sclerostin and myostatin levels as main parameters of study, and total OC, P1NP, CTX and PTH as secondary measurements, at first week, first and third months.

Serum ucOC levels were significantly lower in Denosumab group and significantly higher in Teriparatide group at first and third month compared to baseline (12.4, 47.8% vs 117, 87% respectively; $P<0.05$); serum sclerostin levels were increased but not significantly in Denosumab group and decreased in Teriparatide group at first week, first and third months (2.9, 10.6, 8.5% vs 0.7, 3.8, 1.9% respectively; $P>0.05$); however, there were significative differences between groups at first month ($P<0.05$). Serum myostatin levels remained unchanged. Bone markers were significantly decreased in Denosumab group and increased in Teriparatide group at all measurements with significative differences between groups ($P<0.05$). PTH levels were significantly increased at first and third month in Denosumab group (65 and 21.5% respectively, $P<0.05$) with significative differences between groups ($P<0.001$).

These preliminary results show an opposite effect of denosumab and teriparatide on Wnt signaling leading to a decrease or increase in serum ucOC levels and bone turnover markers respectively.

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## PP373

**Involvement of Gla rich protein with pathological calcification during osteoarthritis. Insights into its γ-carboxylation status**

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Gla-rich protein (GRP) is a vitamin K-dependent protein, characterized by a high density of γ-carboxylated Glu residues and high calcium binding affinity. It was shown to accumulate in mouse and sturgeon cartilage and in sites of skin and vascular calcification in humans. Four alternatively spliced transcripts of the *GRP* gene (GRP–F1, F2, F3 and F4) were described in mouse chondrocytes and zebrafish. Osteoarthritis (OA) is a common degenerative joint disease, and reported to be associated with basic calcium phosphate crystals deposition either in the articular cartilage, synovial fluid or synovial membrane, which lead us to investigate the relation between calcium mineral deposition and GRP expression/accumulation during OA.

Comparative analysis of GRP patterning at transcriptional and translational levels was performed between controls and OA patients. We have first identified novel alternative splice variants in humans by RT-PCR and the respective protein

isoforms are characterized by the loss of full γ-carboxylation and secretion functional motifs. These findings led us to produce an overexpressing human cell system to further understand the GRP secretory and γ-carboxylation potentials. Using newly developed and validated GRP conformation-specific antibodies we determined the differential accumulation pattern of human γ-carboxylated GRP (cGRP) and under-carboxylated GRP (ucGRP) in healthy and OA tissues by immunohistochemistry. Furthermore we used a cell-free *in vitro* assay to evaluate the calcium/phosphate (Ca/P) mineral-binding capacity of cGRP and ucGRP protein forms.

Our results show that GRP–F1 appears to be the predominant splice variant expressed in mouse and human adult tissues, particularly in OA cartilage, while the overexpressed protein appears to be γ-carboxylated. Immunohistochemistry results using the conformational-specific antibodies show a preferred cGRP accumulation in controls, whereas ucGRP was the predominant form in OA-affected tissues, co-localizing at sites of ectopic calcifications in OA. Overall our results indicate an association of under-carboxylated GRP with OA.

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## PP374

**Clinical presentation of Paget's disease: evaluation of a contemporary cohort and systematic review**

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Background

The prevalence and severity of Paget's disease of bone (PDB) have fallen over recent years but it is unclear if this has impacted on disease presentation. Here we evaluated the presentation of PDB in a contemporary cohort and conducted a systematic review of MEDLINE on the mode of presentation.

Methods

The presenting features of PDB were recorded in 87 patients who had presented to a specialist clinic between 2005 and 2013. The systematic review was conducted using the MESH terms 'Paget's disease of bone' or 'osteitis deformans' and the keyword 'clinical'. The abstracts of these citations ($n=415$) were analysed and relevant data extracted.

Results

The incidence of clinically diagnosed PDB in our region was 0.13 cases/10 000 population per year compared with an age-adjusted incidence of 0.70 cases/10 000 per year in the general population. Based on this we estimate that 18% of patients had come to medical attention. Skeletal pain was the presenting feature in 75% of cases but only 36% were thought to have pain caused by PDB. About 5% presented with a pathological fracture; 14% with bone deformity and 1% with deafness. Only 21% of patients were asymptomatic. The patients with bone pain thought to be due to PDB were treated with bisphosphonates and 58% reported an improvement but no tangible improvement was noted in deformity or deafness. The systematic review also showed that bone pain was the most common presenting feature in other case series with variable frequencies of other complications.

Conclusion

Most patients with PDB who come to clinical attention are symptomatic and about 15% have complications by the time they present. Although PDB has become less common in recent years, there remains a clinical need to develop biomarkers for early detection of PDB so that treatment can be initiated before irreversible skeletal damage has occurred.

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## PP375

**Osteoprotegerin and bone-like vascular calcification are predictive markers of vulnerable carotid plaques**

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Vascular calcification has a marked impact on arterial heterogeneity and plaque stability. Based on a cohort of 116 patients (carotid and femoral endarterectomies), the aim of the present study was to determine the influence of the bone-like vascular calcification and of osteoprotegerin (OPG) on plaque stability.

Seventy-three carotid and 43 femoral plaques along with plasma were harvested and analyzed in a single center study. The presence of osteoid metaplasia (bone-like vascular calcification) was significantly higher in femoral compared to carotid plaques ($P<0.01$) and in asymptomatic vs symptomatic carotid plaques ($P<0.01$). OPG staining was significantly higher in carotid than in femoral plaques ($P<0.05$) and in asymptomatic vs symptomatic carotid lesions ($P<0.05$). Circulating OPG levels were significantly higher in the asymptomatic carotid group compared to the symptomatic carotid group. *In vitro*, human pericytes secreted high amounts of OPG and differentiated in osteoblasts. In pro-inflammatory conditions, pericytes induced an imbalance between osteoblast- and osteoclast-precursor differentiation towards mineral formation in which OPG appeared strongly involved.

These results indicate that circulating OPG should be considered as a promising predictive marker for carotid plaque vulnerability. Furthermore, the presence of intense intraplaque vascular pericytes and OPG infiltration was associated with a higher presence of OM and with a significantly more stable phenotype of carotid plaques. Our *in vitro* findings indicate that pericytes may be strongly implicated in bone-like vascular formation.

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## PP376

**Blocking β-adrenergic signaling attenuates calorie alteration- induced bone marrow adiposity**

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We sought to elucidate the effects of dietary caloric alterations on bone marrow adiposity and the effects of β-adrenergic signaling on marrow stromal cells' adipogenic differentiation. Male 6-week-old C57BL/6 mice were assigned into three groups: an *ad-libitum* fed control diet (CON; 10 kcal% fat), a high calorie diet (HIGH; 60 kcal% fat) and a low calorie diet (LOW; 30% kcal restriction vs CON diet). In each diet group, mice were treated with vehicle (VEH: DI water) or propranolol, a β-adrenergic receptor antagonist (BB; 0.5 g/l in drinking water). Over 12 weeks, the number of adipocytes in the bone marrow area significantly increased in LOWVEH and HIGHVEH mice compared with CONVEH mice. Propranolol significantly mitigated the increased number of adipocytes in the bone marrow area seen in both LOWVEH and HIGHVEH mice. Isopreterenol, a major β-adrenergic receptor (βAR) agonist, increased while propranolol suppressed lipid droplet accumulation and adipogenic marker gene expressions, including adiponectin, adipocyte protein 2, pparγ, in 3T3L1 preadipocytes and bone marrow stromal cells (bMSCs). Next, we performed two compartment co-cultures of 3T3L1 preadipocytes and MC3T3E1 osteoblasts to elucidate the role of sympathetic nervous system (SNS) in osteoblasts mediated regulation of marrow adipogenesis. Isopreterenol led to an increment of adipogenic marker gene expressions while propranolol mitigated isoproterenol induced increase of adipogenic gene expressions in 3T3L1 cells co-cultured with osteoblasts. Levels of adipogenic marker gene expressions decreased when 3T3L1 cells were co-cultured in the presence of conditioned media from MC3T3E1 cells. However, the decreased adipogenic marker gene expressions were elevated when 3T3L1 cells cultured with conditioned media from the isopreterenol treated MC3T3E1 cells. Collectively, these data suggest that SNS activation through βAR signaling is critical for the regulation of marrow adipogenesis and the SNS regulation of marrow adipogenesis occurs in part via the osteoblasts.

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## PP377

**Fine-needle aspiration with PTH measurement facilitates minimally invasive parathyroidectomy – report of two cases**

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Objective
Minimally invasive parathyroidectomy (MIP) has become a frequently used strategy but it requires a precise preoperative localization and the use of intraoperative PTH to fulfill its benefits. The current localization techniques (ultrasonography, MIBI scan) have shortcomings and intraoperative PTH is not available in our country.
Case presentation
We report the cases of two patients, females (63 and 55 years old), with clinical and biochemical features of primary hyperparathyroidism (total calcium: 10.1 and 12.4 mg/dl; PTH: 171 and 257.5 pg/ml). In the first case, neck ultrasonography (USG) revealed a mixed hypoechoic tumor along the posterior aspect of the left thyroid lobe of 15×8 mm, been difficult to say if it was intrathyroidal or not. There was only minimal residual uptake in the left thyroid lobe at 4 h on $Tc^{99m}$ sestamibi imaging. She underwent USG-guided fine-needle aspiration (FNA) of the suspected tumor followed by PTH measurement from the needle washing (FNA-PTH). The cytology was non-diagnostic but the level of PTH in the aspirated fluid was 10.000 pg/ ml. In the second case, a CT scan performed elsewhere described two tumors with similar appearance, one (17×16 mm) in front of the other (20×15 mm), located posterior to the left thyroid lobe, suggesting one was a thyroid nodule and the other a parathyroid adenoma. USG-guided fine-needle aspiration of each tumor revealed PTH concentrations of 100.000 and 32.980 pg/ml respectively. MIP was performed in each case, and was curative. Histopathology revealed a left superior parathyroid adenoma included in the thyroid capsule in the first case, and a double left superior parathyroid adenoma in the second.
Conclusion
This case report highlights the importance of FNA-PTH in the localization of functionally parathyroid tissue in difficult cases, where a clear target exists, and facilitates MIP.

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## PP378

**Zoledronic acid induces apoptosis on osteoblast and inhibits RANKL-induced osteoclast differentiation**

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Bisphosphonates (BPs) are widely used as antiresorptive drugs. However, one of most potent BPs, zoledronic acid (ZA) can cause BP-related osteonecrosis of the jaws (BRONJ) with a poorly understood pathophysiology. The aim of this study was to find a clue for the development of BRONJ by evaluating the cytotoxic effects of ZA on osteoblasts and examining the action mechanism of ZA on osteoclast differentiation. Jaw bone osteoblasts (JB-OBs), long bone osteoblasts (LB-OBs), and bone marrow macrophages (BMMs) were isolated from Balb/C mouse. Osteoblasts were treated with ZA at increasing doses (0.01, 0.1, 1, 5, 10, 50, and 100 μM) for 7 days. The cytotoxic effects of ZA appeared from day 3 at high dose of 50 and 100 μM with increase of apoptosis in both types of osteoblasts, of which JB-OBs were more sensitive than LB-OBs. Western blotting showed increased expression of apoptotic markers, p21 and p53 at 50 μM of ZA. BMMs were pretreated with various concentrations (0, 0.01, 0.1, and 1 μM) of ZA for 1 h before stimulating with receptor activator of nuclear factor-κB ligand, the osteoclast differentiation factor. ZA attenuates osteoclast differentiation by suppressing the development of multinuclear cells. ZA decreased the transcriptional expression of osteoclast transcription factor, NFATc1 as well as ATP6v0d2 gene which was involved in cell-cell fusion during osteoclastogenesis. These findings revealed that ZA at high dose induces apoptosis, more apparently in JB-OBs than LB-OBs, and attenuates osteoclast differentiation, suggesting that chronic administration of ZA might exert adverse effect on bone metabolism.

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## PP379

**Effect of mesenchymal stem cells and platelet-derived growth factor transplantation on the localized radiation-induced ulcerative lesion in rats**

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Purpose
Osteoradionecrosis (ORN) of the mandible is a serious complication of radiation therapy, and accompanies soft tissue damage before bone loss appears. However, there is still no adequate treatment to heal the soft tissue damage of ORN. This study investigated the effect of platelet-derived growth factor (PDGF) or rat mesenchymal stem cells (rMSCs) on the healing of radiation-induced soft tissue injury by varying administration timing.
Methods
Rats were bilaterally irradiated in both right and the left flanks at a dose of 50 Gy. Experimental groups were randomly divided into three groups, and received PDGF (8 μg), rMSCs ($10^6$ cells) or PDGF + rMSCs in left side next day (Group 1) or at three weeks (Group 2) after irradiation, while the right side each was used as vehicle control. Each wound was analyzed by defining the percentage of ulcerated area to the irradiated during five weeks healing period after administration of PDGF or rMSCs, and histological observation.
Results
Greater than 60% of skin within each irradiated zone underwent ulceration within 16 days after irradiation, and reached peak ulceration above 50% around 3 weeks after irradiation. There was no any improvement of wound healing in all treatments of Group 1. However, the combined treatment of PDGF and rMSCs of Group 2 significantly reduced the wound size by 50.5%, compared to non-treated control while either rMSCs or PDGF alone of Group 2 showed no significant healing effect. PDGF treatment (PDGF alone or PDGF and rMSCs mixes) of Group 2 exhibited highly organized collagen fiber deposition in full-thickness, compared with vehicle control.
Conclusions
These results suggest that the combined administration of MSCs and PDGF efficiently enhanced the healing of radiation-induced skin ulceration when administered at time point when ulceration reaches peak in comparison with non-effect with immediate injection after irradiation.

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## PP380

**TNSALP influences neurogenic differentiation by altering gene expression in SH-SY5Y cells**

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Hypophosphatasia (HPP) is a rare disease characterized by low enzymatic activity of tissue-nonspecific alkaline phosphatase (TNSALP) resulting in an accumulation of its endogenous substrates like pyridoxal phosphate (PLP) and inorganic pyrophosphate ($PP_i$). The ectoenzyme plays an important role during bone mineralization and might contribute to proper function of kidney and muscle. Neurological symptoms of HPP like seizures, anxiety disorders and depression provide an indication of the enzyme's relevance in the nervous system. The severity of symptoms varies strongly depending on the respective mutation in the *ALPL* gene and the residual alkaline phosphatase activity.
The neuroblastoma cell line SH-SY5Y was chosen as an *in vitro* model for unraveling the role of TNSALP in the nervous system and SH-SY5Y–TNSALP which is stably overexpressing TNSALP was created by lipofection. A comparative microarray in combination with Gostat analysis highlighted differences in genes relating to neurogenesis and axonal growth. Cells with higher TNSALP activity reacted quicker to treatment with differentiation media containing 1 mm all-*trans* retinoic acid and showed a stronger growth of projections compared to cells with lower activity. A possible explanation might be the differential expression of neuropilin1 (Nrp1). Quantitative real-time PCR confirmed the microarray analysis which indicated a negative correlation of Nrp1 and TNSALP expression. Nrp1 is a receptor for semaphorin3A and located in the plasma membrane. Nrp1 forms a complex with plexinA in order to transfer the extracellular signal towards a modulation of the cytoskeleton, finally resulting in an inhibition of neurite outgrowth.

As a conclusion the comparison of neuronal cell lines with different expression levels of tissue-nonspecific alkaline phosphatase reveals possible molecular explanations for its role during brain development and the transmission of neuronal signals in addition to its function during bone mineralization. Therefore the described data might indicate putative targets for therapies of the neuronal symptoms caused by HPP.

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## PP381

**The influence of black and white tea on bone development of rat exposured to cadmium and lead**

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Tea is the second most consumed beverage in the world, next to water. The popularity of tea is determined by healthy properties connected with the presence of antioxidants, hence tea can be classified as functional food. Cadmium (Cd) and lead (Pb) are toxic metals commonly occurring in the human environment with exposure $\sim$5 and 35 mg/kg Bw per week respectively.
Thirty growing male Wistar rats (12 weeks old, 326.4$\pm$31.0 g) were used. Rat were fed *ad libitum* with common laboratory animal feed mixed with 7 mg of Cd ($CdCl_2$) and 50 mg of Pb ($(CH_3COO)_2Pb$) per kg of feed. The level of metals supplied in the feed was calculated to ensure that the daily supply of Cd and Pb did not exceed the environmental exposure of humans. Then, rats were divided into the control group ($n=10$) and black tea (BT; $n=10$) or white tea (WT; $n=10$) supplemented groups. The rats were euthanized at the age of 24 week to analyze femora in bone morphometry and geometry (A, cross section area; IC, cortical index; and MRWT, mean relative wall thickness) as well as maximum elastic strength (Wy) and ultimate strength (Wf).
Obtained results showed significant increase of IC (about 21.7% in both groups). Moreover, Wy increased by 37% and 65% in BT and WT group respectively. Similarly, Wf increased by 30 and 25% in BT and WT group respectively. While, MRWT decreased by 21.7% in both, BT and WT groups when compared to the control bone (all $P<0.05$).
All these results indicate that the administration of black or weight tee improves geometrical and mechanical properties of bones in growing rats exposed to cadmium and lead.
Declaration of interest
There is no conflict of interest.

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## PP382

**Osteogenic differentiation of fibroblast derived from patients with fybrodisplasia ossificans progressiva**

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Fibrodysplasia ossificans progressiva (FOP) is a rare, extremely disabling genetic disorder characterized by progressive heterotopic ossification preceded by episodic inflammatory soft tissue swellings (flare ups) leading to early death. There is no proven effective treatment yet. We aimed to develop an *in vitro* system to investigate the working mechanism of flare ups induced ossification.
Skin biopsies were obtained from four patients with FOP. Dermal fibroblasts were cultured in Ham F10 media until passage 3. Fibroblast cell lines from four age- and sex-matched healthy individuals were used as controls. Osteogenic trans-differentiation was induced by culturing for 21 days in osteogenic medium containing beta glycerol phosphate, ascorbic acid and 5% platelate lysate. Osteogenic differentiation was determined by mRNA expression of runx2, alkaline phosphatase, and osteocalcin. Mineralization was detected after 21 days using Alizarin red staining.
In all four cell lines the classical mutation in the activin receptor-like kinase2 (Alk2) receptor was confirmed. Runx2 and alkaline phosphatase mRNA expression started to increase 3 days after addition of osteogenic medium with a maximum increase at day 7, both in control and FOP cell lines. Osteocalcin mRNA expression was not increased until day 14 of culture. After 21 days of culture calcium deposits were detected with alizarin red staining in FOP cell lines, similar to the control cell lines.
We demonstrated osteogenic differentiation in fibroblasts form FOP patients. This *in vitro* system can be used to test the mechanism of inflammation related flare ups but also enables to test inhibitors of the ossification process.

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## PP383

**MicroRNAs as new biomarkers for monitoring of vascular calcification in CKD patients**

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Introduction
Calcification of vessels, especially media calcification, in combination with bone demineralization and disturbed bone metabolism is abundant in patients suffering from end stage renal disease (ESRD). In this project, we compare biomarkers of calcification with a focus on microRNAs from ESRD patients listed for renal transplantation (RTX) and healthy controls.
Methods
Samples are collected from kidney transplant patients. At the same time this patients are examined clinically to establish the degree of calcification. Systemic biomarkers as indicators of calcification mechanisms are analyzed with a focus on micoRNAs, new important regulators of gene expression. Clinical characterization including documentation of the vessel calcification status, go along with analysis of biomarkers of bone metabolism. MicroRNA profiles are measured in serum using the nCounter analysis system (Nanostring) and qPCR.
Results
Several regulators important for mineralization, such as FGF23, PTH, TRAP or bALP, show different levels comparing healthy controls and ESRD patients.
Further microRNAs have been isolated from serum of ESRD patients and controls and the presence of U6snRNA as well as hsr-miR23a has been checked as a control. 15 samples from patients with a glomerular filtration rate $<20$ and nine healthy controls have been used for the profiling with the Nanostring-Technology, evaluating the concentration of 800 different microRNAs. Differentially expressed microRNAs are further tested by qPCR.
Conclusion
MicroRNAs playing a role during vascular calcification and osteoblast-like differentiation of VSMCs are identified in samples from ESRD patients. They are potential new biomarkers for cardiovascular and osteological complications. Specific microRNA profiles from ESRD patients may be early diagnostic markers indicating the risk for vascular calcification and demineralization of bone, and serve as putative targets for therapy options in the future.

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## PP384

**Wnt16 as a new regulator of vascular calcification**

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Objective
Vascular calcification contributes to the pathogenesis of atherosclerosis, hypercholesterolemia, end stage renal disease, and diabetes, and is caused by genetic ablation of matrix Gla protein (MGP) in mice. Genetic ablation of MGP in mice results in excessive calcification of the vessel wall associated with a chondrocyte-like trans-differentiation in smooth muscle cells (VSMCs). Canonical β-catenin signaling is activated in the calcified arterial $Mgp^{-/-}$ tissue, however expression of Wnt16 is down-regulated almost 20-fold. The expression pattern of Wnt16 in embryonic bone development suggests its antagonistic function in chondrogenic differentiation and recent studies indicate a role for Wnt16 as a regulator of Notch signaling. The objective if this study was to determine whether and how Wnt16 controls phenotypic stability in VSMCs.

Methods
Primary WT and MGP$^{-/-}$ VSMCs were induced to undergo chondrogenesis in high-density micromasses in the presence or absence of overexpressed Wnt16.
Results
Expression of Wnt16 decreases with chondrogenic transformation in WT VSMCs. Primary MGP$^{-/-}$ VSMCs that have reduced levels of Wnt16 expression undergo spontaneous chondrogenesis in the absence of common inducer TGFβ. Overexpression of Wnt16 prevents chondrogenesis in both WT and MGP$^{-/-}$ VSMCs. Accordingly, Notch signaling is inactivated with chondrogenesis and loss of MGP expression in VSMCs, and forced expression of Wnt16 activates this signaling.
Conclusions
The Wnt16–Notch signaling network supports phenotypic stability in VSMCs and prevents their chondrogenic transformation. These results identify Wnt16 as a new therapeutic approach to vascular calcification, and may contribute to the understanding of its role in embryonic bone development.

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## PP385

### The effect of vitamin D treatment on pain, fatigue and muscular strength in women with vitamin D deficiency

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Objective
To find whether treatment with cholecalciferol and calcium in a group of vitamin D deficient women is associated with changes in muscular function, musculoskeletal pain, and fatigue.
Design
Prospective interventional cohort study.
Sample
A study group of 25 post partum women recruited and diagnosed with hypovitaminosis D during pregnancy.
Setting
The study group was recruited 2006–2009 at two mother care units in the south of Stockholm, Sweden. The investigations took place at the Karolinska University Hospital Huddinge. The study has been approved by the Local Ethics Committee and conducted in accordance with the Declaration of Helsinki.
Methods
Investigations of serum 25-hydroxyvitamin D (25(OH)D), parathyriod hormone (PTH), performance on chair stand test, pain as measured by visual analogue scale (VAS), bone tenderness by pressure algometer, and self assessed fatigue, before and after a treatment period of three months. The treatment consisted of 800–1600 IU cholecalciferol and 500–1000 mg calcium. Statistical analysis compared the results before and after treatment.
Main outcome measures
Differences of means, and *P* -values from the analysis of the investigations.
Results
Of the 25 women 21 attended in the treatment and follow-up. Following treatment the women had lower pain assessed on VAS, and higher performance on chair stand test, lower PTH and higher 25(OH)D, compared to before treatment.
Conclusions
In this small cohort of vitamin D deficient immigrant women treatment was associated with higher muscular performance and less musculoskeletal pain. In young and healthy women a short treatment period of a moderate dose of cholecalciferol can lead to improved vitamin D status. Vitamin D treatment should be considered in risk groups.

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## PP386

### Bone mineral density and micro-architectural changes in advanced chronic kidney disease

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Background
Risk of fracture in chronic kidney disease (CKD) population is high and it is associated with increased mortality. CKD affects bone quality through changes in bone turnover, microarchitecture, and mineralization. Secondary hyperparathyroidism has different effects on cortical and trabecular bone but dual-energy X-ray absorptiometry (DXA) is unable to effectively differentiate these bone compartments. High resolution peripheral quantitative computed tomography (HRpQCT) can overcome this limitation.
Aim
To compare bone mineral density (BMD) and micro-architectural parameters in CKD stages 4–5D (i.e. including dialysis) patients with controls.
Methodology
This is a cross-sectional study of CKD stages 4–5D patients and their age- and sex-matched controls with estimated glomerular filtration rate (eGFR) $>60$ ml/min per1.73 m$^2$. We used DXA to measure areal BMD (aBMD) of LS, hip and forearm. XtremeCT was used to obtain HRpQCT images of distal radius and tibia. This study has the Local Research Ethics Committee approval (ref 13/YH/0078).
Results
This is preliminary results from 20 CKD patients (13 pre-dialysis, and seven dialysis) in our study and their controls. This is an ongoing cross sectional study examining imaging and bone turnover markers utility to predict bone histomorphometry. Mean eGFR was 12 ml/min per 1.73 m$^2$ for pre-dialysis patients and 78 ml/min per 1.73 m$^2$ for controls. 25% of each group were female. We found that CKD patients had lower BMD measured by both techniques compared to controls. CKD patients had lower aBMD by DXA but this was only significant at the hip.
At the tibia, total vBMD was significantly lower in CKD compared to controls and there was a trend towards lower cortical and trabecular vBMD but this was not significant. At the radius, total vBMD was lower in CKD but trabecular vBMD was significantly lower compared to controls. Meanwhile, cortical vBMD in CKD at this site was similar to controls. We also found thinner cortical bone at the tibia and thinner trabeculae at the radius in CKD.
Conclusion
Preliminary data suggests that CKD is associated with different effects in trabecular and cortical bone. HRpQCT is a useful research tool in detecting bone changes in CKD although this needs to be verified in larger study and with bone histomorphometry.

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## PP387

### Heterotopic ossification in 453 chronic spinal injury patients

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Introduction
Heterotopic ossification (HO) means deposition of bone within the soft tissue around peripheral joints, first described by Guy Patin in 1692. This may occurs in up to 50% of spinal cord injury (SCI) patients. HO begins at mean of 12 weeks after injury. Only 10–20% of patients have clinical symptoms with decreased range of motion and inflammatory symptoms in the affected joints. The large joints below the levels of injury are typically affected, most commonly the hip. HO classified to four classes according to Booker's intensity of ossification, Class 1: a few small islands of periarticular soft tissue bone formation, Class 2: more than 1 centimeter distance between two adjacent bones, and Class 3: $<1$ centimeter distance between two adjacent bones, Class 4: complete bony ankylosis of the subjacent joint.
Materials and methods
Pelvic plane radiography of 453 patients with chronic SCI that admitted in the hospital for check up, studied in years 2010–2013.
Results
The age of patients was between 25–82 years with mean of 50 years. 97% of patients were males and 3% was females. The most common cause of injury was bullet and quiver in 77.7% of patients and the most injury level in thoracic vertebra (in th12). 91% of patients were paraplegic and 9% quadriplegic. The mean time of post spinal injury was 26 years. 35.3% of patients had HO that 11.3% in Class 1, 11.5% in Class 2, 5.3% in Class 3 and 7.3% in Class 4.
Conclusions
In our study the incidence of HO in SCI patients was 35.3% that was higher than Guttman (1976) 5%, Freehafer (1966) 17%, Scher (1976) 19%, Warton & Morgan (1977) 20%, Soulie (1927) 27% and lower than Dejerine and Callier (1919) 48.7%, Abramson & Kamberg (1949) 41%, and Paeslack (1965) 40%.

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## PP388

### Late onset presentation of osteogenesis imperfecta with additional mutation on GNAS gene: case report

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Aim
We present the case of a 36y female patient with multiple fragility fractures after the age of 21 and mutations in COL1A1, COL1A2 and GNAS genes.
Material and methods
A 36y female patient with multiple fractures of the axial and appendicular skeleton was referred to us for consultation. The patient was born with hexadactyly of the left foot and had a history of mild thoracolumbar scoliosis (10°) and medium height (165 cm) with no other history of medical conditions throughout childhood and adolescence. At the age of 21 she suffered a low energy fracture of her right femur and DXA scan revealed L2-L4 BMD 0.715, $z$-score $= -3.81$, Left Neck BMD 0.474, $z$-score $= -4.2$. Laboratory evaluation for bone metabolic disorders at that time was normal, the patient was diagnosed with Juvenile Osteoporosis and commenced therapy with Alendronate 10 mg per os daily and calcium/vitamin D supplementation. A year later she suffered a new fracture of her right femur, and after 2 years multiple vertebral fractures were diagnosed, followed by low energy fractures of right tibia, left femur, left shoulder and wrist. We performed laboratory examinations, peripheral quantitative computed tomography (pQCT) of the right tibia and proposed a bone biopsy as well as genetic testing for osteogenesis imperfecta and fibrous dysplasia.
Results
Bone biopsy and subsequent histomorphometry provided evidence that the patient suffered from Osteogenesis Imperfecta. Gene analysis with PCR sequencing proved that the patient was homozygous for polymorphism Sp1 and heterozygous for a Gly382Cys mutation of COL1A1, and had also the mutations Gly586Val, Gly646Cys, Gly661Ser, Gly1012Arg of COL1A2 gene, as well as the mutation R201C of GNAS gene.
Conclusion
This female patient with late-onset multiple fragility had mutations of COL1A1, COL1A2 as in osteogenesis imperfect but also of the GNAS gene, usually encountered in McCune Albright syndrome.

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## PP389

### Changing clinical profile of primary hyperparathyroidism in Indian patients

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Background
Primary hyperparathyroidism (PHPT) has evolved into an asymptomatic disease in the west. In contrast classic symptoms of PHPT have been reported to be common in the East with as many as 80–100% of PHPT patients presenting with bone manifestations in India.
Objective
To describe clinical and biochemical profile of patients diagnosed with PHPT between years 2009 and 2012.
Methodology
This was a retrospective study conducted at two tertiary care centres in India. All patients who underwent evaluation and surgery for PHPT from January 2009 to December 2012 were included in the study. Ethical clearance was obtained from Institutional Review Board.
Results
A total of 50 patients with PHPT were admitted between years 2009 and 2012. Among them, 31 (62%) were symptomatic and 19 (38%) were asymptomatic. Mean age (S.D.) was 48.3 (15.8). Female: male ratio was 1.9:1. Skeletal manifestations in total cohort were bone pains in 30% and fracture in 16%. Renal calculi were present in 20% of patients. The asymptomatic group had significantly lower median adenoma weight (0.57 vs 3.4, $P<0.05$), higher mean age (57.3 vs 42.8 years, $P<0.05$) and lower median intact parathormone (iPTH) level (254.5 vs 295 pg/ml, $P<0.05$) as compared to symptomatic group. Parathyroid adenoma weight was positively correlated with baseline serum calcium, iPTH and alkaline phosphatase levels. No correlation was found between serum 25-hydroxyvitamin D3 level and any of the biochemical parameters or adenoma weight.
Conclusions
In contrast to earlier reports, asymptomatic form of PHPT was found in significant percentage of Indian patients in this study. Asymptomatic PHPT patients were older in age and had lower parathyroid adenoma weight as compared to symptomatic PHPT patients. Positive correlation was found between parathyroid adenoma weight and serum calcium, iPTH and alkaline phosphatase levels.

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## PP390

### Bone marrow densitometry by clinical high resolution computed tomography of human vertebrae

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Introduction
Gaucher disease (GD), the most prevalent glycolipid storage disease, is an autosomal recessive metabolic disorder that is caused by an inherited deficiency of the lysosomal enzyme, glycocerebrosidase. This defect leads to reduce enzyme activity, resulting in the accumulation of glucosylceramide in cells of the monocyte-macrophages linage, known as Gaucher cells. Common presenting features include anemia, thrombocytopenia, hepatosplenomegaly and bone abnormalities. Skeletal disorders include osteopenia, bone pain crisis, bone infarctions, avascular bone necrosis (of the proximal and distal end of femur, proximal end of tibia and humerus), osteolytic lesions and fractures. Currently enzyme replacement therapy (ERT) has demonstrated a fast recovery of the cytopenias and visceromegalies. Besides, it has shown beneficial effects in both bone pain and the development of osteoporosis.
Objectives
The objective of this work was to analyze the clinical characteristics and bone involvement of GD patients diagnosed and controlled in our department.
Methods
Descriptive study including GD patients diagnosed in our department. In all patients we analyzed clinical and laboratory data (including PTH, 25OHD, P1NP and βCTX); bone mineral density of lumbar spine and femur and MRI of spine, femur, tibia and humerus bilaterally.
Results
Nine GD patients (six men and three women) were studied, with a mean age of 48 years (34–70), with an average time of evolution of the illness of 21 years (2–43). Currently, all patients receive ERT, with a mean duration of 10,5 years (1–16). Most of them ($n=6$) started with bone symptoms such as pain and bone crisis. Before received ERT, patients developed the following bone abnormalities: bone infarctions in eight patients (89%), Erlenmeyer flask deformity in two patients, femur avascular necrosis in five patients, 80% of them required hip replacement (one of them bilaterally).
Also four patients had been splenectomized. The study with serial MRI demonstrated that once the ERT is initiated none of the bone manifestations (bone infarctions and avascular necrosis) progressed in any of the patients.
GD patients present mean values of 25OHD of $27.4 \pm 10.5$ ng/ml. Insufficient vitamin D levels (25OHD $<$ 30 ng/ml) were observed in most GD patients (87%), 14% showed deficient levels (25OHD $<$20 ng/ml). As for bone remodeling markers we found values of P1NP $60.75 \pm 34$ ng/ml and βCTX $552 \pm 240$ pg/ml. None of the patients received supplementation with calcium and vitamin D.
According to densitometry criteria 22% of the patients have osteoporosis and 22% are in the range of osteopenia. One pathological fracture was registered (vertebral).
Conclusions
ERT prevents progression of bone abnormalities in GD. Vitamin D insufficiency is frequent in GD and almost half of the patients have decreased bone mass.

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## PP391

**Excess dietary calcium intake associated with higher Framingham risk score in 25-hydroxyvitamin D deficient male; analysis of the Korea National Health and Nutrition Examination Survey (KNHANES 2008–2011)**

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Background and objectives
The association between excess calcium intake and cardiovascular mortality has been reported, but the association has not yet been explored according to serum vitamin D status. Thus, we investigated the relation of dietary calcium intake and Framingham risk score (FRS) according to serum 25-hydroxyvitamin D (25(OH)D) status.
Methods
A total of 7809 subjects (3452 males and 4357 female) aged over 40 years from the data of the Korea National Health and Nutrition Examination Survey (KNHANES, 2008-200-11) were selected for this cross-sectional study. Daily dietary calcium intake was categorized into $<300$, 300–600, 600–900, 900–1,200, and $>1200$ mg/day and serum 25(OH)D concentration classified into $<50$, 50–75, and $>75$ mmol/l. The FRS was compared by the daily dietary calcium intake categories according to 25(OH)D concentration after adjustment with relevant variables in both genders.
Result
Higher FRS was observed in both $<300$ and $>1200$ mg of dietary calcium intake groups of males and $<300$ mg of females without adjustment. The significantly higher FRS were remained in the $<300$ and $>1200$ mg of dietary calcium intake in both genders after relevant variables adjustments. FRS was significantly higher in the group of $>1,200$ mg of dietary calcium intake group and serum 25(OH)D $<50$ nmol/l, which is vitamin D deficient group, only in male, not in female.
Conclusion
Very low ($<300$ mg/day) and excess ($>1200$ mg/day) dietary calcium intake was related with higher FRS in both genders, in particular, higher FRS was observed in the excess ($>1200$ mg/day) dietary calcium intake under vitamin D deficient ($<50$ nmol/l) males.

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## PP392

**Serum concentration of bone tissue metabolism markers in 28 and 180-day-old Polish Large White pigs**

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Evaluation of time-related changes of serum biochemical bone metabolism markers was performed in male pigs. Control group ($n=7$) received saline. NanoCa group ($n=7$) received nanopartical calcium *per os* (Ace Nano Calcium, NanoTechWorld, Korea). Dex group ($n=7$) received dexamethasone (1 mg/kg/48 hr *i.m.*). NanoCa/Dex group ($n=6$) received simultaneously nanopartical calcium and dexamethasone the same as the groups NanoCa and Dex. Nanopartical calcium was administered at two different dosages; 250 mg/pig/day (1-120 day) and 500 mg/pig/day (121-180 day). Dexamethasone and nanopartical calcium were administered throughout 6 months to accelerate bone metabolism. Blood was collected from 28- and 180-day-old piglets. Bone-specific alkaline phosphatase (BAP) concentration was determined using an immunoenzymometric assay (Ostease®BAP, IDS Ltd., UK). Osteocalcin (OC) concentration was assessed using MicroVue Human Osteocalcin EIA Kit (QUIDEL, USA). C-terminal telopeptide of type-I collagen (CTX-I) was evaluated using Serum CrossLaps® ELISA (IDS Ltd., UK). Insulin-like growth factor-1 (IGF-1) was determined using OCTEIA IGF-1 (IDS Ltd., UK). Parathormone (PTH) was determined using Porcine Intact PTH Elisa Kit (Immunotopics Inc., U.S.A). Statistical comparison was performed using Student *t*-test and $P<0.05$ was statistically significant. Serum concentrations of BAP, OC and PTH were lowered by 54, 20 and 16% in 180-day-old pigs when compared to 28-day-old group ($P\leq0.001$). Serum concentrations of CTX-I and IGF-1 were increased by 181 and 60% in 180-day-old pigs when compared to 28-day-old group ($P<0.001$). In conclusion, this study has shown higher levels of bone formation markers such as BAP and OC in younger pigs confirming intensive skeletal formation in rapidly growing pigs. Bone resorption marker (CTX-I) level in serum was nearly three-fold higher in the older group of pigs when compared to the younger group, confirming higher resorption rate of bone tissue in animals with significantly higher skeletal bone mass. IGF-1 concentration was elevated in the older group while an opposite results were obtained for PTH.

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## PP393

**Adult Niemann–Pick disease type B with myositis ossificans: a case report**

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Introduction
Niemann–Pick disease (NPD) is a rare autosomal recessive lysosomal lipid storage disorder. It is caused by mutations of genes which products are involved in the metabolism of sphingolipids. Their dysfunction causes sphingomyelin to accumulate in different organs which leads to progressive multisystemic disorder. Types A and B NPD are caused by mutations in sphingomyelin phosphodiesterase-1 gene with deficiency of acid sphingomyelinase (ASM). Types C and D NPD have normal or reduced sphingomyelinase activity but differ pathogenetically from types A and B. The various types share common clinical features and the severity of the disease varies depending on the gene mutation, enzyme deficiency and the system involved. The estimated incidence of types A and B NPD is 1:25 0000 and of type C is 1:150 000 live births.
Methods
A 34 year-old man with a family history of NPD type B was observed for pain and limited range of motion in both hip and knee joints.
Results
The clinical, biochemical and imaging data showed reduced ASM activity, hepatosplenomegaly, neurological deficiency, bone abnormalities, joint contractures not due to synovitis and myositis ossificans. The latter were seen on X-ray as massive ossifications with amorphous character around the hip joints and the CT images showed multiple massive ossifications and exostoses around the iliac bones and greater trochanter bilateral. The ossifications were visible in the internal and external obtorator muscles, gluteus medius and minimus and more severe in quadratus femoris muscle. This confirmed the combination of NPD type B with myositis ossificans.
Conclusion
It is important to raise the awareness of this debilitating condition and the need of a multidisciplinary management of such patients. As there is no recognized effective treatment for this disorder the possibility for prenatal diagnosis through amniocentesis or chorionic villus sampling especially in familial cases is of great importance.

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## PP394

**The influence of organic and inorganic Zn supplementation on bone development**

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Zinc (Zn) has a number of key roles relating to cell signalling, cell activation, gene expression, protein synthesis, apoptosis and is crucial for the development of immune cells. Damage to the linings of the gastrointestinal tract is observed during Zn deficiency. Since a few years it has been suggested the existence of the novel interrelationship between bone and gut:gut–bone axis.
The aim was to establish changes of morphological, geometric and mechanical parameters of femur in chickens supplemented with organic and inorganic Zn.
One-day-old Ross 308 male chickens were split into three groups each of 20 chickens. The birds were fed *ad libitum* with basal diets adequate to respective growth periods and had free access to water. Chickens were divided into three groups: the control (Zn-deficient group) and two groups supplemented with organic (zinc oxide) or inorganic (glycine chelate) Zn in the premix, which contained about 100% of the daily need of Zn. The requirement of mineral components in diet was based on nutritional recommendations for Ross 308

broiler chickens, amounting to 100 mg/kg$^{-1}$ of Zn. The birds were slaughtered at the age of 42 days. Tibiae were isolated and their weight and length were measured. Geometric (cross section area; cortical index; and mean relative wall thickness) and mechanical parameters like maximum elastic strength and ultimate strength, were determined. Additionally, the concentration of IGF, GH, osteocalcin and leptin was determined.
Obtained results showed that there was no diffrence between Zn-deficient group and supplemented with organic or inorganic groups of chickens.
Diet supplementet with Zn in organic or inorganic form did not influence bone development in chickens.
Conflict of interest
There is no conflict of interest.

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## PP395

**Assessment of vitamin K status by fully automated IDS-iSYS ina*Ktiv* MGP**

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Poor vitamin K intake is associated with markedly increased cardiovascular risk and mortality. The molecular mechanism underlying this association is suggested to be the vitamin K-dependent carboxylation of vascular matrix Gla-protein (MGP), a potent calcification inhibitor. The carboxylation step is essential for its activation, and uncarboxylated MGP, produced during poor vitamin K status, is inactive.
The IDS-iSYS ina*K*tiv MGP assay is the automated version of a microtiter-plate sandwich ELISA for desphospho-uncarboxylated MGP (dp-ucMGP) developed and reported by VitaK. The ina*K*tiv MGP assay was demonstrated to respond to variations of vitamin K status, and the normal range in the healthy population is 200-800 pmol/l. During recent years we have analyzed dp-ucMGP levels in a number of cohorts at high-risk for cardiovascular mortality. It was found invariably that subjects with dp-ucMGP levels above the upper-normal range were at two to fivefold increased risk of all-cause mortality. This was especially so in patients with end-stage kidney disease and peripheral artery disease. Human intervention studies have demonstrated that high vitamin K intake both decreases dp-ucMGP to normal or sub-normal levels and also decreases arterial stiffening. We found a high correlation between the microtiter-plate assay and the IDS-iSYS ina*K*tiv MGP assay ($R^2 = 0.95$) and the variation coefficient of the automated assay was $<5\%$.
Therefore, we propose the ina*K*tiv MGP assay as the method of choice for quantifying circulating dp-ucMGP, an independent biomarker for vascular vitamin K status and cardiovascular risk. Poor vitamin K status is a modifiable risk factor for arterial calcification, and the IDS-iSYS ina*K*tiv MGP assay is the most direct way to quickly identify subjects at risk and to monitor the effect of vitamin K intervention.

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## PP396

**Melorheostosis: succesfull conservative treatment of polyostotic skeleton affection**

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Melorheostosis together with osteopoikilosis, ostepathia striata and Buschke–Ollendorfov's syndrom belongs to mesodermal sclerotic dysplasia. Melorheostosis is a very rare disease with an incidence of 1:1 000 000, which was firstly described by French neurologist Léri in 1922. Hyperdense bands prominent upon the outer cortex niveau are visible on X-rays of long bones diaphysis reminding flowing wax of a candle. This disease is connected with contractures of soft tissues, limited motion, intermittent edemas of joints, limb deformities and especially algesia. Etiology is still unknown. Dysfunction of gene LEMD3 with simultaneous inhibition of TGFβ and BMP is considered. On the contrary, a lot of cases were reported without detection of mutation or gene function impairment. It is possible to detect three forms of melorheostosis: monostotic, monomelic and polyostotic. Polyostotic form is sometimes described as a generalized form. It represents combined monomelic and osteopoikilotic affection of other parts of skeleton. Standard X-ray is very important for differential diagnosis. For the further examination three-phase scintigraphy, CT and MRI are needed. CT and MRI are necessary for surgery extend planning, which is considered especially in case of central neurovasal oppression or release of extreme joint restriction. Peripheral vascular disturbances may be responsible for the pain associated with this disorder and vascular abnormalities could possibly be related to the pathogenesis of this disease.
Authors present a case of 26-year-old female. Despite the severe restriction of shoulder and elbow joint movement, palmar flexion of the wrist and concomitant affection of vertebral body of cervical spine and collarbone the diagnosis was confirmed as late as her age of 25 as a consequence of injury. Differential diagnosis, conservative as well as surgery treatment is further discussed.
After the diagnose confirmation the patient was treated with 70 mg once weekly alendronate, nifedipine and non-steroidal antirheumatics. There was a quick improvement in pain and vasomotor functions. Further medication was needed to solve the sleep disturbances. Due to the successful management of subjective symptoms there was no need for the patient to undergo surgery for nerve oppression.

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## PP397

**Serum 25-hydroxyvitamin D may have an association with lower coronary artery calcification score and higher bone mineral density against osteocalcin**

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Background and objective
Recent studies have demonstrated that higher serum 25-hydroxyvitamin D (25(OH)D) had a favorable effect on bone health. Osteocalcin, however, according to its serum concentration showed different outcomes for bone and aortic calcification. The aim of this cross-sectional study was to compare the bone mineral density (BMD) and coronary artery calcification score (CACS) according to the serum concentration of 25-hydroxyvitamin D and osteocalcin.
Methods
A total of 241 subject's data of the Health Promotion Center of CHA Anti-aging Institute, CHA University, Seoul, Republic of Korea, were selected. All data had serum 25(OH)D, osteocalcin, BMD, and CACS. We divided serum 25(OH)D and osteocalcin into two groups by their median concentration. BMD and LogCACS were compared according to four different groups of 25(OH)D and osteocalcin; low 25(OH)D with low osteocalcin, high 25(OH)D with low osteocalcin, low 25(OH)D with high osteocalcin, and high 25(OH)D with high osteocalcin.
Results
Serum glucose and triglyceride concentration was significantly low in the high 25(OH)D with high osteocalcin group. On the contrary, femur neck BMD (FNBMD) and total hip BMD (THBMD) were the highest in the high 25(OH)D with low osteocalcin group. As the same manner, LogCACS was the lowest, but not significant ($P = 0.072$), in the high 25(OH)D with high osteocalcin group and the FNBMD ($P = 0.025$) and THBMD ($P = 0.008$) were the highest in the high 25(OH)D with low osteocalcin group by ANCOVA test after adjustment with relevant variables.
Conclusion
Higher serum 25(OH)D with low osteocalcin showed significant association with higher FMBMD and THBMD. In addition, the high 25(OH)D with high osteocalcin group had a trend to have low LogCACS, which may mean high serum 25(OH)D may have favorable effect on LogCACS against high osteocalcin.

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## PP398

**A study on the effect factors on BMD of affected femur neck in patients with hemiplegia**

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The aim of this study was to investigate BMD of affected femur in patients with hemiplegic stroke.
Medical records of 153 patients with stroke who admitted a rehabilitation clinic from January 2011 to March 2013 were retrospectively reviewed. We excluded the patients with non-hemiplegia, diseases which can affect the BMD such as diabetes mellitus, thyroid disease and anti-epileptic drugs. We also excluded the patients who did not check both femur BMD. Total 68 subjects were finally enrolled in the study (38 males, 30 postmenopausal females). We measured BMD at affected and unaffected femur using dual energy X-ray absorptiometry, bone turn-over markers and activity levels. The influences of factors on affected femur neck BMD were investigated by linear regression test.
31 subjects showed decreased BMD of affected femur neck (45%). In 16 males ($61.9 \pm 10.5$ years), the mean duration after stroke was $51.3 \pm 31.5$ days, BMI was $22.4 \pm 3.5$ (kg/m$^2$) and wheel chair ambulators were 12. The BMD ratio of affected and unaffected femur neck was $0.915 \pm 0.07$. The 25-hydroxy vitamin D was $8.2 \pm 12.3$ (ng/ml), serum osteocalcin was $5.25 \pm 6.67$ (ng/ml) and serum CTX was $0.315 \pm 0.42$ (ng/ml). In 15 postmenopausal females ($68.3 \pm 10.4$ years), the mean duration after stroke was $31.1 \pm 19.7$ days, BMI was $22.6 \pm 3.0$ and wheel chair ambulators were eight. The BMD ratio of affected and unaffected femur neck was $0.926 \pm 0.083$. The 25-hydroxy vitamin D was $10.6 \pm 10.5$, serum osteocalcin was $7.81 \pm 8.23$ and serum CTX was $0.44 \pm 0.56$. In linear regression test, ambulation classification was a only effect factor on the BMD ratio of affected and unaffected femur neck ($\beta = 0.38$, $P = 0.034$).
The femur neck BMD of hemiplegic side were not decreased in all stroke patients. This study showed BMD changes in affected femur neck were link to ambulation classification than bone turnover marker.

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## PP399

**Effect of vitamin D treatment on bone mineral density in deficient immigrant women**

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Background
The optimal level of 25-hydroxyvitamin D2 (25(OH) D) for a healthy bone is not clear, nor the effects of treatment with vitamin D. Few previous studies have measured treatment effect on BMD in young deficient individuals.
Materials and methods
A treatment group of vitamin D deficient immigrant women and a control group of ethnic Swedish women were recruited during pregnancy. A treatment of 800–1600 IU cholecalciferol and 500–1000 mg calcium per day started *post partum*. Examinations of S-25(OH) D, serum intact parathyroid hormone (S-iPTH), bone density by dual X-ray absorptiometry (DEXA) and peripheral quantitative computed tomography (pQCT), took place at the start of treatment, and again after 1 year. Statistical analysis compared the baseline examinations and the follow up. The study has been approved by the local ethics committee and conducted in accordance with the Declaration of H-elsinki.
Results
The treatment effect could be followed in 12 immigrant women. At the baseline all immigrant women were deficient, and none of the controls. At the follow up the immigrant women had normalized S-25(OH) D and S-PTH. In both groups BMD measured by DEXA was higher at the follow-up. The two groups did not differ in BMD at the two time points or over time.
Discussion
We observed a normalization of 25(OH) D and PTH in the group of deficient young immigrant women treated with moderate dose vitamin D and calcium. This can indicate a positive effect of vitamin D treatment in young individuals.
Conclusion
Treatment and testing should be considered for risk groups.

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## PP400

**Pseudoseptical myositis as the prodrome of myositis ossificans**

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A 20-year-old male presented following a fall down. He was diagnosed with a C5–C7 burst fracture and underwent a cervical fixation surgery. He was transferred to inpatient rehabilitation with C6 ASIA A tetraplegia.
70 days after the operation, he began to have intermittent mild fever with a temperature of 37.4 °C. On postoperative day 72, left thigh was noted to be edematous. The circumferential difference between the lower extremities measured at 10 cm above the knee was 5 cm. CT chest thromboembolism demonstrated no evidence suggesting deep vein thrombosis. A thigh contrast MRI showed intramuscular fluid collections that appeared hyperintense on T2WI. Diffusely increased signal intensity on T2WI was noted in left iliacus, iliopsoas, vastus medialis and intermedius, gluteus medius and minimus, part of vastus lateralis. This finding suggested myositis or less like infection. At bone scan, there were uptake signals on left femur, great trochanter. And ALP was 80 IU/l and calcium was 8.9 mg/dl (after 20 days from initial, calcium level was within normal limit, but ALP was increased at 190). There were no other conditions triggered inflammatory reactions. NSAIDs and etidronate were used for 2 weeks. Although fever subsided and inflammatory markers normalized, the swelling and limitation of motion persisted. On follow-up X-ray two weeks after the initial, there were no calcified lesions. Seven weeks after initial, at the follow-up bone scan, there were high uptake signals on left femur, great trochanter. ALP was 391 and X-ray at this time, there was no calcified lesion.
When patients present with numerous medical comorbidities predisposing them to infectious conditions, immature myositis ossificans can be extremely difficult to distinguish from deep infections such as pyomyositis, cellulitis and osteomyelitis. In the case of after neurogenic and traumatic insults which include brain injury and spinal cord injury, we should suspect myositis ossificans and should not miss that treatment of choice is NSAIDs, not the antibiotics.

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## PP401

**Relationship between history of pregnancies and bone mineral density**

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Objectives
We investigated bone mineral density (BMD) in women as this is related to a history of previous pregnancies and miscarriage and according to age.
Materials and methods
We performed retrospective study on 1043 women who had their BMD in a university hospital. We analyzed the BMD and the previous obstetric history according to age and the clinical characteristics.
Results
The mean age of the study subjects was $56.7 \pm 5.9$ years. The gravida based on ages is not statistically related to the BMD ($P$ value $= 0.578$), except for the spine BMD ($P$ value $< 0.001$).
Conclusions
Obstetric history of previous child birth and/or miscarriages, did not effect on bone mineral density, but further prospective multicenter study about obstetric history should be considered for prevention of osteoporosis before menopause.

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## PP402

**Clinical case of a patient with severe tertiary hyperparathyroidism, osteitis fibrosa cystica and osteomalacia as a consequence of severe vitamin D deficiency**

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Vitamin D (vit D) plays an important role in the regulation of mineral homeostasis. Vit D insufficiency leads to decreased calcium and phosphorous intestinal absorption, parathyroid glands (PG) stimulation with consequent development of secondary hyperparathyroidism (HPT) and bone mineralization defect. We present a clinical case of a patient with tertiary HPT as a consequence of severe vit D deficiency.

A clinical case

A woman 59 years old considered herself ill since childhood after nutritional rickets. She was receiving vit D occasionally, and for a long period of time was suffering from the pain in the lumbar spine, other bones, large joints, and progressing chest deformity. Between 50 and 56 years the above mentioned complaints intensified, which forced her to use a walking stick. Three Colles' fractures also occurred. In 2012 a significant decrease in BMD was noted (*T*-score) at the radius −9.6 SD and the spine −8.1 SD, as well as an increased PTH level and hypercalcemia (verified twice). The diagnosis workup for primary HPT included Physical examination revealed kyphoscoliotic chest, bow-shaped thigh and crura deformities, and bone excrescence on the anterior surface of the left tibia. There were normal serum calcium and phosphorous levels and 24-h urine calcium, vit D deficiency 9.1 ng/ml, PTH 828.8 pg/ml (15.0–65.0), alkaline phosphatase 236 IU/l (10.0–150.0), osteocalcin 188.6 ng/ml (11.0–43.0) and b-crosslaps 2.29 (0.01–0.69). Ultrasound and CT imaging detected the enlargement of the upper left PG. Radiographs of the hands revealed osteoporosis, bow-shaped first metacarpal deformities, severe osteoporosis in the spine, compression fractures of all thoracic vertebrae, and kyphoscoliotic chest. CT imaging of the bones showed bow-shaped curvature of proximal thirds of both femoral bones, multiple Looser's zones combined with bone cysts of the pelvis, both greater and lesser trochanters, right femoral bone and left tibia diaphyses. The condition was considered as tertiary HPT in the setting of severe vit D. Parathyroidectomy was performed. Follow-up in 6–12 months showed improvement in medical condition, normalization of mineral homeostasis, and significant increase in BMD.

Conclusion

The case presented is the outcome of non-compensated nutritional rickets since early childhood and illustrate the need most of early diagnosis and adequate treatment of deficiency of vitamin D.

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## PP403

**Adsorption salivary proteins on dental materias**

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The aim of this study was to evaluate the adsorption of salivary proteins on giomer (Beautifil II) and composite resin (Filtek Z350).

Three discs (5 mm diameter and 1 mm height) were prepared for each material. Three discs were immersed in 1 ml? of human saliva within polyethylene tube and stored at room temperature. Evaluations were performed by Bradford method and SDS gel electrophoresis for analysis of salivary protein adsorbed on giomer and composite resin.

The results can be summarized as follows:

1. The mount of salivary proteins adsorbed on giomer was more than that of adsorbed on composite resin.
2. These proteins of 14, 20, 25, 55 and 66 kDa molecular weight were different between giomer and composite resin.
3. Lysozyme/cystatin and PRPs are more adsorbed on composite resin than giomer.
4. α-amylase and albumin are more adsorbed on giomer than composite resin.

There are quantitative and qualitative differences of salivary protein adsorbed on between giomer and composite rein. These data offer understanding of relationship between salivary proteins and dental mateials. We will conduct further study to determine the correlation of salivary protein and bacterial adhesion.

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## PP404

**Effects of up to 15 years of recombinant human GH replacement therapy on the skeleton in adult GH deficiency: the Leiden Cohort Study**

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Background

Adult GH Deficiency (GHD) is associated with decreased bone mass and increased fracture risk. Recombinant human GH (rhGH) replacement therapy leads to progressive increases in bone mineral density (BMD) for up to 7 years of treatment, but little is known on longer term effects of rhGH therapy on bone mass or fracture risk.

Methods

230 GHD patients (mean age 47.1 years, 52.6% female) on rhGH replacement therapy for ≥5 years were included in the study. BMD measurements were evaluated at the lumbar spine (LS) and femoral neck (FN) at baseline and at 5, 10 and 15 years after start of therapy. Clinical fracture incidence was also assessed over the period of follow-up. All patients received hormone replacement therapy for other pituitary deficiencies and calcium and/or vitamin D supplements as required, and a number additional bisphosphonate treatment.

Results

211 patients completed 5 years, 98 patients 10 years, and 43 patients 15 years of rhGH therapy. Ten patients (4.3%) received bisphosphonates at baseline and 12.2, 19.4 and 18.6% received these agents after respectively 5, 10 and 15 years of starting rhGH. Mean duration of treatment with bisphosphonates was 6.9±4.3 years. Mean LS BMD remained stable in women, but demonstrated a significant 4% increase in men after 15 years of rhGH therapy. There was no additional benefit of bisphosphonate therapy on BMD. 15 patients (7%) sustained a clinical vertebral fracture during follow-up. the incidence rate of fractures during rhGH replacement was 46/2288.5 (mean duration of rhGH therapy 9.95 years ×230 patients) =20.1/1000 py in our GHD cohort.

Conclusions

In adult GHD, longterm rhGH replacement therapy stabilises BMD. Bisphosphonate therapy does not appear to confer additional beneficial effects on BMD or fracture risk.

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## PP405

**Innovative cell-based strategy for systemic delivery of soluble RANKL in RANKL-deficient osteopetrotic mice**

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In autosomal recessive osteopetrosis due to mutations of the TNFSF11 gene, deficiency of the pro-osteoclastogenic cytokine RANKL prevents osteoclast formation. RANKL is a membrane-bound protein cleaved into active soluble (s)RANKL by various enzymes, including metalloproteinase 14 (MMP14). We created a bio-device that released sRANKL and induced osteoclastogenesis in tnfsf11−/− mice. We tested various RANKL cell sources, and used mouse primary calvarial osteoblasts, which are readily available, easy to handle and express RANKL in large amount and in a PTH-regulated fashion. Cells were cultured on 3D-hydroxyapatite scaffolds (3D-HASs) adsorbed with the catalytic domain of MMP14, creating a device enzymatically stable over time, that enhanced sRANKL release. These conditions allowed to achieve a free sRANKL concentration, calculated on the basis of 1:1 molar ratio with released OPG, three times higher than in standard cultures, seemly for osteoclastogenesis. These 3D-HASs were sealed in diffusion chambers (DCs) that isolate cells, preventing immune responses, but let molecular flow and release of soluble factors into the circulation. They were implanted in tnfsf11−/− mice of various ages (21–40 days) and genetic background C57BL/6 and C57BL/6-CD1. Mice were sacrificed after 1 or 2 months from implants and received 1 or 2 DCs, once or twice. An increase of overall survival and body weight was observed in all implanted groups compared to non-implanted mice. Histological sections of tibias of non-implanted mice were negative for the osteoclast marker TRAcP, consistent with the lack of the osteoclast lineage. In contrast, tibias excided from implanted mice showed TRAcP-positive cells both in the bone marrow and on the bone surface, these latter morphologically similar to mature osteoclasts. Improved outcome was observed in longer treatments and double implants. We suggest that engineered DCs delivering sRANKL support the feasibility of an innovative

experimental strategy to deliver the soluble cytokine and treat systemic deficiency.

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## Paediatric bone disease

### PP406

**Early intervention with anti-resorptives is essential in OI: a longterm murine study**

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The *oim/oim* mouse resembles type III OI. Our study mimics clinical questions: is RANK-Fc as efficacious as ALN in reducing fracture number? Is RANK-Fc safe to administer to children? Is there any advantage to receiving RANK-Fc treatment post adolescence, following no treatment or ALN treatment from infancy through adolescence?

All animal work was done under an IACUC-approval. Mice – $n=200$, WT or *oim/oim*. Treatment spanned age 2–26 weeks. Five study arms ($n=20$/group/genotype): i) saline – 24 weeks ii) ALN – 24 weeks iii) RANK-Fc – 24 weeks iv) saline – 12 weeks then RANK-Fc for 12 weeks and v) ALN – 12 weeks then Rank-Fc for 12 weeks.

At transition, all treatments had reduced fracture number. From transition to sacrifice, *oim/oim* mice treated with saline averaged 0.9 new fractures. At sacrifice, all *oim/oim* treatment groups had reduced fracture number. The saline + RANK-Fc group averaged 0.3 new fractures since transition whereas RANK-Fc 24 weeks, ALN + RANK-Fc, and ALN 24 weeks had no new fractures. Delayed fracture remodeling was observed in treated and untreated *oim/oim* mice.

For *oim/oim* mice, all four treatments resulted in increased BV/TV% compared to saline 24 weeks.

Micro Ct: in *oim/oim* mice, treatment with RANK-Fc, ALN + RANK-Fc, and ALN 24 weeks increased cortical BVF compared to saline 24 weeks. In trabecular parameters changes in the WT mirrored those in the *oim/oim*, treatment with RANK-Fc 24 weeks, ALN + RANK-Fc, and ALN 24 weeks increased trabecular number and BVF and decreased trabecular spacing compared to saline 24 weeks, without changing trabecular thickness. In the *oim/oim* saline + RANK-Fc reduced trabecular separation compared to saline 24 weeks.

The conclusion is early intervention with antiresorptive agents at a young age is imperative for significant reductions in fracture incidence and sustained increased BVF into adulthood.

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### PP407

**Glucocorticoid-treated boys with Duchenne muscular dystrophy DMD and osteoporosis have higher bone matrix mineralization before and after i.v. bisphosphonate therapy**

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Duchenne muscular dystrophy (DMD) causes progressive muscle weakness and loss of ambulation. While glucocorticoid (GC) therapy improves motor function, many boys sustain fractures due to osteoporosis. Recently, i.v. bisphosphonate (i.v.BP) therapy has shown promise in the treatment of DMD-related osteoporosis. At the same time, bone histomorphometry revealed lowered bone volume and significant reductions in bone formation pre-i.v.BP treatment, and a further drop after 2 years' therapy.

Given these findings, the purpose of this study was to evaluate the bone matrix mineralization density distribution (BMDD) in cancellous (Cn.) and cortical (Ct.) compartments on paired transiliac bone biopsies from five boys with DMD using quantitative backscatter electron imaging compared to healthy controls. The baseline biopsy was performed at a mean $\pm$ S.D. age of $12.8 \pm 1.2$ years, and the second was carried out between 2.1 and 2.8 years after the start of i.v.BP (pamidronate or zoledronic acid) therapy.

The typical (mode) Ca concentration in cancellous bone was high at baseline (Cn.CaPeak + 5.6%, $P<0.05$) and even higher after i.v.BP treatment (Cn.CaPeak + 7.2%, $P<0.001$) compared to reference, while the mineralization heterogeneity was normal before and decreased after i.v.BP (Cn.CaWidth − 15%, $P<0.01$). Paired sample comparisons after i.v.BP showed non-significant increases in Cn.CaPeak in all but one boy, and decreases in Cn.CaWidth in all patients. Ct.BMDD variables were not different from controls at both timepoints. Our findings suggest higher degrees of bone matrix mineralization in boys with DMD even before i.v.BP therapy, which we hypothesize is due to GC therapy. Although i.v.BP treatment effects failed statistical significance (low sample size), the comparison to normal suggests i.v.BP treatment further increases the magnitude, but decreases the heterogeneity of mineralization consistent with the earlier histomorphometric bone formation findings. Bone fragility in these boys with DMD appears to result mainly from reductions in bone volume and is further associated with elevated bone matrix mineralization.

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### PP408

**Effect of GOS/FOS on calcium and phosphorus absorption and retention during recovering from undernutrition: experimental model in normal growing rats**

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During early life undernutrition impairs normal weight and height gain that affects bone health. During recovering it is required an extra-amount of Ca to ensure the high demands of bone mineralization. A mixture of galactooligosaccharides (GOS) and fructooligosaccharides (FOS) would be a useful tool to increase mineral intestinal absorption to enhancing the amount of Ca to be retained into bone. Our objective was to evaluate, in an experimental model of protein restriction (PR) the effect of a mixture of GOS/FOS during recovering. Undernourished male rats were obtained by feeding from weaning a low protein diet (casein: 4%) during 7 days. Then, they were divided in two groups receiving AIN'93G (UA) or AIN'93G + 5% GOS/FOS (UP) till day- 50. Normal growing rats received from weaning to the end of the study, one of the two mentioned experimental diets (NA and NP groups respectively). Body weight (BW) and length (BL), and intestinal lactobacilli (DL) colonies development were measured weekly. At T50: caecum pH, Ca and phosphorus (P) absorption, femoral Ca and P content, total skeleton bone mineral content and density (BMC and BMD) (Lunar DXA), bone volume (BV/TV), growth plate cartilage thickness (GPC.Th), hypertrophic zone thickness (HpZ.Th) and tibia length (TL) were evaluated. There was a stop in growth during the PR period. After recovering, UA and UP increased their BW and BL without reaching NA and NP values. UP and NP increased DL and decreased caecum pH ($P<0.0001$). UP showed a significant increase in: Ca and P absorption ($P<0.05$), femur Ca and P content ($P<0.01$) and BMC ($P<0.05$) compared to UA. UP showed significantly higher BV/TV, GPC.Th, HpZ.Th and TL compared to NA ($P<0.05$)

Conclusion

The consumption of the GOS/FOS mixture allowed increasing Ca and P absorption and their retention in bone, suggesting its usefulness to give an extra amount of Ca and P not only during normal growth but also during nutritional recovery from undernutrition.

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### PP409

**Ibandronate in the treatment of pediatric osteoporosis**

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Background

Orally administered ibandronate is an effective agent in the treatment of postmenopausal osteoporosis. There are only scarce data regarding its use in children with impaired bone health.

Objectives
To evaluate the effect of oral ibandronate on bone health in osteoporotic children and adolescents.
Patients, materials and methods
We enrolled six patients (all boys; mean age 15.7±3.8 years; range 8–18 years) with low bone mineral density (BMD) (mean −3.5±1.6 S.D. *Z*-score) and with prevalent fractures (mean 4.5±4.4 S.D.). Oral ibandronate (150 mg/tablet) was administered once-a-month in full accordance with current recommendations. All patients were receiving oral calcium (1000–1500 mg/day) and vitamin D (cholecalciferol, 1000–1500 IU/day). Laboratory parameters (biochemical: S-Na, K, Cl, Ca, P, ALP, AST, ALT, urea nitrogen, creatinine, and parathyroid hormone and bone markers: S-osteocalcin, Crosslaps; hematologic–blood count) were assessed on baseline and were further checked every 3 months within the first year of therapy, and every 6 months thereafter. Lumbar spine BMD was assessed by DXA (Lunar) at the baseline and every 12 months of the treatment. New fractures and adverse events were recorded in the course of the treatment.
Results
Mean duration of the treatment was 2.0±0.8 years: 1 year ($n=2$ patients), two years ($n=3$), 3 years ($n=1$). In three patients the treatment is still ongoing. After 1 year there was a 13% increase in BMD (*Z*-score −2.5±1.5 S.D.; $P=0.0001$), after 2 years of treatment the 13% increase in BMD was maintained (*Z*-score −2.4±2.5 S.D.; $P=0.02$) compared to baseline values. The values of laboratory parameters were within reference ranges at the baseline and in the course of the treatment. No new fractures occurred. Only 1 adverse event/reaction was recorded in 1 subject: transient epigastric pain and myalgia after the first dose of ibandronate.
Conclusion
Orally administered ibandronate significantly increased BMD and decreased fracture incidence in pediatric patients with osteoporosis. Oral ibandronate can be helpful in the treatment of pediatric osteoporosis.

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## PP410

**Bone health in children with hemolytic anemia: does the pathogenesis of hemolysis determine the phenotype of bone alteration?**

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Introduction
Bone health in patients with sickle cell disease and thalassemia is impaired. These patients feature altered parameters of bone metabolism and bone mineral density.
Aim and design
To investigate bone health in patients with hemolytic anemia we conducted a cross-sectional analysis in our Hematology Outpatient Clinic at the Children's Hospital Essen. The largest subgroups within our cohort are patients with homozygous sickle cell (HBSS) disease and patients with spherocytosis.
Clinical and biochemical parameters of growth, puberty, bone turnover, and vitamin D metabolism were obtained, as well as bone densitometry using DXA scans in a subgroup of patients. Additionally a questionnaire was developed assessing life style parameters including calcium and vitamin D intake.
Patients ($n=46$, 25 females) with the following diagnoses were recruited at regular visits: HBSS disease ($n=16$), HBSS-α thalassemia ($n=1$), HBSC disease ($n=2$), HBS-β thalassemia ($n=1$), β thalassemia major ($n=6$), β thalassemia minor ($n=1$), hereditary spherocytosis ($n=14$), glucose-6-phosphate deficiency ($n=2$), paroxysmal nocturnal hemoglobinuria ($n=1$), and hemolytic anemia of unknown origin ($n=2$). The study was approved by the Local Ethics Committee.
Results
Mean serum 25-OH vitamin D was 12.0 (1–30.2) ng/ml, BAP was 137.5 (37.3–531.4) U/l, PTH 49.5 (17.3–239.6) pg/ml, N-telopeptide in urine 776.6 (117–1994) mmol/mg creatinine and calcium:creatinine ratio in urine was 0.07 (0.01–0.32) mg/mg.
Vitamin D deficiency (25 OH-vitamin <20 ng/ml) was observed in 78% and secondary hyperparathyroidism in 23%. 19% of patients reported bone pain after physical activity, 12% experienced fractures. Osteopenia (*Z*-score <−2) was detected in 14% of the patients screened.
No correlation between bone health and markers of disease activity was observed but patients with spherocytosis showed less impairment of bone health than patients with HBSS disease.
Conclusion
Bone health is impaired in patients with hemolytic anemia, especially in children with HBSS disease. Clinically this is reflected by bone pain and/or fractures in some patients. Identification of children at risk is difficult and requires assessment of additional measures. Vitamin D and calcium deficiencies are frequent findings in this group and adequate monitoring and supplementation is recommended.

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## PP411

**Tricho-rhino-phalangeal syndrome TRPS: the new three cases from Poland**

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Tricho-rhino-pharyngeal syndrome (TRPS) is a rare genetic disorder, which is characterized by craniofacial and skeletal abnormalities. This report presents three cases of TRPS (two sporadic and one familial). Clinical presentation included typical facial features (pear-shape nose, long flat philtrum, thin upper vermilion border and protruding ears), thin, sparse scalp hair and different skeletal abnormalities with normal mentation. Case 1: 5.5-year-old boy with short stature (GH deficiency excluded), clinically no skeletal abnormalities, but radiological assessment confirmed cone-shaped epiphyses at the phalanges. Moreover his father manifested the disease phenotype (not genetically confirmed yet). Case 2: 12-year-old girl hospitalized with growth velocity retardation, with clinical and radiological epiphyseal deformities in phalanges, right humerus and brachydactyly of toes. Genetic analysis confirmed interstitial heterozygous microdeletion of 8q24. Case 3: 13.5-year-old with short stature (GH deficiency excluded), typical epiphyseal deformities in phalanges and Perthes-like left hip deformation. Interestingly, none of TRPS features were found in her twin sister. Both girls (Case 2 and 3) had also mild vitamin D insufficiency. The wide variability in clinical expression of TRPS can mimic the other pathology (i.e. GH deficiency). The cooperation of multidisciplinary team seems to be essential in the proper management of these patients.

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## PP412

**Team management of young persons with osteogenesis imperfecta**

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The pediatric osteogenesis imperfecta (OI) team at our university hospital was established in 1991. The multi- and inter-disciplinary team consists of: pediatric neurologist, nurse, nursing assistant, physiotherapist, occupational therapist, orthopedic surgeon, orthotist, radiologist, dentist and geneticist. We also have a close collaboration with other specialists.
Our assignment is high qualified diagnostics, functional assessments and also individualized evaluation and advice in order to optimize treatment, medications and other interventions. A special concern is to assess untreated children with different types of OI to find criteria for and monitoring treatment. The team follows most children and youths with OI, from an early age and does regular follow-ups until 18 years of age, when transition to adult care is made.
We follow growth, abilities and disabilities using appropriate radiology, dual-energy X-ray absorptiometry (DXA), bone markers, physical examinations, dental development, range of movements (ROM), gross motor function measure (GMFM) and patient reported measurements as activities scale for kids (ASK) and Pediatric evaluation disability Inventory (PEDI). Genetic analyses are now also performed.
After each individualized assessment by the team the results and advice are rapidly reported to the child's local hospital, rehabilitation team and dental specialist.
The team has in total during the years assessed >300 children and adolescents. We prescribe and monitor bisphosphonate treatment to children with either severe OI (type III/IV) or milder forms with skeletal pain and vertebral compression fractures. Advanced surgery by pediatric orthopedic surgeons is also performed. Information brings knowledge and confidence both to the patients and to their local teams.. Early diagnosis, care and treatment can also prevent some skeletal deformities and fractures.

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## Steroid hormones and receptors

### PP413

**Chondroitin sulfate chains are co-receptors for interleukin- 34**

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Interleukin- 34 (IL34) is a new, challenging cytokine discovered in 2008. It promotes the proliferation, survival and differentiation of the monocyte/macrophage lineage with almost the same efficiency as the macrophage-colony stimulating factor (M-CSF). IL34 has already been described to play a key role in various musculoskeletal bone diseases such as bone giant cell tumors or in rheumatoid arthritis. These 'twin' cytokines share a lot of functional similarities which were explained by their ability to bind and activate the M-CSF receptor (M-CSFR). The aim of this work was to search for an alternative binding of IL34 on cells.
M-CSFR expression was analyzed by flow-cytometry, and intracellular pathways were studied by western blot. Signaling was also investigated after enzymatic treatment of cells. To characterize IL34 binding to various cell lines (myeloid cell lines, ostesarcoma and embryonic cells), Scatchard and binding inhibition assays were carried out using $^{125}$I radiolabelled IL34 and M-CSF. Molecular interactions were also studied using surface plasmon resonance (Biacore).
M-CSF and IL34 induced a different pattern of phopshorylations in cells overexpressing the M-CSFR, hence suggesting the existence of an alternative receptor for IL34. Biacore and scatchard experiments confirmed the binding of IL34 on cells in which the M-CSFR was lacking. Enzymatic treatments of the cells and binding assays of glycosaminoglycans on immobilized IL34 identified the chondroitin sulfate chains as co-receptors for IL34.
This work evidences an alternative binding of IL34 to chondroitin sulfate chains, on cells lacking the known IL34 receptors, the M-CSFR and the receptor protein tyrosine phoshatase β/ζ (RPTP β/ζ). In fact, recent data show that RPTP β/ζ is another receptor for IL34 in the central nervous system, and that this receptor needs its chondroitin sulfate chains to work. Consequently, these glycosaminoglycans could control IL34 bioavailability at the cell surface, thereby modulating the M-CSFR activations.

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### PP414

**Glucocorticoids suppress inflammation in arthritis via the glucocorticoid receptor in non-hematopoietic cells**

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Owing to their anti-inflammatory effects, steroid therapy using glucocorticoids (GCs) is still part of the treatment of rheumatoid arthritis (RA), despite several severe side effects like glucocorticoid-induced osteoporosis (GIO). Until now the molecular mechanisms underlying the beneficial and side effects of GC therapy are poorly understood. GCs exert their actions via the glucocorticoid receptor (GR) that alters gene expression by either binding as a dimer to GC response elements in the promoter region of target genes or by interacting with and thus interfering with other transcription factors.
Using conditional and functional GR mutant mice, we previously showed in antigen-induced arthritis (AIA) that GCs reduce acute inflammation via the dimerized GR in IL17 producing T cells (*PNAS* 2011, **108**:19317).
Now, we demonstrate that in the chronic, bone destructive K/BxN serum transfer induced arthritis, a T cell independent model of arthritis, unexpectedly, dimerization of the GR in non-hematopoietic cells contributes to the anti-inflammatory effect of GCs. Currently we test, which type of mesenchymal cells mediate the immunosuppressive effects of glucocorticoids in arthritis. Thus, for immunosuppression of arthritis GR dimer dependent gene regulation is decisive in distinct cell types, partly of non-hematopoietic origin. Our findings of GC action in arthritis have consequences on new concepts for anti-inflammatory therapies.

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### PP415

**Vitamin D deficiency and prevention; what is the position of European Calcified Tissue Society?**

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Vitamin D deficiency is common within Europe and the Middle East, especially in risk groups. While treatment is simple, there is no consensus on the diagnostic threshold for deficiency and the required dose. The Institute of Medicine and the Endocrine Society have established guidelines on the required 25-hydroxy-vitamin D (25(OH)D) levels, supplementation doses and (extra)skeletal effects of vitamin D, coming to very different conclusions. The European Calcified Tissue Society has instituted a Working Group on Vitamin D to prepare a position statement regarding various aspects of vitamin D deficiency and prevention. The members agreed that a European statement outlined in a position paper would be appropriate after the guidelines and the discussions in the American, European and international journals.
The statement will include an overview of the vitamin D status in different European and Middle East countries, the prevalence of vitamin D deficiency according to different thresholds, the optimal/minimal required 25(OH)D levels and required doses to prevent vitamin D deficiency. Data on food fortification policy and the availability of supplements are included. Strategic options, implementation strategies, and public health options will be discussed. The statement will finish with a research agenda and a conclusion.
It will be a tremendous effort to improve vitamin D status in Europe and reduce the percentage of the population with serum 25(OH)D below 50 nmol/l. The Working Group will consider prudent sun exposure, adequate nutrition, food fortification and vitamin D supplementation.

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### PP416

**Novel mechanisms of action and new target genes of the glucocorticoid receptor in inflammatory bone disease and bone loss**

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Glucocorticoids (GCs) are widely used to treat chronic inflammatory diseases such as rheumatoid (RA) and lead to multiple side effects including glucocorticoid induced osteoporosis (GIO). Our work challenges the dogma that transrepression of pro-inflammatory genes by the glucocorticoid receptor (GR) is solely responsible for reducing inflammation, whereas transactivation of genes is causing side effects.
Using conditional and function selective mutant mice for the GR we recently revealed that transrepression of genes by the GR is not sufficient to suppress inflammation in mouse models of arthritis *in vivo* (*PNAS* 2011 **108**: 19317). In contrast transactivation of anti-inflammatory genes by GR dimerization is absolutely required, since mice carrying a GR with disturbed dimerization interface do not respond to GCs to reduce inflammation in antigen-induced arthritis and K/BxN serum induced arthritis. Whereas in antigen-induced arthritis GR function in T cells is essential we surprisingly discovered that in K/BxN serum induced arthritis GR expression in non-hematopoietic cells suppress inflammation.

In contrast to suppression of inflammation, the induction of 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). Here mice with attenuated GR dimerization reacted completely normal in inhibition of bone formation and decrease of osteoblast differentiation. 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

**Endochrondral ossification, mesenchymal stem cell and Wnt pathway specific loci predict differential skeletal effects in high bone mass**

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Extreme high bone mass (HBM) may be monogenic (e.g. *LRP5* mutations) or polygenic, due to variants in the same genes determining bone mineral density (BMD) as found in the general population. We aimed to determine how variation in established BMD loci, in different functional pathways, explains the HBM phenotype.

241 unexplained HBM cases (lumbar spine(LS)1+total hip(TH) *Z*-scores $\geq$ +4.4) were recruited from 15 UK centres, by screening 335 115 DXA scans. Established *LRP5* mutations were excluded by Sanger sequencing ($n=6$). Infinium OmniExpress-12v1.0 genotypes were imputed to UK10K; 57 SNPs had certainty $>0.8$. Using Estrada's Femoral Neck(FN) or LS Betas, we calculated weighted genetic risk scores ($GRS^{FN}$/$GRS^{LS}$) for total ($n=57$), Wnt ($n=12$), OPG-RANK-RANKL ($n=3$), endochrondral ossification ($n=6$) and mesenchymal stem cell (MSC) differentiation ($n=4$) annotated loci.

A one SD increase in total $GRS^{FN}$ was associated with a 0.13 s.d. (−0.01, 0.27), $P=0.06$, increase in TH $BMD^{TH}$ and 0.13 (0.01, 0.25), $P=0.04$, increase in total body $BMD^{TB}$, explaining 1.6 and 3.2% phenotypic variance respectively however, $GRS^{LS}$ was independent of LS $BMD^{LS}$ (0.12 (−0.05, 0.29), $P=0.17$, $r^2=0.8\%$).

Wnt GRS was associated with a 0.15 s.d. (0.01, 0.29), $P=0.04$, increase in $BMD^{TH}$ ($r^2=2\%$), but was independent of $BMD^{TB}$ (0.03 (−0.08, 0.15), $P=0.57$, $r^2=0.3\%$) and $BMD^{LS}$ (−0.02 (−0.20, 0.15), $P=0.79$, $r^2=0\%$). Conversely, the endochrondral ossification GRS was independent of $BMD^{TH}$ (0.01 (−0.13, 0.15), $P=0.85$, $r^2=0\%$) and $BMD^{LS}$ (0.14 (−0.03, 0.31), $P=0.11$, $r^2=1.2\%$), but explained 2.3% of variance in $BMD^{TB}$ (0.11 (−0.01, 0.23), $P=0.08$). Whereas, MSC GRS was associated with a 0.19 s.d. (0.02, 0.37), $P=0.03$, $r^2=2.2\%$) increase in $BMD^{LS}$, but was independent of both $BMD^{TB}$ and $BMD^{TH}$ ($P>0.8$, $r^2=0\%$). All BMD sites were independent of OPG-RANK-RANKL GRS.

In conclusion, BMD in HBM appears driven by osteoblast rather than osteoclast pathways. A greater proportion of phenotypic variance in i) hip BMD is explained by wnt pathway loci, ii) total body BMD by endochrondral ossification loci and iii) lumbar spine BMD by mesenchymal stem cell differentiation loci, suggesting differential genetic regulation of individual skeletal sites.

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## LB2

**Bisphosphonates for osteoporosis and risk of breast cancer: misleading results from observational studies**

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Purpose
To test whether bisphosphonates for osteoporosis reduce the risk of breast cancer.
Background
Observational studies and meta-analyses find that women taking bisphosphonates have about a 1/3rd reduction in risk of breast cancer as soon as 1 year of treatment. This is plausible because bisphosphonates have *in vitro* anti-tumor activity and high doses of zoledronate reduce recurrence in women with breast cancer. However, observational studies may be confounded because low estradiol levels decrease risk of breast cancer and also decrease BMD, increase fracture risk, and leading to bisphosphonate treatment. Thus, the issue can only be tested with randomized trials.
Methods
We ascertained cases of breast cancer in two randomized trials of bisphosphonates. FIT randomized 6459 postmenopausal women aged 55–81 years to alendronate or placebo and 103 cases of invasive breast cancer occurred during 3.8 years. HORIZON-PFT randomized 7765 postmenopausal women aged 65–89 years with osteoporosis to 5 mg of zoledronate or placebo and 62 cases of invasive breast cancer occurred during 3 years.
Results
Alendronate did not reduce the risk of breast cancer: hazard ratio = 1.24 (95% CI, 0.84–1.83; $P=0.28$). Zoledronate also did not reduce the risk of breast cancer: HR = 1.15 (95% CI, 0.70 1.8; $P=0.59$). There was no evidence of reduction in risk with longer duration of use.
Conclusion
Alendronate and zoledronate for osteoporosis do not reduce the risk of breast cancer. Observational studies produced misleading answers perhaps because low levels of estradiol lead to both low risk of breast cancer and prescription of treatments for osteoporosis.

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## LB3

**Meta-analysis of randomised trials shows limited evidence to support the use of bisphosphonates for fracture prevention in osteogenesis imperfecta**

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Osteogenesis imperfecta refers to a group of inherited disorders characterised by increased bone fragility, low bone mass and fragility fractures. Most patients carry mutations in the genes that encode type I collagen or other proteins involved in its post-translational modification. Bisphosphonates are widely used in the treatment of osteogenesis imperfecta but clinical trials have been powered to detect effects on BMD rather than fracture. In order to gain better understanding of the effects of bisphosphonates on fracture in OI we conducted a meta-analysis of randomised placebo controlled trials in which fracture data had been reported. Relative risks (RR) and 95% CI were calculated under fixed and random effects models and heterogeneity assessed by the $I^2$ statistic We identified six eligible studies which had included 424 subjects with 721 patient-years of follow-up. Most had Sillence type I OI (59.9%) followed by type IV (22.1%) and type III (14.6%). In 3.3% of cases the type was unknown. The bisphosphonates used were oral risedronate, oral alendronate, oral olpadronate and intravenous pamidronate. Only one study was conducted in adults with OI. The proportion of patients who experienced a fracture was not significantly reduced by bisphosphonate therapy (RR 0.83, 95% CI (0.69–1.00), $P=0.052$) with no heterogeneity between studies ($I^2=0$). Total fracture numbers were reduced by bisphosphonate treatment (RR 0.71 (0.52–0.97) $P=0.031$, but with considerable heterogeneity ($I^2=39\%$). This was explained by one study in which a substantial effect on fracture numbers was driven by a small number of placebo-treated patients who suffered multiple fractures. When this study was excluded, bisphosphonates had no effect on fracture number (RR 0.79 (0.62–1.02), $P=0.07$, $I^2=0\%$). We conclude that there is limited evidence to support the use of bisphosphonates for fracture prevention in OI and that further adequately powered trials with a fracture endpoint are urgently needed.

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## LB4

**History of cardiovascular events and risk factors for cardiovascular events among osteoporotic patients initiating strontium ranelate in the UK**

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Objective
To estimate the proportion of patients who experienced cardiovascular (CV) events or were at risk for CV events prior to initiation of strontium ranelate among osteoporotic (OP) patients.
Methods
This was a retrospective database analysis using the Clinical Practice Research Datalink (CPRD) database. Patients were included if they had $\geq 1$ prescription (Rx) of strontium from 9/1/2008 to 8/31/2013, were aged $\geq 50$ years as of the index date, and had $\geq 1$ year of medical records pre-index. A CV event was identified any time pre-index and included uncontrolled hypertension, or diagnosis (Dx) of ischemic heart disease, peripheral arterial diseases and cerebrovascular disease. CV risk factors assessed included i) Dx or Rx for type 2 diabetes mellitus or hypertension any time pre-index; ii) Dx or Rx of

hyperlipidemia in 12 months pre-index; or iii) Dx of obesity or BMI $\geq 30$ kg/m$^2$ in the 12 months pre-index.

Results

7474 patients (patients) were included: 90.4% were female with an average age of 76.5 years (s.d. = 10.3), 63.5% had record of OP Dx, and 84.5% used bisphosphonate (BIS) (27.2% used $\geq 2$ different BIS) or non-BIS medication prior to strontium initiation. 23.6% of patients (20.8% of patients with OP Dx) experienced $\geq 1$ CV events prior to strontium initiation; the rate was lower among female patients than that of male patients (22.4 vs 35.3% $P < 0.01$). 45.9% had risk factors for CV events (without CV event history).

Conclusion

More than one fifth of UK OP patients who used strontium had CV event history, and one half of them had CV risk factors and no previous CV event prior to strontium initiation. In response to the EMA (European Medicines Agency) recommendation related to strontium use for patients with history of the included CV events, OP treatment among this cohort may be re-considered.

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## LB5

### Early exposure to extra vitamin D from food fortification and bone fractures in adolescents: results from the D-tect study

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Background

Improving the general population's vitamin D status through food fortification is part of an ongoing debate. Vitamin D status during pregnancy may influence the long-term bone health of offspring; yet conflicting results have been reported and none of the studies have examined paediatric fracture as outcome.

Method

The influence of extra vitamin D exposure during prenatal life and risk of fracture during adolescence was determined by comparing subjects born before and after the termination of a mandatory vitamin D fortification program, applied in Denmark from 1961 to 1985, the effect of which has never been evaluated. For subjects born in 1983–1988, civil registration numbers were linked to the National Patient Registry for incident and recurrent fractures at ages 12–18 years. Semi-parametric multiplicative models for mean functions were used to assess the association between vitamin D exposure and occurrence of fractures, accounting for season of birth.

Results

A total of 103.569 exposed and 114.210 unexposed subjects were identified. Among those 11.693 exposed and 11.427 unexposed subjects sustained fractures. Within each season of birth, the wrist/forearm and ankle fracture rates in the exposed individuals were significantly greater than the rate for the unexposed group, e.g. the estimated rate ratio for wrist/forearm fracture comparing exposed to unexposed individuals born November–January: RR = 1.20; 95% CI: 1.12, 1.28. There was no significant association between exposure and the rate of clavicle fractures (Table 1).

**Table 1** Fracture rate per 1000 person-years by gender and exposure status.

| | Girls | | Boys | |
|---|---|---|---|---|
| Fracture Type | D+ | D− | D+ | D− |
| Clavicle | 0.9 | 0.9 | 2.5 | 2.5 |
| Wrist/forearm | 10.0 | 8.8 | 16.4 | 15.0 |
| Ankle | 2.5 | 2.1 | 3.3 | 2.9 |
| Total | 13.4 | 11.8 | 22.2 | 20.4 |

Conclusion

Among adolescents exposure to extra vitamin D from food fortification during prenatal life, seems related to an increased risk of fractures, in particular wrist/forearm and ankle.

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