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Woodlands
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Opening Ceremony
ECTS 50th Anniversary Symposium: 50 years of research in bone and mineral metabolism

OPC1.1

Abstract unavailable.

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OPC1.2

50 Years of bone imaging
Harry Genant
University of California, San Francisco, California, USA.

Considerable progress has been made over the past half century in the development of imaging methods for assessing the skeleton noninvasively or nondestructively, so that osteoporosis can be detected early, its progression and response to therapy monitored, and the risk of fracture determined. Clinicians and researchers can now evaluate the peripheral, central, or entire skeleton as well as the trabecular, cortical, and endosteal envelopes with a high degree of accuracy and precision, and they can reliably estimate bone strength and the propensity to fracture. The purposes of this presentation are to review the historical evolution of the methods for bone imaging and bone densitometry, and to assess their current capabilities.

The numerous methods for noninvasive assessment of the skeleton began their evolution in the 1960s and 1970s, with simple X-ray-based radiogrammetry, high-resolution fine-detail radiography, radiographic absorptiometry, and single-photon absorptiometry (SPA), all emerging as research tools, with only the latter becoming a clinical tool available in Europe and North America. Supporting and driving these methodological advances were the growing interests in postmenopausal osteoporosis and in bone loss during space flight, as evidenced by the first NIH, NASA and University sponsored bone density workshops, and the initiation of the biennial series of International Bone Density Workshops (IBDW). During this early period the nascent foundations were laid for what has evolved as our most eminent bone mineral societies, namely, the ECTS, IBMS, ASBMR, and IOF.

During the 1980s and 1990s, the medical imaging technologies exploded with the advent of computed tomography (CT) and then a decade later, with magnetic resonance imaging (MRI), each opening new perspectives with in vivo three-dimensional sectioning of the body. Quantitative computed tomography (QCT) was extensively investigated both for central and peripheral (pQCT) skeletal imaging. The early isotope-based pQCT systems were converted to X-ray based systems in the late 1980s. Similarly, during this period, the SPA technique was advanced to dual photon absorptiometry (DPA), permitting quantitative imaging of the central skeleton, mainly the spine and hip. At the same time pharmaceutical interest expanded in the area of osteoporosis therapeutics with exploration of a variety of estrogen and HRT regimens and the development of first and later generation bisphosphonates. Dual-X-ray absorptiometry (DXA) represented a major technological advance, replacing isotope-based DPA, and improving the speed, precision and spatial resolution, thus thrusting it into routine clinical practice.

During the most recent 20 years, the DXA technology has further advanced from pencil-beam, single-detector to fan-beam multi-detector array, further improving performance. Similarly, CT has evolved dramatically, to a high-resolution, fan-beam, multi-detector, spiral-scanning mode, greatly enhancing speed, coverage and efficiency. Special purpose advanced Micro-CT systems have also been developed, these for imaging small animals and bone specimens, or for in vivo imaging of the quasi-micro-structure of the distal radius and tibia. Simultaneously over this time, MRI technology has continued its remarkable evolution, through many configurations, becoming today the most diverse and powerful medical imaging system. For both MRI and CT, the equipment and hardware advances have been accompanied by impressive developments in computer sciences and image processing, which have facilitated applications for analysis of skeletal macro and microscopic structure, extending well beyond simple BMD measures.

In summary, the past 50 years has witnessed tremendous progress in the development and application of bone imaging and bone densitometry techniques, currently providing a vast array of exquisite research and clinical tools to examine and explore the depths and boundaries of the skeleton in health and disease.

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OPC1.3

Abstract unavailable.

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Clinical Update
Osteoporosis in premenopausal women

Erik Fink Eriksen
Oslo, Norway.

Osteoporosis in premenopausal women is dominated by secondary causes, among which anorexia nervosa, the female athletic triad, celiac disease, and glucocorticoid-induced osteoporosis (GIO) constitute the most frequent conditions. Stress fractures of the lower extremities and low energy fractures of the ribs, are also frequent. Various genetic causes like osteogenesis imperfecta tarda are probably underdiagnosed and various inflammatory conditions also play a role. A rare, but often severe form is pregnancy-associated osteoporosis with multiple fractures of the spine and transient osteoporosis of the hip, the causes of which are still unknown. A large group of young women are considered for treatment due to osteopenia, but due to the lower risk of fracture and high NNT in this population, most guidelines agree that specific osteoporosis treatment is only indicated if low energy fractures are demonstrable or risk estimates (e.g. using FRAX) show very high probability of fracture over the next 10 years. Also in young males secondary causes mainly GIO alcohol abuse, hypogonadism, celiac disease, and malignancy dominate. A subgroup of males, however, show no secondary causes and are classified as idiopathic osteoporosis. Usually they present with very low bone mass and multiple spine fractures. Histomorphometry analysis usually shows a low turnover osteoporosis. The etiology is, however, still poorly defined. A subgroup of males also present with multiple stress ad rib fractures. The treatment options in these age groups are mainly sex hormone replacement and bisphosphonates. I.e. bisphosphonates constitute an attractive option, because they can often be given every second year, thus limiting exposure during long-term treatment. Anabolic therapy with PTH should be reserved for severe cases but a more liberal prescription practice in younger people is probably warranted. DOI: 10.1530/boneabs.1.CU1.1

Management of osteoporosis in pre-menopausal women

Jennifer Walsh
Academic Unit of Bone Metabolism, Sheffield, UK.

Low bone density in younger women is often due to underlying conditions such as eating disorders, premature ovarian failure or glucocorticoid treatment. It may also be due to genetically low peak bone mass. In general, absolute fracture risk in young women is low, even in the context of low bone density. Management should begin with treatment of underlying causes where possible, and lifestyle modification where appropriate. The evidence base for the pharmacological treatment of young women is quite limited. In women who undergo early menopause, many clinicians would recommend oestrogen replacement until the usual age of menopause, but there is uncertainty as to the best form of oestrogen treatment. There have been several clinical trials in anorexia nervosa. Combined treatment approaches with transdermal or oral oestrogen and DHEAS or IGF1 may be effective and are attractive because they aim to decrease bone resorption and increase bone formation. In glucocorticoid-induced osteoporosis there is some evidence for the use of bisphosphonates or teriparatide in young women. It is important to consider potential pregnancies when treating women of child-bearing age, and there are case reports of congenital malformations and neonatal hypocalcaemia in association with bisphosphonates during pregnancy. Bisphosphonates which may have a quicker offset of action may be preferable in young women. Patients should be informed if use of osteoporosis drugs is outside the licence. In general, pharmacological treatment of osteoporosis in young women should be reserved for women at high current fracture risk. DOI: 10.1530/boneabs.1.CU1.2

Genetic determinants of serum sex steroids and bone health in males

Claes Ohlsson
Institute of Medicine, Gothenburg University, Gothenburg, Sweden.

Osteoporosis in men causes significant morbidity and mortality. Considerable progress has been made in understanding the pathophysiology and management of osteoporosis, though it remains under-diagnosed and under-treated, particularly in men. Osteoporosis is widely considered to be more prevalent in women, even though at least one-third of all osteoporotic fractures occur in men. A major difference between the male and the female skeleton is the larger bone dimensions in the males and an important determinant of this sexual dimorphism of the skeleton is the differential sex steroid exposure during lifetime in males and females. Studies in twins indicate that there is a strong heritability of serum sex steroids as well of computed tomography (CT)-analyzed bone parameters such as cortical bone dimensions and volumetric (+) BMDs. The present lecture will summarize recent genome-wide association studies (GWAS) aiming to characterize the genetic determinants of serum sex steroids and bone health in men. A large scale testosterone GWAS identified a polymorphism near FAM9B on the X chromosome that was strongly associated with serum testosterone concentrations. Interestingly, this testosterone-associated locus was also strongly associated with BMD in men but not women. Recent large-scale GWAS of CT-analyzed bone parameters demonstrated that the genetic variants associated with cortical bone dimensions as well as of cortical and trabecular vBMDs differed, underscoring the complexity of the genetics of bone parameters. Cortical bone thickness was mainly associated with a genetic variant in the WNT16 locus. The cortical vBMD and cortical porosity were mainly associated with a genetic variant in the RANKL locus while the trabecular vBMD was associated with a genetic variant in the FMN2/GREM2 locus. The effect sizes for some of the identified genetic variants differed significantly between men and women, demonstrating that the genetic determinants of male and female bone health, at least partly, differ. DOI: 10.1530/boneabs.1.CU1.3

Medical management of osteoporosis in men

Steven Boonen
Leuven University, Leuven, Belgium.

Awareness of osteoporosis in men is improving, although it remains under-diagnosed and under-treated. Empirical data in men display similarities with data acquired in women, despite pathophysiological differences, which may not be clinically relevant. Men should receive treatment at a similar 10 years fracture probability as in women. Bisphosphonates inhibit osteoclastic bone resorption and are the most widely used drugs in male osteoporosis. The treatment response to oral bisphosphonates in male osteoporosis is similar to that observed in postmenopausal osteoporosis, in terms of bone density and bone remodelling. To date, conclusive anti-fracture evidence with alendronate and risedronate is unavailable in men, but fracture reductions are very consistent. With i.v. zoledronic acid, recent fracture endpoint data in osteoporotic men indicate that zoledronic acid anti-fracture efficacy in men mirrored that observed in women. Denosumab, a monoclonal antibody that binds and neutralises the activity of human receptor activator of nuclear factor-kB ligand (RANKL), a key osteoclast cytokine, has been shown to increase bone density and reduce fractures in men with prostate cancer on hormone ablation therapy. The efficacy and safety of denosumab in men with low bone mass at risk of fracture were recently confirmed to be similar to the effects in postmenopausal women with osteoporosis. In line with these findings with antiresorbatives, teriparatide and strontium ranelate studies concluded that the changes in biochemical markers and bone density in men were essentially the same as in women. It would seem therefore that the approaches developed to treat and identify women at high risk (e.g. the FRAX approach) is equally useful in m. DOI: 10.1530/boneabs.1.CU1.4
CU1.5

Glucocorticoid-Induced Osteoporosis
Cyrus Cooper
MRC Lifecourse Epidemiology Unit, University of Southampton; and Institute of Musculoskeletal Science, University of Oxford, UK.

The ECTS and IOF have recently constructed a framework for the development of national guidelines for the management of glucocorticoid-induced osteoporosis in men and women aged 18 years and over in whom oral glucocorticoid therapy is considered for three months or longer. These review the epidemiology of GIO; assessment of risk utilises a fracture probability-based approach and intervention thresholds are based on 10 year probabilities using FRAX. National guidelines derived from this resource need to be tailored within the national healthcare framework of each country.

Oral glucocorticoids are prescribed for a wide variety of medical disorders, most commonly musculoskeletal disease and obstructive pulmonary disease. Up to 4.6% of postmenopausal women are reported as currently taking oral glucocorticoids, and fracture risk increases during the first three to six months of glucocorticoid therapy. An increase in fracture risk occurs with low doses and rises further with increasing daily dose: the greatest increase in risk is seen for vertebral fracture where patients taking prednisolone >7.5 mg daily have a relative risk of 5.18 (95% CI 4.25–6.31).

The management of GIO in premenopausal women and men is complicated by a dearth of evidence addressing the epidemiology, risk assessment, and therapeutic interventions. Premenopausal women and younger men have a lower risk of fracture than older individuals, although there is evidence that glucocorticoid-treated premenopausal women fracture at higher BMD than their postmenopausal counterparts. Data on the effects of pharmacological interventions in this population are sparse, particularly with regard to fracture risk. In large, randomised, controlled trials in which subsets of premenopausal women and men were studied, therapy with alendronate, risedronate and etidronate has been shown to result in larger increases in BMD than alendronate in premenopausal women and men with GIO.

Despite the lack of evidence for fracture reduction in glucocorticoid-treated premenopausal women and younger men, bone protective therapy may be appropriate in some cases, particularly among patients treated with high doses of glucocorticoids and in those with a previous history of fracture. The long term use of bisphosphonates and the potential for side effects remains a concern; caution is advised to women of child bearing age as bisphosphonates cross the placenta and of bisphosphonates and the potential for side effects remains a concern; caution is advised.

CU2.2

Osteoporosis and fragility fractures in rheumatoid arthritis
Glenn Haugeberg1,2
1Hospital of Southern Norway Trust, Kristiansand, Norway; 2NTNU University, Trondheim, Norway.

In rheumatoid arthritis (RA) bone is affected by erosions, periarticular- and generalised osteoporosis, the latter leading to increased risk of both vertebral and non-vertebral fractures. A twofold increase in osteoporosis has been found in the RA population compared with healthy controls. In the RA population the relative risk of hip fracture has been reported to be up to five times higher and vertebral fractures up to three times higher than controls. Osteoporotic fractures are not only associated with increased morbidity and impaired quality of life but also with increased mortality.

Generalised osteoporosis in RA is frequently associated with simultaneous presence of primary osteoporosis risk factors and the disease related risk factors: inflammation, immobilisation, and treatment with corticosteroids (CS). In the WHO fracture risk assessment tool (FRAX) RA is also recognised as an independent risk factor for future fractures.

Previously, the three bone manifestations were thought to be caused by different mechanisms. However, recent studies suggest that both bone erosion and osteoporosis (peri-articular as well as generalised osteoporosis) are mediated by the cellular action of osteoclasts. Tumor necrosis factor α (TNFα), interleukin 1 (IL1) and IL6, which play a pivotal role in the pathogenesis of synovitis in RA, are also found to be regulators of osteoclastic bone resorption, mediated through interactions with the receptor activator of NF-κB ligand (RANKL). This common cellular osteclast pathway, being a direct consequence of the inflammatory disease process, invites opportunities for both new treatment strategies and new ways of assessing patients with RA which includes both aggressive anti-inflammatory treatment and the use of potent osteclast inhibitors, e.g. denosumab and bisphosphonates.

Doctors should be aware of this increased risk of osteoporosis and fragility fracture in RA patients and strengthen their effort in reducing fracture risk.

DOI: 10.1530/boneabs.1.CU2.2

CU2.3

Osteoporosis in SLE
Irene Bultink
VU University Medical Center, Amsterdam, The Netherlands.

Systemic lupus erythematosus (SLE) is a chronic multisystem autoimmune disease that usually affects women during the childbearing ages. The disease can affect any organ system and varies in its clinical manifestations and severity between individuals. The disease course is characterized by relapses and remissions.

Because survival of SLE patients has improved dramatically over the last decades, attention is now more focused on complications of the disease and/or its treatment, that contribute to increased morbidity and mortality.

Osteoporosis and fractures are important disease complications in patients with SLE. In recent studies, a high frequency of low bone mineral density and both peripheral and vertebral fractures has been demonstrated in SLE patients. The incidence of symptomatic fractures is increased five fold in women with SLE. In addition, prevalent vertebral fractures are present in 20–26% of these relatively young patients1.

The etiology of bone loss in SLE is supposed to be multifactorial, involving traditional osteoporosis risk factors, inflammation, metabolic factors, hormonal factors, and medication-induced adverse effects.

A recent 6 years follow-up study in Dutch SLE patients revealed, that low 25-hydroxyvitamin D serum levels at baseline, reduction of BMI and baseline use of antimalarial drugs were associated with bone loss2. In addition, a dose-dependent relationship between glucocorticoid use and spine bone loss was demonstrated in this study. The results of this study have implications for daily clinical practice, because ultraviolet light intolerance (and subsequently low 25-hydroxyvitamin D levels) is highly frequent in SLE patients, antimalarials are ‘anchor drugs’ for the treatment of SLE, and the majority of SLE patients is on chronic glucocorticoid treatment.

Importantly, several risk factors associated with osteoporosis and fractures in SLE are modifiable by lifestyle measures or medication.


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CU1.6

Abstract unavailable.

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Clinical Update 2

CU2.1

Abstract unavailable.

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CU2.4
Osteoporosis in ankylosing spondylitis
Christian Roux
Hôpital Cochin, Service de Rhumatologie, Paris, France.

Ankylosing spondylitis is a chronic inflammatory rheumatic disease, characterized by axial pain and osteoproliferation, leading to painful rigidity of the spine and disability. In contrast with this bone formation, bone loss is an early event in this disease, and an increased vertebral fracture risk (but not non-vertebral fracture risk) has been reported in these patients.

Prospective studies have shown that potent anti-inflammatory drugs, such as anti-TNF therapies, can prevent bone loss and low bone density without effect on bone proliferation, i.e. without evidence of prevention of ossification of ligamentous structures. Ankylosing spondylitis is a relevant model for assessing the effect of inflammation on bone. Data suggest that low sclerostin levels may participate to the structural change. Recent evidence of the presence of enthesis-resident T cells which can be activated by IL-23 and promote lesions that are characteristic of ankylosing spondylitis can open new therapeutic pathways.

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CU2.5
The effect of anti-inflammatory treatments (except GC) on bone
Willem Lems
VU University Medical Centre, Amsterdam, The Netherlands.

Inflammatory joint diseases like rheumatoid arthritis (RA), as well as other rheumatic conditions such as ankylosing spondylitis and systemic lupus erythematosus, comprise a heterogeneous group of joint disorders that are all associated with extra-articular side effects, including bone involvement. Disease activity, immobility and treatment with (high dose) glucocorticoids are the main factors that increase the risk of osteoporotic fractures, on top of the background fracture risk based on, amongst others, age, BMI, and gender (Bultink 2012).

Although systemic osteoporosis and an elevated vertebral and nonvertebral fracture rate can be found in RA, the disease is mainly characterized by the presence of inflammatory synovitis and pannus, leading to destruction of joint cartilage and (local) bone loss. In general, both the generalized and the local bone loss are larger in patients with active RA. Adequate control of disease activity, for instance with TNF-blocking agents or other biologics prevents, bone loss.

In RA patients the effect of TNF blockade on bone has been studied by Vis et al., who showed in a cohort of 102 RA patients (median age 53 years and median disease duration 8 years) that treatment with infliximab in combination with a stable dosage of MTX led to a statistically significant decrease ($P<0.05$) of 20% in serum CTX levels (bone resorption), whereas PINP levels (bone formation) were increased slightly at 46 weeks. RANKL levels also significantly decreased by 33% ($P<0.001$) in this study, while OPG more or less remained stable, leading to an improvement of the RANKL/OPG ratio. The changes in markers of bone resorption paralleled the decrease in disease activity (Vis 2006)

The favorable changes in BMD were also resulted in the absence of the usually occurring bone loss at the spine and hips in RA during treatment with infliximab and MTX, which was later confirmed in a study with adalimumab (Wijbrants 2009). Recently, favorable changes in bone markers in treatment of RA were also observed in RA patients treated with rituximab and tocilizumab. All these data point in the same direction: with biologics both local and generalized bone loss can be prevented in patients with active RA.

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CU2.6
Abstract unavailable.

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AHP1.1

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AHP1.2

Abstract unavailable.

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AHP1.3

Treatment compliance in osteoporosis
Adolfo Diez-Perez
Department of Internal Medicine, Hospital del Mar, Barcelona, Spain.

Compliance with prescribed drugs is poor in most chronic conditions and osteoporosis is no exception. Compliance integrates the concepts adherence (how much drug is taken) and persistence (for how long) and also if the patient follow the instructions for a correct use of the medication. Between 50 and 75% of patients initiating antosteoporosis drugs are not taken the treatment 1 year later. Obviously, this problem significantly decreases the effect of drugs. A smaller increase in BMD and less reduction in fracture risk are the immediate consequences. The burden of wasting medicines that will not reach the therapeutic goals is also significant and it has been estimated that doubles the cost of one quality-adjusted year of life obtained with treatments. The reasons for stopping medications are numerous and not well explored. Side effects is one of the most common. Fears or beliefs about the drugs, large number of concomitant medications, lack of awareness of the consequences of the osteoporosis, low priority of the disease among health problems or the debate about osteoporosis as an ‘invented disease’ are other reasons invoked. In this respect, recent reports on safety problems associated to the use of antosteoporosis medications may be behind the decrease in the use of these drugs in the EU, in spite of the fact that the at-risk population is growing.

A number of strategies have been used in an attempt of improving adherence. Behavioural interventions, interactions between the doctor or nurse with the patient, leaflets, reminders by phone or e-mail, use of laboratory parameters or educational programs have been only partially successful in improving the current situation. Longer intervals between doses are also another widespread approach, with medications used weekly, monthly, every 6 or every 12 months. The problem then can be that one missed dose is associated with a longer period without therapeutic effect.

In summary we are still far from a fully successful strategy. In the meantime, the communication with the patient, with a detailed and clear explanation, addressing their doubts, concerns and uncertainties and explaining the treatment and their objectives is possibly the best system to mitigate the problem.

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AHP1.4

Abstract unavailable.

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Main Symposium
**Developmental origins of metabolic bone disease**

**$1.1$**

**Developmental epigenetics and the intrauterine origins of chronic disease**

Keith Godfrey$^{1,2}$

$^1$University of Southampton, Southampton, UK; $^2$NIHR Southampton BRC, Southampton, UK.

Experimental studies in animals indicate that particular maternal exposures during pregnancy can have specific effects on body composition in the offspring, with long-term implications for subsequent metabolic phenotype and cardiovascular risk. In animals the environment during early life induces altered phenotypes in ways which are influenced or mediated by epigenetic mechanisms, but until recently there has been little direct evidence in humans and understanding of which developmental influences can alter body composition in the offspring is incomplete. To define maternal exposures associated with offspring adiposity and elucidate underlying epigenetic mechanisms we have undertaken follow-up studies within the Southampton Women’s Survey (SWS), in which the pre-pregnant characteristics of a large group of women were assessed at recruitment; 3160 of these women have subsequently become pregnant. Body composition by dual energy X-ray absorptiometry is assessed in samples of the offspring at birth, and at 4, 6, and 8 years; we have shown greater adiposity in the offspring is associated with higher maternal adiposity, poor quality maternal diets in pregnancy, low maternal vitamin D status, excess gestational weight gain, and short duration of breastfeeding. Using Sequenom MassARRAY we have found that greater methylation of a single CpG within the RXRA promoter measured in umbilical cord was strongly associated with greater adiposity in later childhood. Perinatal measurements of DNA methylation explained >25% of the variance in childhood adiposity. Findings were replicated in a second independent cohort and preliminary data link perinatal epigenetic marks with the child’s bone mineral accrual. Our data provide the first human evidence that epigenetic processes in non-imprinted genes have an important role in early growth and later body composition. Understanding the associations with maternal exposures and direct mechanisms of adiposity provides insights into the aetiology of childhood body composition, with implications for the design of intervention studies.

Reference


DOI: 10.1530/boneabs.1.S1.1

**$1.2$**

**Maternal environment and intra-uterine skeletal development**

Muhammad Kassim Javaid

Oxford University Hospitals Trust, University of Oxford, Oxford, UK.

Fragility fractures including hip fracture are a significant global burden. There is a growing body of evidence that the early environment influences an individual’s risk of fracture. Evidence from longitudinal studies have demonstrated the relationship between measures of body size in early life with later bone mass and risk of fragility fracture. These observations have been extended by parent/offspring cohorts with detailed examination of the maternal environment and specific effects on foetal and neonatal bone size and post natal trajectories. The mechanism for persisting effects on an individual’s bone phenotype are likely to involve epigenetic changes of key regulators of bone mass. Current work has focused on CpG methylation of the vitamin D/RXR and eNOS pathways and offer potential insights as well as surrogate outcomes and therapeutic targets for future studies.

DOI: 10.1530/boneabs.1.S1.2

**Muscles and bone**

**$2.1$**

**Skeletal muscle loss: sarcopenia and inactivity**

Anne McArdle

Liverpool, UK.

Age-related loss of skeletal muscle mass and function is a major cause of loss of mobility, increased frailty and falls in the elderly and impacts profoundly on the quality of life of older people. Modified reactive oxygen species (ROS) generation has been implicated in the mechanisms by which muscle function is lost with increasing age. ROS are increased in skeletal muscles of adult mice following a period of isometric contractions and this is associated with adaptive increases in transcription of a number of cytoprotective proteins in muscle including the heat shock proteins (HSPs). In contrast ROS generation and the ability to activate this adaptive stress response are modified in skeletal muscle of old mice, which also demonstrate a chronic activation of NFkB, associated with a pro-inflammatory environment. Transgenic studies have demonstrated that this blunted adaptive response plays a key role in development of age-related functional deficits. Lifelong overexpression of cytoprotective HSPs results in improved muscle function and a reduction in the accumulation of markers of oxidative stress in muscles of old mice. Studies have demonstrated that mice lacking Cu,Zn superoxide dismutase showed an accelerated loss of skeletal muscle and bone mass and function and examination of adaptive responses in muscles of adult Sod1$^{-/-}$ mice show aberrant DNA binding activity of NFkB similar to that observed in muscles of old WT mice. These data demonstrate a role for aberrant ROS generation in age-related loss of muscle mass and function, that the development of age-related muscle weakness and atrophy are not inevitable and strengthen the hypothesis of the involvement of failed adaptive responses in the development of these deficits.

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**$2.2$**

Abstract unavailable.

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**Extreme bone phenotypes**

**$3.1$**

**Diagnosis and clinical management of genetic skeletal disorders**

Yasemin Alanay

Pediatric Genetics Unit, Department of Pediatrics, Acibadem University, Istanbul, Turkey.

Today, there are more than 450 well-characterized genetic skeletal disorders classified primarily on the basis of clinical, radiographic, and molecular criteria. Although individually rare, the overall birth incidence is estimated to be 1/5000 live births. Half a century ago, in the 1960s, individuals with disproportionate short stature were diagnosed either with achondroplasia (short-limbed dwarfism) or Morquio syndrome (short-trunked dwarfism). In time, delineation of numerous entities not fitting these two ‘disorders’ led experts to come up with a systematic approach. The ‘International Nomenclature of Constitutional Diseases of Bone’ group first published in 1970 and has intermittently classified these disorders (1970–1977–1983–1992–2001–2005–2009). In the 1970s the categories were purely clinical and descriptive. This later evolved into a combination of clinical, radiological, and molecular knowledge as the pathogenetic mechanisms of various entities have been revealed. In the latest 2010 revision of the Nosology and Classification of Genetic Skeletal Disorders, an increase from 372 to 456 disorders in the four years since the classification was last revisited in 2007. Of these conditions, 316 are associated with one or more of 226 different genes. This increase reflects the continuing delineation of unique phenotypes among short stature conditions, which in aggregate represent about 5% of children with birth defects.

In daily practice however, clinicians dealing with patients with short stature may be confused with the molecular listings. It is therefore important to remember that an accurate diagnosis of a genetic disorder of skeleton is still based on detailed evaluation of clinical and radiographic (as well as chondro-osseous) findings. This lecture aims to outline the diagnostic approach to disproportionate short stature with emphasis dysmorphic features and radiological findings.

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**S3.2**

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**Energy metabolism and bone**  
**S4.1**

Abstract unavailable.

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**S4.2**

*Sweet bones: the effect of diabetes on bone*

Jochen Seufert  
University Hospital of Freiburg, Freiburg, Germany.

Diabetes mellitus is the most common metabolic disease affecting more than 300 million people worldwide with a constantly growing prevalence mainly due to an increase of obesity associated type 2 diabetes. Serious macrovascular (myocardial infarction, peripheral artery disease, and stroke) and microvascular (retinopathy and nephropathy) complications account for substantial morbidity and mortality in diabetes patients. Somewhat underestimated chronic complications are the negative effects of diabetes on bone health. Typical well known skeletal complications of poorly controlled diabetes mellitus include the diabetic foot syndrome and Charcot neuroarthropathy. However diabetes mellitus is also associated with an increased risk of osteoporosis and fragility fractures. The mechanisms underlying low bone strength in diabetes mellitus are not well understood. While high glucose itself has been reported to induce premature senescence in mesenchymal stem cells (MSC), we have demonstrated that glucose may also positively affect proliferation and differentiation of MSC. Type 1 diabetes mellitus (T1DM) affects the skeleton more severely than type 2 diabetes mellitus (T2DM), probably because of the lack of the bone anabolic actions of insulin. Also, diabetic nephropathy and reduced mobility may contribute to diabetic bone disease. It is important to note that a normal or even high bone mass in diabetic patients may not protect against fractures, because usually bone quality is impaired. In T2DM, oral thiazolidinedione treatment for hyperglycemia has further been associated with an elevated fracture risk. A physically active lifestyle and calcium and vitamin D repletion are effective as a therapeutical basis for elevated fracture risk in patients with T1DM or T2DM. Taking into account BMD and other risk factors can help to identify patients who require more intense pharmaceutical therapy that should be individually tailored. All osteoporosis medications are effective in patients with diabetes mellitus. Increased awareness of osteoporosis associated elevated fracture risk is needed in patients with diabetes mellitus.

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**Uncoupling of resorption and formation**  
**S5.1**

Abstract unavailable.

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**S5.2**

Abstract unavailable.

DOI: 10.1530/boneabs.1.S5.2
Clinical Debate
D1.1
For the motion (ECTS)
John Campbell
Otago University, Otago, New Zealand.

The incidence of hip fractures is declining in later cohorts of older people but, if the cohort effect is controlled for, the period effect shows a steady increase in incidence. This is almost certainly because we are seeing the survival of an increasingly frail group of older people with comorbidities. The great majority of hip fractures result from falls. There is strong research evidence that falls can be prevented. Proven strength and balance programmes reduce the rate of falls by around a third in community at-risk populations. The strength and balance retraining also improves cardiorespiratory function and cognition. Other proven fall prevention strategies include reduction of psychotropic medications, home modification, correction of visual problems, attention to foot problems, and multifactorial interventions. Prevention of falls through strength and balance programmes has advantages in addition to injury prevention. The programmes increase confidence, outside social activity and reduce institutional admission. Although it is certainly important to treat osteoporosis in older people to help prevent fractures there are even more reasons to prevent falls. Treatment of the whole person requires a programme to prevent falls, increase confidence in activities, maintain independence in daily living activities, and encourage social interaction. Proven programmes to improve strength and balance have been shown to have these multiple benefits and are being promoted and funded internationally.

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D1.2

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Workshops
Fat and bone

W1.1

Obesity, bariatric surgery and bone
Nuria Guanabens
Hospital Clinic, University of Barcelona, Barcelona, Spain.

For many years obesity has been considered to be protective against fragility fractures, since low BMI contributes to fracture risk. However, recent studies indicate that obese women represent a subset of patients with low-trauma fractures. In fact, obesity increases the risk of fracture at specific sites such as ankle, upper and lower leg and predictably, proximal humerus. By contrast, wrist, hip and pelvis fracture rates are lower in obese women. Interestingly, the majority of fractures occur in spite of a very low rate of osteoporosis by DXA measurements, although obese women who sustaın fractures usually have lower BMD than those without fractures. The increased risk of falling, the different patterns of falls and the higher impact of the fall due to the high body weight may be related to this site-dependent increased fracture risk. Conversely, greater soft tissue padding may reduce skeletal trauma protecting against fractures in well-padded central body sites. The effects of fat on bone may differ according to its distribution. Thus, high visceral adipose tissue is detrimental to bone, unlike subcutaneous fat, reasonably because of lower levels of leptin and higher levels of adiponectin and pro-inflammatory cytokines. In addition, visceral fat is associated with decreased GH and testosterone in males, with deleterious effects on microarchitecture. Decreased levels of vitamin D and high levels of PTH contribute to the picture of bone disease in obesity.

Bariatric surgery, which includes restrictive, malabsorptive and combined procedures, is the most effective route to weight loss in morbid obesity. Bariatric surgery has been linked to a reduction of BMD, without a significant effect on fracture risk for the first few years after surgery. Of interest, frequent nutritional and metabolic deficiencies have been observed, particularly in malabsorptive procedures, including calcium and vitamin D deficiency.

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W1.2

Anorexia nervosa and bone
Madhusmita Misra
Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, USA.

Anorexia nervosa (AN) occurs in 0.2–1% of adolescent girls and is characterized by physiological and adaptive changes in the various endocrine axes. Changes also occur in many hormones secreted or regulated by fat (a reflection of energy stores) that can impact bone. The physiological changes observed in various endocrine axes in AN in turn cause impaired bone accrual rates, low bone density (associated with increased marrow fat) and impaired bone microarchitecture. This raises concerns regarding attainment of peak bone mass, an important determinant of bone health and fracture risk in later life. Weight and menses recovery are associated with some improvement in bone accrual, but residual deficits persist. Therapeutic strategies to improve bone accrual in AN are limited, and include physiologic estrogen replacement in adolescents, and use of bisphosphonates in adults.

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W1.3

Effects of fat on bone: location and age matter
Jennifer Walsh
Academic Unit of Bone Metabolism, Sheffield, UK.

The epidemiological evidence is clear that higher body weight is protective against most fractures in adults. However, the same may not be true in children. Understanding the mechanisms of interaction of fat and bone may give useful insights into underlying physiology for prevention of fracture. In general, higher body weight in adults is associated with higher bone mineral density and is protective against fracture, but may be associated with an increased risk of some fractures. Possible mechanisms for higher bone density include mechanostat response to increased loading and increased oestrogen production by adipocyte aromatase. Increased soft tissue padding may also contribute to reduced fracture risk, particularly at the hip.

In children, obesity may be associated with impaired bone accrual and obese children with fracture have a bone mass deficit relative to their body size and lean mass. It has been increasingly recognised that bone interacts with other organs and tissues (such as fat, the gastrointestinal tract and the CNS), and also that fat is not just a passive energy reservoir but an endocrine organ with regulatory functions. Fat may have effects on bone through the central and peripheral actions of leptin and other adipokines.

Higher body weight may result from more muscle mass or more fat mass. For a given body weight, greater adiposity may be associated with lower bone density. Subcutaneous and visceral fat may have differing effects on bone; visceral fat produces inflammatory cytokines which may have pro-resorptive effects on bone, and higher visceral fat mass has been associated with increased bone turnover and lower bone density.

These results suggest that the relationship between fat and bone may be more complex than first thought.

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Developmental biology and bone

W2.1

Fish as a model organism for mineralization related pathologies
M Leonor Cancela1,2
1University of Algarve, Faro, Portugal; 2CCMAR, Faro, Portugal.

Department of Biomedical Sciences and Medicine and Centre of Marine Sciences, University of Algarve, Faro, Portugal

In the last decade there has been a growing interest towards the use of fish as models to understand the basic mechanisms of cartilage and bone formation, maintenance and regeneration. In particular, zebrafish and medaka have become accepted models for human skeletal development and associated pathologies such as craniofacial dysplasia, osteoporosis or osteogenesis imperfecta. The availability of an increasing set of molecular and cellular tools, as well as the development of genetic mutants and transgenic fish such as those expressing fluorescent markers specific for a given cell type or tissue, associated to the easiness in observing its internal skeleton in the transparent larval stages and in transduscl adult mutant fish such as casper, contributed decisively to establish zebrafish and medaka as relevant biomedical models to analyse skeletal and mineralization-related pathologies. Another important feature of these models is the possibility of visualizing in vivo the development of the skeletal structures and thus assessing the physiological effects of a given mutation in real time. For example, by crossing a zebrafish exhibiting a mutation in the mef2c gene (mef2cb1086, which leads to cranial malformations among other problems) with a sox10-gfp fish we are able to clearly visualize the sites of skeletal malformations appearing during development. Because of the many applications of these fish models and the overlapping interest of many disciplines such as evolutionary and developmental biology, medicine, genetics, systematics, functional morphology, physiology, nutrition and skeletal pathologies in the fish skeleton, we believe that the use of fish can provide data relevant to further understand bone biology in health and disease.

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W2.3

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Incidence of vertebral fractures in the EU has been estimated in 520,000 for the year 2010. Clinical vertebral fractures cause most of the impact in terms of morbidity, quality of life and economic burden. However, even the sub-clinical ones are not neutral in these aspects. Unlike hip fractures, their incidence is less dissimilar across Europe. Mortality in the first year after a vertebral fracture is higher than for hip fracture, especially in the younger age groups. Vertebral fractures have been linked to 6000 deaths/year in women and 8000 in men, directly attributable to fracture in the EU27. This risk is clearly increased in individuals with three or more fractures.

Reasons for mortality are not fully elucidated. Vertebral fracture can be a marker of poor health and, therefore, comorbidities have been invoked as the reason for decreased survival. As a consequence, mortality studies may have significant bias. Besides the widely known effects on pain and quality of life, VFs have been associated with decreased pulmonary function. Frailty syndrome may be a common driver for VFs and death. Increased cardiovascular risk is associated to osteoporotic fractures and stroke occurs at a increased rate after a VF.

Paradoxically, obese individuals show reduced mortality risk. In any event, a residual mortality effect of VFVs seems to remain after adjusting by comorbidities and other prognostic factors.

Efficacy of interventions to reduce mortality after VFs is not fully assessed. Use of bisphosphonates and SERMs decrease overall mortality but no direct attribution to decreased fracture incidence can be drawn for most of the effect. Kyphoplasty (not vertebroplasty) has been associated with a 35% decrease in mortality and a median life expectancy increase of 3.0-9.5 years. Vertebral fractures are under diagnosed. Presence of fractured vertebrae in lateral chest X-ray films is systematically ignored. Back pain may indicate the presence of VFs but, with little doubt, kyphosis developed during late life and height loss must alert the clinician. Treating spinal osteoporosis is obliged to decrease the impact of VFVs in pain, quality of life and, eventually, in decreased life expectancy.

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Workshop 3: how are vertebral fractures best detected and diagnosed?
Emma Clark
University of Bristol, Bristol, UK.

Less than one-third of osteoporotic vertebral fractures are correctly identified and managed. This is due to a variety of reasons including lack of clear clinical triggers of who to refer for diagnostic spinal radiographs. However, there is increasing evidence for strategies to identify which older people have existing vertebral fractures. One such strategy combines four clinical triggers in a screening tool that has been shown in a large RCT to effectively increase appropriate bisphosphate prescribing in the community. However, there are still unresolved issues around the detection and diagnosis of vertebral fractures, as this screening tool will not identify everyone with an osteoporotic vertebral fracture. In addition, there are important difficulties with interpretation and reporting of the results of spinal imaging. Finally, newer imaging techniques performed at the same time as traditional DXA scans (vertebral fracture assessment, VFA) are being increasingly used in the clinic, without good evidence for change in management as a result.

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Treatment of vertebral fractures
Viviana Tavares
Lisbon, Portugal.

Vertebral fractures are an important cause of pain and disability in osteoporotic patients. The main goals of treatment of vertebral fractures are to alleviate pain, stabilize the fracture, prevent new fractures and reduce comorbidities. Although vertebral fractures are frequently asymptomatic most patients will require analgesic medication for acute pain related with vertebral compression or chronic pain due to kyphosis and posture related problems. Spinal bracing is helpful in controlling pain and stabilizing the fracture during the weeks following an acute vertebral compression. In patients with persistent pain vertebroplasty or kyphoplasty may be indicated and has shown good results in pain control and mobility and function improvement.

Pharmacologic treatment to prevent new fractures is mandatory in patients with vertebral fractures. Available options include antiresorptive (bisphosphonates, SERMs, strontium ranelate, calcitomin, estragen and denosumab) and anabolic agents (teriparatide). All these options have shown to reduce incidence of new vertebral fractures and choice of agent will depend on osteoporosis severity (several vertebral fractures, high fracture risk), drug availability, comorbidities, cost and patient preference.

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Osteoclast activity and haematopoiensis

The relationship between osteoclasts and haematopoiensis
Nikki Horwood
Kennedy Institute of Rheumatology, Oxford, UK.

Over the past decade the importance of the bone marrow environment has been recognized for the development and maintenance of the haematopoietic stem cell (HSC) niche. Both osteoblasts and endothelial cells have been shown to provide a home for HSC within the bone marrow. This interaction is not a one sided affair and recent work has shown that HSC and myeloid cells are capable of driving osteoblast development thus perpetuating the niche itself. The coupled relationship between osteoblasts and osteoclasts has led researchers to question the importance of bone turnover for HSC numbers. The majority of studies to date, but not all, have shown that osteoclasts are required for the mobilization of HSC and more recent work has shown a requirement for osteoclasts in the maintenance of the HSC niche. We have shown that blocking osteoclast activity leads to HSC entering the cell cycle instead of remaining in a quiescent state within the bone marrow. The impact of osteoclast activation and the cancer stem cell niche will be discussed for the progression of leukaemia, multiple myeloma and other bone cancers.

Whether the role of the osteoclast is to direct the formation of new, less mature osteoblasts or to create new spaces in the bone remains to be fully elucidated. What is clear is that active cross-talk between HSCs, their progeny and bone cells determines the HSC niche – knowledge that can be harnessed for obtaining optimal HSC numbers for transplantation and the treatment of residual disease in bone cancers.

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Are osteoclasts dispensable for haematopoietic stem cell maintenance and mobilization?
Claudine Blin-Wakkach
LP2M, CNRS, University of Nice Sophia-Antipolis, Nice, France.

Haematopoietic stem cell (HSC) niches are complex structures located in the trabecular regions of the bone in association with bone-lining osteoblasts (endosteal niches) or with perivascular primitive mesenchymal cells (perivascular niches). These cells provide molecular signals that control HSC fate in terms of self-renewal, proliferation, apoptosis, differentiation, homing, quiescence, etc. Osteoclasts have been implicated in HSC mobilization in response to stress or pharmacological treatments. The mechanisms involved are poorly characterized and raise some controversy, essentially because other monocyctic cells have also been implicated in HSC mobilization. However, our results and those from the literature showed that modulation of osteoclast activity alters the interaction between HSCs and their niches. Moreover, we have demonstrated that functional osteoclasts are required for the formation of the HSC niche by controlling the maturation of osteoblasts that participate in this niche. Lastly, our recent data revealed that osteoclasts also control other cell types involved in the regulation of HSC niches. In conclusion, osteoclasts are not only required for carving space for HSCs in the BM but they also regulate mesenchymal cells for their niche function. These data and more recent ones will be discussed during the presentation.

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W4.3

Osteoclasts and hematopoietic stem cell transplantation in clinical practice
Ansgar Schulz
University Medical Center Ulm, Ulm, Germany.

Dysfunctions of osteoclasts are the pathophysiological hallmark of osteopetrosis (OP). OP is a group of rare inherited human diseases caused by mutations in at least seven different genes. All OP forms are clinical characterized by enhanced bone density. The most severe form infantile OP usually presents with hematological impairment, in particular anemia and thrombocytopenia associated with extra medullary hematopoiesis, leukocytosis and hepatosplenomegaly. Treatment of OP has to consider the variable phenotype and the involvement of multiple organ systems. Symptomatic conservative treatment in less severe cases has to deal with disturbed calcium homeostasis (usually hypocalcemia with secondary hyperparathyroidism) and bone metabolism leading to pathological fractures. In severe infantile cases, hematopoietic stem cell transplantation (HSCT) is a curative approach, since osteoclasts are derived from the hematopoietic progenitor cell compartment. HSCT in OP is associated with specific complications as for instance delayed hematopoietic engraftment and venous occlusive disease. Furthermore, severe hypercalcemia may develop after HCT particularly in older patient. Life threatening hypercalcemia can be treated successfully by Denosumab, an inhibiting antibody to RANK ligand. Following successful HSCT most patients are free of hematological problems and pathological fractures, but develop growth retardation and a short stature. The complex interaction of osteoclasts and hematopoesis in vivo will be illustrated by typical cases of patients with OP before and after HSCT. In particular actual treatment options and site effects will be discussed.

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W5.1

Anabolic bone therapies

Abstract unavailable.

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W5.2

Wnt signalling in and out of bone
Venkatesh Krishnan

The Wnt pathway engages both canonical and non-canonical signalling to accomplish a salutary benefit of increased bone mass, as evidenced by the presence of individuals with high bone mass, who exhibit specific functional variants in members of the pathway. This talk will highlight the importance of the influence on Wnt signalling being received by the bone in response to loading and the signals emanating from bone that influence overall metabolism and health in the whole animal.

The talk will focus on the microenvironment immediately surrounding the bone, both at the periosteum and the endosteum as it prepares to orchestrate the maintenance of bone mass during aging and in the context of bone healing or fracture repair as a result of trauma. The Wnt pathway has distinct effects on bone derived mesenchymal stem cells in terms of affecting their cell fate leading to adipogenesis, chondrogenesis and osteogenesis. Furthermore, access to pluripotent satellite cells from injured muscle near the periostium in the context of bone and muscle trauma, may also provide additional opportunity to influence myogenesis. The talk will highlight the importance of the bone microenvironment in the context of aging and repair from trauma. It will also discuss some new ideas on the consequences of Wnt signalling, as factors derived from bone influence broader metabolism within the whole body.

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W5.3

Therapeutic targeting of activin signalling
Marco Eijken
Erasmus Medical Center, Rotterdam, The Netherlands.

Recent studies have demonstrated that activin signalling plays a crucial role in the skeleton. Activins control both osteoblast and osteoclast function and are present in the bone extracellular matrix. This makes activin signalling an important new therapeutic target for a dual anabolic antiresorptive intervention in osteoporosis. Activins belong to the large TGF-β superfamily that also includes BMPs, TGFβs and GDFs. Like other TGF-β members, activins elicit their biological responses by binding to type I and type II serine/threonine kinase receptors at the cell surface. Upon ligand binding, activin signalling is further transduced by phosphorylation of Smad2/3 intracellular signalling proteins.

In human osteoblast cultures activins strongly suppress in vitro bone formation in an auto/paracrine manner. Subsequent mechanistic studies in human osteoblasts demonstrated that activins elicit their inhibitory effect by altering the bone extra cellular matrix and by limiting the production of bone matrix vesicles.

Although also opposing effects have been reported using other cell models, the inhibitory effects of activins on bone are supported by studies in rodents and primates. In these studies neutralisation of activin signalling using activin type IIA decoy receptors strongly increased trabecular bone volume due to enhanced bone formation and decreased bone resorption.

At the tissue level activins are locally antagonised by follistatin and inhibins. Follistatin is an extracellular protein that besides activins also binds to and neutralises other TGF-β members including myostatin. Myostatin is well known for its inhibitory effect on muscle growth and myostatin neutralization has been shown to lead to enhanced muscle strength. Therefore a follistatin-based therapy might be an unique approach that offers the potential to reduce the risk for fractures in osteoporosis by increasing both bone and muscle strength.

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W6.1

Cancer cells and Bone

How do cancer cells home to and engage in bone?
Peter Croucher
Sydney, Australia.

A number of cancers develop in the skeleton or will metastasize to bone, including multiple myeloma and solid tumours such as breast and prostate cancer. Once established in the skeleton, cancer cells have the ability to modify the environment and cause devastating bone disease. The last decade has seen considerable progress in defining the cellular and molecular mechanisms responsible and also identified new roles for the cells of bone in the pathogenesis of metastasis process.

Tumour cells produce molecules, including parathyroid hormone-related protein, macrophage inhibitory protein 1α, and in some cases the ligand for receptor activator of NFκB (RANKL), and induce RANKL in cells of the bone environment, to promote osteoclastic bone resorption. Tumour cells also produce molecules to either suppress bone formation, which is typically seen in osteolytic disease, or promote bone formation, which leads to osteosclerotic disease. Promoting bone formation and retaining the coupling between resorption and formation prevents osteolytic disease suggesting osteoblasts are in a pivotal position in determining the nature of the bone disease.

In addition, there is now increasing evidence that osteoblasts and osteoclasts play a critical role in supporting the growth and survival of cancer cells in the skeleton. Colonising cancer cells locate to dedicate niches in the skeleton and may compete with haemopoietic stem cells (HSC) for the HSC niche. Cells of the osteoblast lineage play a key role in the HSC niche and may support the immediate homing of cancer cells to the skeleton, their survival and long-term quiescence. Furthermore, switching on bone turnover increases the number of tumour lesions in the skeleton and inhibitors of resorption stop this process, arguing for a key role for the osteoclast in activating tumour cells. These data suggest that bone cells have unique relationship with tumour cells, supporting their colonization and activation as well as mediating the skeletal effects. Understanding these interactions, is likely to result in new approaches to preventing tumour growth in the skeleton.

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W6.3

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Meet The Professor
Epigenetic regulation: what and why?
Keith Godfrey1,2
1MRC Lifecourse Epidemiology Unit, Southampton, UK; 2NIHR Southampton BRC, Southampton, UK.

Recent evidence demonstrates that the environment in early life can have important effects on fetal and postnatal growth, on later body composition and on risk of developing common non-communicable diseases in later life. In animals, the environment during early life induces altered phenotypes in ways which are influenced or mediated by epigenetic mechanisms. The latter include DNA methylation, covalent modifications of histones and non-coding RNAs. Most is known about DNA methylation changes, which are gene-specific, include effects on non-imprinted genes and function at the level of individual CpG dinucleotides to alter gene expression. Preliminary evidence from human studies suggests a similar important role for epigenetic processes. Tuning of phenotype by the developmental environment has adaptive value because it attempts to match an individual’s responses to the environment predicted to be experienced later, hence such processes have been selected during evolution as conferring fitness advantage. When the phenotype is mismatched, e.g. from inaccurate nutritional cues from the mother or placenta before birth, or from rapid environmental change through improved socio-economic conditions, risk of non-communicable diseases including obesity and osteoporotic fracture increases. Evidence is accruing that endocrine or nutritional interventions during early postnatal life can reverse epigenetic and phenotypic changes induced, for example, by unbalanced maternal diet during pregnancy. Elucidation of epigenetic processes may enable early intervention strategies to improve early development and body composition.

Clinical utility of bone turnover markers
Nuria Guatavibens
Hospital Clinic, University of Barcelona, Barcelona, Spain.

Bone turnover markers (BTMs) are particularly useful in the early monitoring of the effectiveness of anabolic and antiresorptive therapy in osteoporosis and may help in the assessment of treatment compliance. How and when BTM levels change under antiresorptive or anabolic drugs is a key factor in assessing response to therapy. Thus, changes in BTM levels depend on the dose of the drug and the route of administration, and particularly on the mechanism of action. In this sense, responses to oral antiresorptive drugs may be assessed quickly, within 3 months, by measuring resorption markers, or later, when using formation markers. In the assessment of parenteral bisphosphonates and denosumab, changes in resorption markers are very fast. Bone markers, such as the cross-linked C-terminal and N-terminal telopeptides of type I collagen (sCTX and uNTX), are well positioned in clinical practice for monitoring antiresorptive treatment, and procollagen type I N-terminal propeptide (PINP) is the best marker for assessing response to teriparatide. In addition, BTMs may be useful in the monitoring of treatment discontinuation. When discussing bone markers in clinical practice, some interesting points arise: they may complement fracture risk assessment, and they may help in the identification of secondary osteoporosis. The measurement of most bone markers by automated analysers has improved their laboratory reproducibility and accessibility. Practical considerations for the clinician when using BTMs include the awareness of the pre-analytical variability, taking care of
precise timing and fasting status of the sample collection, in addition to other sources of pre-analytical variability, such as intercurrent diseases or recent fractures. In recent years, the technical advances in their determination, the use of appropriate reference intervals and the minimization of the sources of the pre-analytical variability have improved the performance of BTMs for assessing and treating patients with osteoporosis.

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MTP8

Abstract unavailable.

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MTP9

Assessment of therapeutic response in osteoporosis
Adolfo Diez-Perez
Department of Internal Medicine and Infectious Diseases, Hospital del Mar-IMIM, Autonomous University of Barcelona, Barcelona, Spain.

No treatment for osteoporosis abolishes the risk of fracture. Even under the ‘ideal’ conditions of adherence and monitoring, in the controlled pivotal trials, a significant proportion of individuals receiving the active drug still suffer new fractures. In everyday practice the situation is even more challenging. Adherence to medication is poor, patients are often older than in trials, or suffer from a number of comorbidities that could have excluded them from the original trials. Moreover receive a number of medications that make the treatment cumbersome and potentially interfere with the drug we prescribe for their osteoporosis. Underlying diseases, sometimes not clinically apparent, may also limit or totally cancel the efficacy of the drugs. Therefore, in clinical practice, a proportion of patients are not responding to the treatment in the way that the caregiver or the patient expects.

Bone density is one of the tools for monitoring the response. However, needs long observation periods and the increment in BMD should be superior to the least significant change for the technique. But, even in those that show significant reduction in BMD, the risk of fracture is reduced in some degree. Biochemical markers have experienced recent progress making them more suitable for clinical use. These markers assess the direct ‘tissue effect’ of the drugs, and are modified very early after starting treatment. However, still suffer from some practical limitations as the need for quite large variations, accessibility and biological variability. Finally, incident fractures are probably the most impactful event. Since their avoidance is the ultimate goal of a treatment, both the patient and the physician consider its occurrence as a possible treatment failure. However, since no treatment reduces the risk to zero, one incident fracture may be simply a chance event.

With these premises, treatment failure has been defined as the occurrence of two or more incident fractures in patients with good compliance. BMD and bone markers are also criteria for judgment. The clinician should, in these cases, assess compliance, rule out secondary causes of osteoporosis and other factors interfering with the effect of the drug. In some cases, however, the disease is too advanced and the bone strength so deteriorated that the available treatments are not enough to stop the fracture cascade.

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MTP10

New osteoporosis treatment modalities
Viviana Tavares
Serviço de Reumatologia, Hospital Garcia de Orta, Almada, Portugal.

Pharmacologic treatment of patients with a high risk of fracture is mandatory. Nowadays there are many available options mostly with antiresorptive agents (bisphosphonates, SERMs, strontium ranelate, calcitonin, estrogens and denosumab) but also with anabolic agents (teriparatide) that have shown to reduce incidence of new fragility fractures. In the near future new drugs targeting different pathways and mediators involved in bone remodelling will be available. At present, choice of agent will depend on osteoporosis severity but also on drug availability, comorbidities, cost and patient preference. Controversial and unanswered issues are the risk of possible long term side effects of these drugs, duration of treatment and drug holidays as well as the possible use sequential or combined treatment modalities.

The goal of this session is to discuss present and evolving guidelines and treatment modalities. At the end of the meeting participants will be able to:

- Apply current osteoporosis treatment guidelines in a clinical setting.
- Understand the safety and efficacy issues of current and emerging osteoporosis treatments.
- Recognize and overcome the current gaps in osteoporosis treatment.

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MTP11

Abstract unavailable.

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MTP12

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MTP13

Arthritis, inflammation and bone: from bench to bedside
João Fonseca1,2
1Rheumatology Research Unit, Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal. 2Rheumatology Department, Lisbon Academic Medical Centre, Lisboa, Portugal.

How exactly does inflammation early affect bone properties at rheumatoid arthritis (RA) onset? As we have shown, mechanical bone properties are negatively affected by inflammation. This could be viewed as a logic consequence of the reduction BMD that occurs in RA. However, the increased risk of fractures in RA patients is independent from BMD and this has been recognized by the WHO FRAX tool, where RA has been included as one of the risk factors. Thus, RA in itself is independently associated with the occurrence of bone fractures but the underlying mechanisms are not completely understood. In our view, a clue for this problem can be found in our innovative observations using SHG microscopy suggesting that arthritis affects the density and organization of collagen type I. Our hypothesis is that the initial steps towards bone fracture and joint collapse are determined by early changes in collagen type I organization capable of interfering with the intrinsic bone tissue properties. There are a number of arguments for positioning collagen type I fiber damage as an initial event in RA bone disease. Bone mineral phase is stiff and brittle and thus it is responsible for bone’s stiffness, while the collagen matrix is much softer and is the main provider of ductility and the ability to absorb energy. As a corollary, a high ratio of calcium crystals / – collagen can reduce the ductility and toughness of bone, particularly if the collagen matrix is quantitatively or qualitatively affected. In addition, disturbances in the bone mineralization density distribution can decrease bone strength, without necessarily affecting bone matrix volume and microarchitecture. Finally, mineral particles are strongly oriented in the direction of collagen fibers and may have a distorted distribution if collagen molecules are not adequately organized due to a high turnover rate, affecting toughness and elastic modulus.

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Abstract unavailable.

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MTP14.1

CRC Grants
Joana Camilo
Omar Albagha (Edinburgh, UK).

Turn your challenges into opportunities through EU funding for research and innovation.

The European Union encompasses several funding mechanisms to support research and innovation (R&I). One of its main instruments is Seventh Framework programme (FP7), established for the period 2007–2013, which is now approaching its end.

The successor EU instrument, called Horizon 2020 (H2020), is currently under discussion and preparation and it is scheduled to be launched in January 2014. This 7 years R&I Programme will contribute for turning European challenges into opportunities, through the creation of new knowledge, technologies, and innovations. The H2020 proposed structure (budget proposal of €80 billion) is organised in three main pillars: ‘Excellent Science’; ‘Industrial Leadership’, and ‘Societal Challenges’, which, in turn, include the ‘Health, Demographic Change and Well Being’ Challenge with a multitude of funding opportunities for clinical, pre-clinical scientists and healthcare professionals. H2020 will support disruptive, high-risk research ideas and also the career of both young and established researchers. It will foster the collaboration between academia and industry, while boosting the European industrial leadership. H2020 will pave the way for the exploitation of the research outputs, namely through transnational, inter and multidisciplinary collaborative research, throughout the full innovation cycle.

The R&I community will find ways to support their basic research, programmes from bench-to-bedside, in a personalized health and care approach, as well as the development of innovative applications for health. Furthermore, it will support the EU Health Strategy, and the delivery of the Europe 2020 Flagship Initiative ‘Innovation Union’ goals towards an active and healthy ageing.

One of the cornerstones of H2020 will be the strategic programming, by which the European Commission will launch biannual work programmes for calls for proposals.

With less than one year for the start of H2020, it is time to full speed the preparation of the R&I community for the maximum exploitation of these funding opportunities.

This session will present the major known features and background of the upcoming H2020, and will show how the R&I stakeholders can be prepared for the calls and contribute to the priority setting. This session will be concluded by a landscape of available tools that can support the response to the upcoming calls for proposals.

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MTP14.2

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MTP15

Fraud, scientific misconduct, or just an oversight?
Jane Barrett
London, UK.

There has been much published about the incidence, detection and prosecution of publication fraud, less about fraud and misconduct in clinical research. We should be equally concerned about research fraud. It is clear that all misconduct is not fraud, and sloppiness would not be so labelled, but the protection of patients must be uppermost of all concerns.

Whichever definition is used, the fact that patients have been exploited remains. This exploitation occurs when Ethics Committee authorisation is not sought or is forged, denying patients the protection of review of the safety and ethics of the study. It occurs when safety data are not recorded or when patients are treated with inappropriate drugs.

Distinction must be drawn between clinical research that is of poor quality and that which is fraudulent. Data with minor errors should be identified by trial monitors from the sponsor company or their subcontractors, and the investigator given the chance to correct it. Such errors are common, represent lack of attention to detail, pressure of work and time, inadequate or over-complicated case record forms, or plain carelessness.

The pharmaceutical industry has been extremely active in its efforts to prevent and detect research fraud and misconduct, and most companies are now comfortable taking action when appropriate. The European Directive on clinical trials, with the International Conference on Harmonisation (ICH) have both aided a growing understanding and awareness of the issue, and most pharmaceutical companies now have standard procedures for handling cases of suspected fraud.

Research fraud is a reality, but physicians and academia have sometimes chosen to turn a blind eye. But the climate now is changing, driven largely by the pharmaceutical industry. Medical research is still vulnerable in the absence of effective mechanisms to combat and detect fraud.

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Educational Symposium
Low vitamin D serum levels have been associated with a considerable number of diseases and conditions and have attracted significant attention of the scientific community as well as of health authorities all over the world. However, discussions and controversies are ongoing about the reliability, significance and correct ranges of low vitamin D serum levels. A central goal is therefore the reliable measurement of circulating vitamin D, regarded as the best measure of an individual’s vitamin D status.

In addition to the main analyte 25(OH) vitamin D₃, several other forms of vitamin D and its metabolites has to be taken into account, such as 1,25(OH)₂ vitamin D. A C-3 epimer of vitamin D, metabolites like 24,25(OH)₂ vitamin D and other members of this secosteroid family, as well as the dualism of D₃ and D₂ will be the topic of this lecture.

The technical analysis of vitamin D (and its subforms) started by using radioimmunological measurements. Commercially available enzyme-linked assays followed, either by manual or automated techniques. HPLC and/or including tandem mass spectrometry of several types (MS–MS, LC–MS, GC–MS) provide a differentiated profile of vitamin D measurement.

A number of efforts to evaluate, validate and unify these techniques are on the way. The establishment of standardized measurements is of considerable value for the comparison of vitamin D analyses and their quality check in routine and research labs.

For scientific and practical purposes, the measurement of vitamin D not only requires reliable laboratory methods, but also international guidelines for the interpretation of the results. Furthermore, regular measurements, especially concerning high risk patients as well as a treating-strategy for low vitamin D levels need to be discussed. This lecture will provide insights into the complex analytics of vitamin D as well as the interpretation and consequences of low vitamin D levels.
Oral Communications
Osteoporosis epidemiology and long term treatment complications

OC1.1

Disease-specific perception of fracture risk and incident fracture rates among postmenopausal women: findings from the Global Longitudinal Study of Osteoporosis in Women (GLOW)

Celia Gregson1,2, Elaine Dennison1, Juliet Compston3, Silvano Adami4, Kenneth Saag5, Stuart Silverman2, Ethel Siris6, Nelson Watts7, Allison Wyman8 & Cyrus Cooper9

1Musculoskeletal Research Unit, University of Bristol, Bristol, UK; 2MRC Lifecourse Epidemiology Unit, University of Southampton, Southampton, UK; 3School of Clinical Medicine, Addenbrooke’s Hospital, University of Cambridge, Cambridge, UK; 4Department of Rheumatology, University of Verona, Ospedale, Verona, Vareggio, Italy; 5St Joseph’s Hospital, McMaster University, Hamilton, Ontario, Canada; 6UMASS Medical School, Centre for Outcomes Research, Worcester, Massachusetts, USA; 7Division of Geriatric Medicine, Leuven University Center for Metabolic Bone Diseases, Katholieke Universiteit Leuven, Leuven, Belgium; 8INSERM U813, Université de Lyon, Division of Rheumatology, Hôpital E Herriot, Lyon, France; 9Hospital del Mar-IMIM-Autonomous University of Barcelona, Barcelona, Spain; 10University of Pittsburgh, Pittsburgh, Pennsylvania, USA; 11Fred Hutchinson Cancer Research Center, Seattle, Washington, USA; 12Helen Hayes Hospital and Columbia University, West Haverstraw, New York, USA; 13Department of Endocrinology, UZ University Medical Center, Amsterdam, The Netherlands; 14Department of Internal Medicine III, Alfred Krupp Krankenhaus, Essen, Germany; 15Paris Descartes University, Cochin Hospital, Paris, France; 16University of Alabama-Birmingham, Birmingham, Alabama, USA; 17Department of Rheumatology, Cedars-Sinai/UCLA, Los Angeles, California, USA; 18Department of Medicine, Columbia University Medical Center, New York, New York, USA; 19Bone Health and Osteoporosis Center, University of Cincinnati, Cincinnati, Ohio, USA; 20Institute of Musculoskeletal Sciences, University of Oxford, Oxford, UK.

Patients with improved health understanding have greater autonomy over, and motivation towards, health-related lifestyles. We compared self-perceived fracture risk and 3-year incident fracture rates in postmenopausal women for a range of co-morbid diseases using data from the Global Longitudinal study of Osteoporosis in Women (GLOW).

GLOW is an international cohort study involving 723 physician practices across 10 countries in Europe, North America, Australasia. 60 393 women aged ≥55 years completed baseline questionnaires detailing medical history, including co-morbidities, fractures and self-perceived fracture risk. Annual follow-up determined self-reported incident fractures.

In total, 2945/43 832 (6.7%) sustained an incident fracture over 3 years. All co-morbidities were strongly associated with increased fracture rates, particularly Parkinson’s disease (PD) (hazard ratio (HR) 95% CI: 3.89 (2.78, 5.44)), multiple sclerosis (MS) 2.70 (1.90, 3.83), cerebrovascular events 2.02 (1.67, 2.46), and rheumatoid arthritis 2.15 (1.53, 3.04), all P<0.001. Most individuals perceived their own fracture risk to be similar to (46%) or lower than (36%) women of the same age. Increased self-perceived fracture risk was strongly associated with incident fracture rates. However, only 29% of women who experienced a fracture had perceived their risk as increased. Under-appreciation of fracture risk occurred for all co-morbidities, particularly for women with neurological disease, in whom women with self-perceived low fracture risk had a fracture HR of 2.39 (1.74, 3.29) compared with women without co-morbidities.

Our results suggest postmenopausal women with co-morbidities known to be associated with increased fracture rates tend to under-appreciate their risk, especially in the context of neurological diseases, where fracture rates are highest. Our findings have important implications for health education particularly among women with neurological disease and support updating of relevant guidelines.

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OC1.2

Hip fractures and bone mineral density of the elderly: importance of serum 25-hydroxy vitamin D

Lafeay Steingrimsdottir1,2, Thorhallur Halldorsson1,4, Kristin Siggeirsdottir2, Mary Frances Cotch3, Gudny Eiriksdottir2, Sigurdur Sigurdssoñ2,4, Tamara Harris5, Vilmundur Gudnason3,4 & Gunnar Sigurdssoñ1,4

1Unit for Nutrition Research, University of Iceland and Landspitali Hospital, Reykjavik, Iceland; 2Icelandic Heart Association Research Institute, Kopavogur, Iceland; 3Intramural Research Program, Laboratory of Epidemiology, Demography, and Biometry, National Institute on Aging, Bethesda, Maryland, USA; 4University of Iceland, Reykjavik, Iceland.

Introduction

Vitamin D is known to be important for bone health. Still, the significance of serum 25-hydroxy vitamin D concentrations (s-25OHD) for hip fracture risk of the elderly is uncertain. Discordant findings may in part be explained by difficulties of RCTs or large cohort studies to reach both the frail and the healthy elderly. The objectives of this study were to determine the risk of hip fractures of the elderly related to s-25OHD, including both the frail and the healthy.

Methods/participants

The AGES-Reykjavik Study is a prospective study of 5764 elderly, age 66–96 years, based on a random sample of the population of Reykjavik, participation 71.8%. Incident hip fractures were related to s-25OHD at baseline, average time to event 3.4 years. BMD was measured by quantitative computed topography.

Results

Compared with referent values (50–75 nmol/l), hazard ratios for hip fractures were 2.24 (95% CI 1.63, 3.09) for s-25OHD <30 nmol/l, adjusting for age, sex, BMI, smoking, alcohol intake and season of blood sampling, and 2.08 (95% CI 1.51, 2.87) adjusting additionally for maximal knee extension, time up and go and physical activity. No difference in risk was associated with 30–50 nmol/l, nor with >75 nmol/l in either model compared with referent. Hazard ratios were 2.51 (95% CI 1.47, 4.65) in men and 1.92 (95% CI 1.30, 2.82) in women. Values <30 nmol/l compared with 50–75 nmol/l were associated with slightly lower BMD of femoral neck, reported as T-score, or –0.18 (95% CI –0.31, 0.04) in men and –0.13 (95% CI –0.23, –0.03) in women.

Conclusions

Our study lends support to the prime importance of keeping s-25OHD above 30–40 nmol/l for lowering hip fracture risk of the elderly. While higher levels may be of some benefit for other health outcomes, the main emphasis should be to ensure sufficient vitamin D to maintain adequate status.

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OC1.3

Size at birth is not associated with risk of hip fracture. results from two population-based cohorts

Lisa Byberg1, Karl Michaelsson1 & Ilena Karl2

1Department of Surgical Sciences, Orthopaedics, Uppsala University, Uppsala, Sweden; 2Centre for Health Equity Studies (CHESS), Stockholm University and Karolinska Institutet, Stockholm, Sweden.

Early life growth has been suggested to influence bone health. However, the relationship with risk of hip fracture in old age has not been thoroughly investigated. We therefore studied the association between birth weight and hip fracture incidence after age 50 among 10 893 men and women (48% women) from the Uppsala Birth Cohort Study (UBCoS, born 1915–1929) and 1334 men from the Uppsala Longitudinal Study of Adult Men (ULSAM, born 1920–1924). Birth weight was collected from hospital or midwives’ records and hip fractures were obtained from the Swedish Hospital Discharge Register.

We observed 717 hip fractures in UBCoS (458 in women, 259 in men, end of follow-up: 31 December 2008) and 102 hip fractures in ULSAM (end of follow-up: 31 December 2009). There were no indications of non-linear associations. Results are presented as hazard ratios (HR) and 95% CI per 1 kg increase in birth weight.

The crude HR for 1 kg increase in birth weight on hip fracture rate in UBCoS was 0.99 (95% CI: 0.85–1.14). After controlling for gender and socioeconomic status at birth, the HR was 1.06 (95% CI: 0.91–1.23). Additional adjustment for adult height and comorbidity in a subgroup of UBCoS men (n=1241, 50 hip fractures) gave a HR of 0.97 (95% CI: 0.52–1.90). Parity and gestational age did not largely influence the estimates. Northern birth weight standard for gestational age nor gestational duration was associated with hip fracture rate.

The unadjusted HR in ULSAM was 1.06 (95% CI: 0.73–1.53). After adjustment for adult body mass index, height, social class, comorbidity, and smoking status, the HR was 1.03 (95% CI: 0.70–1.51).

Based on the results from two population-based cohorts with accurate assessment of both birth weight and hip fractures, we conclude that there is no association between birth weight and risk of hip fracture.

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OC.1.4
Intake and serum levels of α-tocopherol in relation to fractures in elderly women and men
Karl Michaeïlsson1, Alicia Wolk1, Liisa Byberg1, Johan Arnlov1 & Håkan Mellström2
1Section of Orthopaedics, Department of Surgical Sciences, Uppsala University, Uppsala, Sweden; 2Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden; 3Department of Public Health and Caring Sciences/Section of Geriatrics, Uppsala University, Uppsala, Sweden; 4Section of Clinical Pharmacology, Department of Medical Sciences, Uppsala University, Uppsala, Sweden.

Recent studies indicate a potential importance of the antioxidant α-tocopherol for bone and the development of sarcopenia. No longitudinal clinical fracture studies have been performed. We aimed to determine whether α-tocopherol intake or serum concentrations are associated with fracture risk in older women and men. We used data from two community-based cohorts, the Swedish Mammmography Cohort (SMC; 61 433 women) and the Uppsala Longitudinal Study of Adult Men (ULSAM; 1138 men). Nutrient intakes were assessed with repeated food frequency questionnaires in the SMC and by dietary food recordings in the ULSAM cohort. Serum α-tocopherol analyses were done by HPLC. During follow-up, 14 738 women in the SMC experienced a first fracture at any site of which 3871 were hip fractures. A gradual increase in hip fracture rate was observed with lower intakes of α-tocopherol. In comparison with the highest quintile of intake, the lowest quintile intake had a multi-variable-adjusted hazard ratio (HR) of 1.86 (95% CI 1.67–2.06). The corresponding HR of any fracture was 1.20 (95% CI 1.14–1.28). Moreover, α-tocopherol-containing supplement use was associated with a reduced rate of hip fracture (HR 0.87; 95% CI 0.65–0.93) and any type of fracture (HR 0.86; 95% CI 0.78–0.94). Compared with highest quintile of α-tocopherol intake in the ULSAM study, lower intakes were associated with a higher rate of hip fracture (multivariable-adjusted HR 3.33; 95% CI 1.43–7.76) and any type of fracture (HR 1.84; 95% CI 1.18–2.88). Each SD decrease in serum α-tocopherol conferred a HR for hip fracture of 1.58 (95% CI 1.13–2.22) and of 1.23 (95% CI 1.02–1.48) for any fracture. We conclude that a low intake and low serum levels of α-tocopherol are associated with an increased rate of fracture in elderly women and men.

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OC.1.6
Femur geometrical parameters in the pathogenesis of atypical femur fractures
Suzanne N Morin1,2, Benoït Godbout1, Michelle Wall1, Etienne L Belzile3, Léa-Léa Michou3, Louis-Georges Ste-Marie3, Andrew C Karaplis4, Jacques A de Guise5 & Jacques P Brown4
1McGill University, Montréal, Québec, Canada; 2Université de Montréal, Montréal, Québec, Canada; 3McGill University Health Center, Montréal, Québec, Canada; 4Université Laval, Québec, Canada; 5Centre de recherche du CHUM, Montréal, Québec, Canada.

Background
Atypical femur fractures (AFF) arise in the subtrochanteric and diaphyseal regions. Because of this unique distribution, we hypothesized that patients with AFF demonstrate specific geometrical variations of their femur whereby baseline tensile forces applied to the lateral cortex are higher and might favor the appearance of these rare stress fractures, when exposed to bisphosphonates.

Methods
Subjects who sustained AFF, as defined by the ASBMR task force, were recruited. Using the EOS low irradiation 2D–3D X-ray scanner, bilateral lower extremities examinations were obtained in the upright weight-bearing position. EOS permits 3D surface images of high resolution from simultaneous two-plane images. We compared the participants’ femur geometrical parameters to those of a normal reference cohort and examined differences between those who sustained diaphyseal vs subtrochanteric AFF.

Results
We identified 25 subjects (21 women; mean age 67 (s.d. 9) years; 23 Caucasian, 2 Asian) with AFF. All were exposed to bisphosphonates (average cumulative duration of use of 10.6 (s.d. 4.6) years). There were 38 AFF (13 bilateral, 15 complete and 23 incomplete; 28 diaphyseal and 10 subtrochanteric). Compared with reference values, our subjects tended to have shorter lower limbs (femur 39.9 s.d. (2.2) cm and tibia 33.9 s.d. (2.2) cm), lesser femur neck-shaft angle (125.5° s.d. (6.5)), wider hip knee shaft angle (7.0° s.d. (1.8)) and higher femoral torsion (15.1° s.d. (10.8)). Compared to women with diaphyseal fractures, those with subtrochanteric fractures had a lesser femur neck-shaft angle (122.8° s.d. (3.8) vs 127.9° s.d. (6.8); P = 0.09) and longer femoral offset (4.2 s.d. (0.2) cm vs 3.8 s.d. (0.6) cm; P = 0.08).

Conclusion
Our data support that subjects with AFF exhibit femur geometry that results in higher mechanical load on the lateral femur, particularly in women with subtrochanteric fractures; this may play an important role in the pathogenesis of AFF.

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Bone quality and fracture repair - animal models
OC2.1
Treatment with soluble activin type IIIB-receptor improves bone mass and strength in a mouse model of duchenne muscular dystrophy
Tero Puolakkainen1, Hongqiang Ma1, Arja Pastermak5, Heikki Kaimlainen1, Olli Ritvos1, Kristiina Heinininen1, Juha Hulmi1 & Riku Kiviranta1,2
1University of Turku, Turku, Finland; 2Turku University Hospital, Turku, Finland; 3University of Jyväskylä, Jyväskylä, Finland; 4University of Helsinki, Helsinki, Finland; 5Department of Oral and Maxillofacial Surgery, University of Turku.

Patients with Duchenne muscular dystrophy (DMD) carry a mutation in the dystrophin gene that leads to progressive muscle degeneration. In addition, DMD patients develop low bone mass especially in long bones and have high incidence of fractures. The underlying mechanisms for decreased bone mass remain unclear but muscle weakness and increased IL6 levels may play a role. Inhibition of activin/myostatin pathway has emerged as a novel approach to increase muscle mass and strength in DMD. The aim of our study was to test whether inhibition of

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this pathway in MDX mice, a model for DMD, would improve bone properties in addition to muscle strength. Sixteen MDX mice were randomised 1:1 to receive either PBS or an in-house soluble activin type IIIB-receptor (sAct-RIIB-Fc) 5 mg/kg i.p. once weekly for 7 weeks. Hind limbs and vertebrae were harvested and subjected to μCT and biomechanical testing.

As expected, treatment of MDX mice with sAct-RIIB-Fc resulted in significantly increased bone formation and muscle weights compared to PBS group. μCT analysis of the femurs showed increased bone volume and trabecular number (BV/TV +70%, Tr.N +60%, P <0.05 in both) in sAct-RIIB-Fc treated group. sAct-RIIB-Fc increased bone mass also in vertebrae (BV/TV +20%, Tr.N +30%, P <0.05 in both) but the effects were more modest in axial skeleton than in long bones. Increased bone mass in femurs translated into enhanced bone strength as the maximum force (+19%, P <0.01) and stiffness (+19%, P <0.01) were significantly elevated in sAct-RIIB-Fc-treated mice.

Our results indicate that treatment of MDX mice with the soluble activin type IIIB-receptor results in a robust increase in both bone mass and strength in long bones but positively affects also axial skeleton. Thus sAct-RIIB-Fc could be an attractive option in the treatment of DMD, addressing both muscular and skeletal sequelae of the disease.

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**OC2.2**

**Intermittent human parathyroid hormone (1–84) treatment bone mass and bone defect healing in rats with type 2 diabetes mellitus**

Christine Hamann1, Ann-Kristin Pick1, Martina Rauner1, Ricardo Bernhardt2, Graeme Campbell3, Claus-Christian Gliüler1 & Lorenz C Hofbauer1

1TU Dresden, Medical Center, Dresden, Germany; 2Max Bergmann Center TU Dresden, Dresden, Germany; 3Christian Albrechts University, Kiel, Germany.

The pathogenesis of skeletal fragility in diabetes mellitus is poorly defined and efficient therapies are limited. Zicker diabetic fatty (ZDF) rats with type 2 diabetes mellitus display low bone mass and delayed bone defect healing. We tested whether intermittent treatment with human parathyroid hormone 1–84 (PTH) increases bone mass and bone defect regeneration in diabetic rats. A subcritical gap defect was created at the femur of 10 weeks old diabetic ZDF♂♂ and non-diabetic ZDF♀♀ rats (n =10/group). PTH (75 μg/kg) or water as control were administered subcutaneously 5 days/week over the course of 12 weeks. Bone mass was assessed ex vivo at the non-operated femur and the lumbar vertebra by μCT, and the filling of the femur gap was analyzed by micro-CT. Diabetic rats had significantly lower total BMD at the metaphyseal area of the femur (−20%) and the lumbar vertebra (−11%) compared to non-diabetic rats. PTH treatment in diabetic rats resulted in increased bone mass at the femur (total BMD +10%, trabecular +46%) and lumbar spine (total BMD +18%, trabecular BMD +36%) compared to control-treated animals. Spinal BMD parameters of diabetic rats receiving PTH treatment were higher than those of non-diabetic rats treated with control. While non-diabetic rats filled 35% of the femoral defect, diabetic rats filled only 25%. PTH treatment increased defect regeneration in the diabetic and non-diabetic groups by 49% and 8%, respectively. Intermittent PTH treatment resulted in increased serum levels of osteocalcin by 33 and 10% in diabetic and non-diabetic animals, respectively, and lower serum levels of CTX (−49 and −19%), consistent with a marked bone-anabolic effect.

In conclusion, intermittent PTH therapy is capable of reversing the adverse effects of type 2 diabetes mellitus on bone mass and delayed bone defect regeneration in rats.

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**OC2.3**

**Low-magnitude high-frequency vibration improves fracture healing in aged, ovariectomized mice**

Esther Wehrle1, Ronny Bindl2, Tim Wehner4, Aline Heilmann1, Lena Fischer1, Jarrod Noland1, Michael Amling2 & Anita Ignatius1

1Institute of Orthopaedic Research and Biomechanics, Centre of Musculoskeletal Research, University of Ulm, Ulm, Germany; 2Department of Osteology and Biomechanics, University Medical Center Hamburg Eppendorf, Hamburg, Germany.

Introduction

Fracture healing is impaired in aged and osteoporotic patients. Because bone formation is tightly regulated by the mechanical conditions in the fracture gap and because suitable mechanical stimuli improve fracture healing (Claes et al., 1998), we investigated whether low-magnitude high-frequency vibration (LMHFV; Rubin et al., 2004) is able to improve delayed fracture healing induced by age and ovariectomy in mice.

Study design and methods

Female C57BL/6Ncrl mice (n = 80) were either ovariectomized (OVX) or sham operated at an age of 41 weeks. Eight weeks later all animals received an osteotomy of the right femur, which was stabilized using an external fixator. Starting on the third postoperative day, all animals were placed on vibration platforms (20 min/day; 5 days/week), and received either a mechanical intervention therapy (f = 45 Hz, Δz = 0.3 g) or no vibration (control groups). The animals were sacrificed on d21 and the femora were analysed by biomechanical testing, μ-computed tomography and histomorphometry.

Results

The vibration provoked different effects in non-OVX and OVX mice. In non-OVX mice, vibration significantly decreased the bending stiffness of the fracture callus in comparison to non-vibrated controls (901 vs 174 Nmm−2) as well as bone formation in the fracture gap (μCT-analysis: BV/TV: 47 vs 16%; histomorphometry: bone fraction in callus: 44 vs 14%). In OVX mice vibration resulted in a significantly improved bending stiffness (47 vs 69 Nmm−2) and bone formation in the fracture callus (BV/TV: 9 vs 36%; bone fraction in callus: 7 vs 49%).

Conclusion

LMHFV significantly improved fracture healing in aged, ovariectomized mice whereas it significantly impaired fracture healing in intact animals of the same age. This might indicate that estrogen plays a major role in the mechanobiology of fracture repair.

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**OC2.4**

**PPARβ deficiency induces muscle and bone loss with aging but does not impair the bone biomechanical response to loading: a sarco-osteopenic mouse model**

Nicolas Bonnet1, Béatrice Desvergne2 & Serge Ferrari1

1Division of Bone Diseases, Geneva University Hospital and Faculty of Medicine, Switzerland, Geneva, Switzerland; 2Faculty of Biology and Medicine, Center for Integrative Genomics, University of Lausanne, Lausanne, Switzerland.

PPARβ is crucial for muscle fatty acid oxidation. Pparβ−/− mice have reduced muscle strength, exercise performance, and also a decreased skeletal response to exercise. Here we investigate the influence of Pparβ on muscle and bone loss with aging, and its role on the bone biomechanical response to loading. Pparβ−/− and Pparβ+/+ mice were monitored at 1, 3 and 18 months of age. Muscle function was evaluated by handgrip and locomotor activity. Six-months-old Pparβ−/− and Pparβ+/+ male were subjected to in-vivo axial compression of the tibia for 2 weeks. At 1-month of age, lean mass, skeletal muscle function, and bone parameters did not differ between Pparβ−/− and Pparβ+/+. From 1 to 3-months of age, Pparβ−/− had lower gain in lean mass (+64 vs +88% in Pparβ+/+), P <0.01) and TB (total body) BMD (+66 vs +73% in Pparβ+/+, P <0.05). Mean force and running distance were significantly lower in Pparβ−/− (0.56 ± 0.02 mm, 0.34 ± 0.01 mm, 19.7 ± 1.3N vs 62.0 ± 0.02 mm, 0.38 ± 0.02 mm, 25.1 ± 1.4N respectively in Pparβ+/+, P <0.05). From 3- to 18-months, differences between genotypes in TB lean and bone mass, mean force and running distance further increased. At 18 months of age, Pparβ−/− had lower BV/TV, CITV, CBV, and strength were also reduced in Pparβ−/− compared to Pparβ+/+.

Circulating myostatin increased more with age in Pparβ−/− (4.4- vs 2.9-fold, P <0.05). However, following axial compression, the increase in BMD, CITV, PsBFR and PsMP/BP was similar in both genotypes. These results indicate that Pparβ plays an important role on the acquisition and maintenance of muscle and bone mass. Hence, in absence of Pparβ, sarcopenia and osteoporosis develop with aging, paralleling an increase in myostatin. However the skeletal response to direct loading is maintained, suggesting that bone alterations are due to the loss of muscle functions and partly reversible.

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Glucagon-like peptide 1 receptor is required for optimal bone strength and quality
Aleksandra Miecikowska¹, Nigel Irwin², Peter R Flatt³, Daniel Chappard¹ & Guillaume Mabileaud¹
¹LUMAN Université, Angers, France; ²University of Ulster, Coleraine, UK.

Objectives
Glucagon-like peptide 1 is secreted by intestinal L-cells into the blood supply in response to nutrients in the intestine. Although osteoblasts express the GLP-1 receptor (GLP-1R), the main action of the GLP-1/GLP-1R pathway in bone physiology and bone quality is unknown. The aim of the present study was to investigate bone strength and quality in a mouse model of GLP-1R deficiency.

Materials/methods
Eight 16-week-old GLP-1R knock-out male mice, with a deletion of two exons of the glp1r gene, were age- and sex-matched with 12 wild-type (WT) mice. Resistance to fracture was studied by three-point bending in femur, whilst cortical microarchitecture was determined by high resolution microCT and quantitative X-ray imaging. Intrinsinc material properties were investigated by nanoindentation on backscattered electron imaging (qBEI) and Fourier-transformed infrared microscopy (FTIRM). Non-parametric Mann–Whitney U test was used to compare differences between groups.

Results
As compared with control mice, GLP-1R KO animals exhibited reduced bone strength as evidenced by significant decreases in ultimate load (−17%) and absorbed energy (−34%). Cortical microarchitecture was also affected in GLP-1R-deficient mice as demonstrated by significant reductions in cortical thickness (−13%) and cross-sectional moment of inertia (−25%). These microarchitectural modifications were accompanied by alterations of intrinsic material properties. Maximal load and hardness as assessed by nanoindentation on hydrated bone were both significantly reduced by 19%. Interestingly, bone mineral density distribution was not affected by GLP-1R deletion, but the ratio of mature/immature collagen cross-links was significantly reduced by 15%.

Conclusion
The inactivation of the GLP-1/GLP-1R pathway resulted in marked alterations of cortical microarchitecture, bone matrix properties and bone strength. Overall these data support an important role for the GLP-1/GLP-1R signalling pathway in bone quality. This is important given the recent introduction of GLP-1 mimetics for the treatment of type 2 diabetes mellitus.

Osteoporosis pathophysiology and genetics
OC3.1
Heavy cannabis use negatively impacts on bone density: a population based prospective study
Antonia Sophocleous¹, James McKenzie¹, Roy Robertson²,³ & Stuart H Ralston¹
¹Rheumatic Disease Unit, Institute of Genetics and Molecular Medicine, Centre for Molecular Medicine, University of Edinburgh, Edinburgh, UK; ²Muriehouse Medical Group, Edinburgh, UK; ³Centre for Population Health Sciences, University of Edinburgh, Edinburgh, UK.

The endocannabinoid system has important effects on bone mass and bone turnover. Mice with targeted inactivation of type 1 (CB1) and type 2 (CB2) cannabinoid receptors develop osteoporosis with increasing age raising the possibility that cannabinoid receptor agonists might protect against age-related bone loss. Since cannabis is the most widely used illegal drug and its main psychotropic component Δ⁹-tetrahydrocannabinol (THC) is an agonist at CB1 and CB2 receptors, we wanted to determine if there was an association between cannabis use and bone mineral density (BMD) in humans. The study comprised 109 regular cannabis users and 71 cigarette-smoking controls, prospectively recruited from the local community. Cannabis users were divided into two groups based on their lifetime exposure (joint-years) into moderate (0.01–57) and heavy subgroups (58–540). Cannabis users were younger than controls by about 10 years. Heavy users had a lower BMI (P = 0.002) and lower fat mass on DEXA (P < 0.001) compared to controls. They had substantially lower BMD Z-score values at the lumbar spine (P = 0.047) and total hip (P = 0.003) than controls with evidence of a dose effect such that heavy users had total hip Z-score values ~0.5 Z-score units lower than controls. A high proportion of heavy users were young men. There was no difference between users and controls in self-reported alcohol intake but heavy users smoked less tobacco (P = 0.025) and had higher dietary calcium intake (P < 0.001) than controls. Multivariate analysis showed that gender and BMI were the most important determinants of spine and hip BMD Z-score in the study cohort indicating that the negative effects of cannabis use on bone health might be due to an effect on BMI. We conclude that cannabis users have low bone mass at spine and hip, demonstrating that in people of this age, heavy cannabis use negatively impacts bone health.

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Design
We aimed to compare associations between intramuscular and subcutaneous fat and cortical bone outcomes in young adults, in cross-sectional analyses based on the Avon Longitudinal Study of Parents and Children.

Method
Data were collected from a research clinic conducted at 17 years of age. Intramuscular fat (IMF) (inverse of muscle density) and subcutaneous fat area (SFA) were estimated using Stratec XCT2000 pQCT, as were periosteal circumference (PC), cortical bone mineral density (BMDc), and cortical thickness (CT). Multivariable linear regression was used to assess the relationship between IMF/SFA and cortical bone outcomes. Interactions were examined with candidate metabolic pathways, i.e., insulin, C-reactive protein (CRP) and β-C-receptor (CTx), as measured on fasting blood samples.

Results
In analyses based on 3946 individuals (boys = 1703), IMF was positively associated with PC (β = 0.07; 95% CI 0.04, 0.1), BMDc (β = 0.21; 0.17, 0.26) and CT (β = 0.37; 0.33, 0.42) (adjusted for age, height, gender and muscle cross-sectional area). SFA was positively associated with PC (β = 0.10; 0.07, 0.12), but no association was seen with BMDc (β = −0.01; −0.05, 0.02) or CT (β = 0.01; −0.02, 0.04). In subsequent analyses (n = 2085, boys = 941)) adjustments for insulin, CRP and CTx were made to assess candidate intermediary metabolic pathways. Similar associations were observed after adjustment for insulin and CRP, but adjusting for CTx attenuated the association between IMF and BMDc by 30% (β = 0.14; 0.20, 0.08).

Conclusion
Although IMF and SFA were positively associated with cortical bone mass, the nature of these relationships differed in that IMF was predominantly associated with CT and BMDc, whereas SFA was mainly associated with PC. Other than a contribution of bone resorption to associations between IMF and BMDc, these relationships were independent of candidate metabolic pathways.

DOI: 10.1530/boneabs.1.OC3.3

OC3.5

Genome-wide association identifies a new susceptibility locus at 4q35 associated with clinical vertebral fractures in post-menopausal women: the GEFOS-GENOMOS consortium


1University of Melbourne, Melbourne, Victoria, Australia; 2Rheumatic Diseases Unit, The Centre for Molecular Medicine, IGMM, Western General Hospital, University of Edinburgh, Edinburgh, UK; 3Departments of Internal Medicine and Epidemiology, Erasmus Medical Centre, Rotterdam, The Netherlands; 4Department of Internal Medicine, Hospital UM Valdecilla, University of Cantabria, Santander, Spain; 5Department of Endocrinology and Internal Medicine THG, Aarhus University Hospital, Aarhus, Denmark; 6University of Queensland Diamantina Institute, Translational Research Institute, Brisbane, Australia; 7School of Medicine and Pharmacology, University of Western Australia, Perth, Western Australia, Australia; 8Department of Medical Biochemistry, Oslo University Hospital, Oslo, Norway; 9Department of Clinical Biochemistry, Lovisenberg Deacon Hospital, Oslo, Norway; 10Department of Internal Medicine, Hospital del Mar-IMIM, RETICEF, Universitat Autonoma de Barcelona, Barcelona, Spain; 11Department of Clinical Biochemistry, Faculty of Pharmacy, University of Ljubljana, Slovenia; 12Unidad de Medicina Molecular, Departamento de Medicina, Universidad de Salamanca, Hospital Universitario de Salamanca, RETICEF, Salamanca, Spain; 13Osteoporosis and Bone Biology Program, Garvan Institute of Medical Research, Sydney, Australia; 14Statens Serum institute, National Institute for Health Data and Disease Control, Copenhagen, Denmark; 15Department of Psychology, Centre for Cognitive Ageing and Cognitive Epidemiology, University of Edinburgh, Edinburgh, UK; 16Unidade de Xenectica, Facultade de Medicina, Instituto de Medicina Legal, Universidade de Santiago de Compostela, Santiago de Compostela, Spain; 17Fundacion Publica Galega de Medicina Xenomica (PGPMX-SERGAS), CIBER Enfermedades Raras, Santiago de Compostela, Spain; 18Grupo de Epidemiologia i Genetica Cardiovascular, IMIM, Barcelona, Spain; 19Medical Research Council, Epidemiology Unit, Institute of Metabolic Science, Cambridge, UK; 20Department of Public Health and Primary Care, University of Cambridge, Cambridge, UK; 21Wellcome Trust Sanger Institute, Cambridge, UK; 22Unidad de Recerca en Lipids i Epidemiologia Cardiovascular (URLEC), IMIM-Hospital del Mar, Barcelona, Spain.

Vertebral fractures (VF) defined by morphometric analysis of spine radiographs are the most common complication of osteoporosis. Those that come to medical attention, with symptoms such as back pain and kyphosis are termed clinical vertebral fractures (CVF) and account for significant morbidity and mortality. Although much progress was made in identifying loci for bone mineral density, the genetic determinants of CVF remain unclear. Here we present the initial results from a genome wide association (GWAS) study involving 1634 postmenopausal women with CVF recruited from 11 centres in Europe and Australia and 4692 regionally matched female controls. Cases were genotyped on the Illumina Omni Express platform whereas various platforms were used for the controls. We analysed 330 365 SNPs which were directly genotyped in both cases and controls. Standard quality control measures were applied. Each study was analysed separately and the results were combined using inverse-variance meta-analysis. The P value thresholds for suggestive and genome-wide significance were set at 1×10−4 and 5×10−8 respectively. We identified nine loci with suggestive association with CVF (P values ranging from 7.43×10−3 to 2.5×10−7) and one locus on chromosome 4q35 which was significantly associated with CVF (P = 7.28×10−5, odds ratio = 1.3 (95% CI 1.18-1.43)), to take account of multiple testing (P < 1.64×10−7). The associated SNP lies within the SORBS2 gene which plays a role in osteoclast and osteoblast activity. Expression of SORBS2 in bone biopsies was found to strongly correlate with that of Runx2 and other genes in the BMP pathway. Work is in progress through imputation and direct genotyping to replicate the associations we have observed in further and independent case-control studies. We conclude that this initial GWAS among postmenopausal women identifies nine suggestive and one significant locus for CVF, within a gene that regulates bone cell function.

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Causal metabolomic pathways to osteoporosis in elderly women
Ariteza Moayyeri, Deborah Hart, Idil Erte, Massimo Mangino, Christopher Hammond & Timothy Spector
Department of Twin Research and Genetic Epidemiology, King’s College London, London, UK.

Background
Recent technological ‘omics’ advances have empowered us to identify associations between genetic markers and various traits. Knowledge of serum metabolites as intermediary phenotypes can help us achieve a better understanding of the causal pathways from genes to complex diseases like osteoporosis.

Methods
In the context of TwinsUK study, serum metabolomic profiles of 6055 participants were assessed using a non-targeted mass spectrometry platform (Metabolon, Inc., Durham, NC, USA). The concentrations of 510 serum metabolites (211 unknown and 299 known molecules including amino-acids, lipids, carbohydrates, vitamins, peptides, and xenobiotics) were measured. Out of 6055 participants, 5224 (86.7%) were female twins with at least one hip and spine DXA measurement (Hologic devices) and 5605 (92.9%) had genome-wide genotyping scans (Illumina platforms imputed to 2.5 million single nucleotide polymorphisms). Genome-wide association studies (GWAS) for all metabolites and direct associations between metabolites and bone phenotypes (femoral neck, total hip, and lumbar spine BMD) were measured using mixed-effects models adjusting for age, weight, height and family-relatedness. Causal associations between metabolites and bone phenotypes were assessed using genetic markers as instrumental variables.

Results
Several metabolites showed significant associations (corrected for multiple testing) with bone phenotypes including prolyl-hydroxy-proline (P = 1.65 X 10^{-17}), pipecolic acid (P = 1.27 X 10^{-12}) and several sulphated steroids. In Mendelian randomisation analysis, epiandrosterone sulphate (encoded by CYP3A5 on chromosome 7q22.1) and 4-androsten-3-one were causally associated to an unknown metabolite encoded by ABCC4 on chromosome 13q32.1. was causally associated with femoral neck BMD. Total hip BMD changes were additionally caused by changes in 17-ketosteroid (encoded by SULT2A1 on 19q13.33) were causally associated with femoral neck, total hip, and lumbar spine BMD) were measured using mixed-effects models adjusting for age, weight, height and family-relatedness. Causal associations between metabolites and bone phenotypes were assessed using genetic markers as instrumental variables.

Discussion
To our knowledge, this is the first metabolome-genome-wide Mendelian randomisation study of human bone mineral density. Causal associations observed in this study can direct us to biological pathways involved in the pathogenesis of osteoporosis. Our results need replication in other studies.

DOI: 10.1530/boneabs.1.OC3.6

Osteoblasts and osteocytes

High-throughput DEXA and micro-CT screening in gene knockout mice identifies bone mass phenotypes
Robert Brommage1, Jeff Liu1, Laura Kirkpatrick1, David Powell1 & Peter Vogel1,2
1Lexicon Pharmaceuticals, The Woodlands, Texas, USA; 2St Jude Children’s Research Hospital, Memphis, Tennessee, USA.

Screening gene function in vivo is a powerful approach to discover novel drug targets in the human genome (Nat Rev Drug Discov 2:38–51, 2003). We present data for 3776 distinct gene knockout (KO) mouse lines with viable adult homozygous mice generated using both gene-trapping and homologous recombination technologies. Bone mass was determined from PIXImus DEXA scans of male and female mice at 14 weeks of age and by microCT analyses of bones from male mice at 16 weeks of age. Wild-type (WT) littermates were examined for each gene KO. For most lines DEXA scans were performed on four KO and two WT mice of each gender. Body BMC, aBMD, vBMD, and BMC/LBM ratio, femur BMD, and spine BMD were monitored. Bone parameters were normally distributed. Volumetric BMD in KOs (n = 3629) averaged 99.5% of WT values with a s.d. of 3.7%. Using a Scanco Medical μCT40, trabecular bone parameters in LV5 were analyzed in 3381 lines and midfemur cortical thickness (CT) in 3345 lines (four KO and two WT). Specially designed plastic inserts held 48 LV5s (scanned overnight with 4 LV5s scanned simultaneously) and 18 femurs (scanned in 30 min with six femurs scanned simultaneously). LV5 trabecular BV/TV in KOs averaged 16.2% (s.d. = 3.9%). Femoral CT averaged 245 μm (s.d. = 16 μm). Since primary high-throughput screens (HTS) are susceptible to false positive findings, additional cohorts of mice from KO lines with intriguing HTS bone data were examined. Aging, ovariectomy, histomorphometry and bone strength studies were performed on lines identifying potentially novel osteoporosis drug targets, and possible non-skeletal phenotypes were explored. Together, these screens identified previously reported (Lrp5, Sost, Wnt10b, Sfrp4, myostatin, Klotho, c-Src, Ostm1, and Crip3) as well as novel (Wnt16, Lrtk1, Fam20c, sphingosine-1P-lyase, and claudin-18) genes regulating bone mass.

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The p38 MAPK pathway in osteoblasts contributes to ovariectomy-induced bone loss by upregulating interleukin 6 expression
Cyril Thourevy & Joseph Caverzasio
University Hospital of Geneva, Geneva, Geneva, Switzerland.

Selective p38 inhibitors have been found to prevent bone loss induced by estrogen deficiency but implicated mechanisms remained to be identified. The p38 MAPK pathway has been suggested to influence bone resorption at different regulatory levels. In osteoblasts, p38α has been reported to be involved in the production of osteoclastogenic interleukin 6 and Rankl in response to various bone-resorptive agents in vitro. Therefore, we investigated whether p38α in osteoblasts may contribute to ovariectomy-induced bone loss in mice.

Mice lacking p38α in osteoblasts (Ocn-Cref; p38α^−/−) and their control littermates (p38α^+/−) were either sham-operated or ovariectomized at 12 weeks of age. Their bone phenotypes were assessed 6 weeks after operations by dXa, micro-CT, histogrammetry and gene expression analyses (n = 7 per group). Data were analyzed by two-way ANOVA and post hoc analyses were performed using the Holm-Sidak method.

Ovariectomy caused a decrease in bone mineral density in the spine (−13.1%; P < 0.001 vs sham) and to a lesser extent in the femur (−1.8%; P < 0.001 vs sham) of control mice but not in Ocn-Cref; p38α^−/− mice (+12.7% in the spine; +8.1% in the femur; P < 0.01 vs p38α^−/−). In addition, ovariectomy decreased vertebral cancellous bone volume (−45.8%; P < 0.01 vs sham), trabecular thickness (−16%; P < 0.01), and trabecular number (−20.6%; P < 0.01) in control mice but not in Ocn-Cref; p38α^−/− mice, indicating that mice lacking p38α in osteoblasts were protected from ovariectomy-induced bone loss. Consistent with this, ovariectomy caused an increase in osteoclast surface (fourfold), osteoclast number (threefold) and serum level of CTX (1.4-fold) in p38α^−/− mice but not in Ocn-Cref; p38α^−/− mice. Finally, ovariectomy induced a twofold increase in interleukin 6 expression in the long bones of p38α^−/− mice (P < 0.05), whereas this osteoclastogenic cytokine was downregulated in Ocn-Cref; p38α^−/− mice. Our findings indicate that the p38α MAPK in osteoblasts contributes to ovariectomy-induced bone loss by upregulating interleukin 6 expression.

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OC4.3

Severe osteopenia, increased bone marrow adipogenesis, and fibroblast matrix changes in mice lacking Tg2 and FxIIia transglutaminases

Aisha Mousa1, Cui Cui2, Aimei Song1, Vamsee Myneni1, Jingjing Li1, Gerry Melino2, Gerhard Dickneite3, Monzur Murshed1 & Mari Kaartinen1
1McGill University, Montreal, Quebec, Canada; 2University of Leicester, Leicester, UK; 3CSL Behring GmbH, Marburg, Germany.

Osteoblasts produce protein-crosslinking enzymes, transglutaminase 2 (Tg2) and factor XIIIa (FxIIia), which regulate fibroblast matrix stabilization and osteoblast differentiation in vitro. To examine if they are important in bone remodeling and in maintenance of bone quality and mass in vivo, we performed skeletal phenotyping of Tg2−/− and FxIIia−/− mice and generated a double-null Tg2−/−FxIIia−/− mouse. Tg2−/− mice showed no loss of bone mass and maintained normal bone mineral density (BMD) to 12 months age. FxIIia−/− mice showed normal BMD values at 3 and 6 months, but significantly decreased BMD (−6.6%) at 12 months. Supportive of synergistic functions, the double-null Tg2−/−FxIIia−/− mice were osteoporotic at 3 months of age, showing a significant decrease in femur BMD (−16.3%). Three-point bending tests showed significantly decreased bone strength. Micro-computed tomography of the Tg2−/−FxIIia−/− double-null mice showed significant alterations in trabecular bone parameters: decreased BV/TV (−57%), decreased Tr.N (−51%) and increased Tr.Sp (+49%). The fibroblast matrix from double-null bone showed significantly increased detergent solubility, suggesting defective matrix stabilization. Osteoblast number was significantly increased (N.Ob/B.Pm +35%); however, mineral apposition rate showed no difference suggesting enhanced cell proliferation but impaired differentiation of preosteoblasts and/or precursors in the double-null mice. Bone marrow adiposity showed large increases in both fat percent (+70.7%) and adipocyte numbers (+65%) suggesting that Tg2 and FxIIia might regulate an osteoblast-adipocyte switch via fibroblast matrix stabilization. The presence of an osteoblast-differentiation defect was further supported by a significantly higher RANKL/OPG ratio, this likely causing the observed increases in osteoclast number (N.Oc/Total (BM) +104%) and the resorption marker (RatLaps; +80%) consistent with the bone loss observed in the double-null mice.

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OC4.4

Glucocorticoid exposure reduces expression of sclerostin in bone marrow stromal cells

Sylvia Thiele1, Alexander Rauch2, Jan P. Tuckermann3, Lorenz C. Hofbauer1 & Martina Rauner1
1Division of Endocrinology and Metabolic Bone Diseases, Department of Medicine III, Technical University, Dresden, Germany; 2Department of Biochemistry and Molecular Biology, University of Southern Denmark, Odense, Denmark; 3Institute of General Zoology and Endocrinology, University of Ulm, Ulm, Germany; 4DFG Research Center and Cluster of Excellence for Regenerative Therapies, Technical University Dresden, Dresden, Germany.

Glucocorticoids (GC) are effective drugs in the treatment of inflammatory diseases, including various forms of arthritis. However, their use is limited by negative effects on bone mass and strength, resulting in increased osteoporotic fractures. Conditional knockout mice demonstrated that the GR in osteoblasts is essential for GC-dependent bone loss. Recent studies show that GC profoundly inhibit Wnt signaling by stimulating the expression of Wnt antagonists such as dickkopf-1 (Dkk-1). Here, we assessed the regulation of sclerostin (Sost), another Wnt inhibitor, by GC. Sost mRNA expression was down-regulated by 75% by pharmacological concentrations of GC, implying that GC might regulate osteoblast maturation in vitro. In contrast, osteoblasts from GR−/− mice exposed normal levels of Sost. However, compared to wild-type osteoblasts, in which Sost mRNA levels were decreased by 49% after DEX exposure, Sost was unchanged in osteoblasts from GR−/− mice, indicating that GR dimerization is a critical mechanism for Sost regulation. Given that we previously demonstrated that GR−/− mice lose bone in response to GC our data strongly suggest that regulation of Sost by GC is not essential for regulation bone mass by GC. In summary, we show that pharmacological concentrations of GC suppress Sost expression in a GR dimerization-dependent manner. Additionally, basic GR signaling seems to be required for Sost expression.

DOl: 10.1530/boneabs.1.OC4.4

OC4.5

Mechanical loading increases the effect of sclerostin antibody treatment in a mouse model of high turnover osteoporosis

Marc von der Mark-Soglio1, Gisela Kuhn1, Michaela Knesel2 & Ralph Müller1
1Institute for Biomechanics, ETHZ Zurich, Zurich, Switzerland; 2Musculoskeletal Disease Area, Novartis Institutes for Biomedical Research, Basel, Switzerland.

Sclerostin, a Wnt signaling antagonist encoded by the SOST gene, negatively regulates osteoblasts and inhibits bone formation. Mechanical loading, which induces bone formation, leads to a decrease in sclerostin levels. Recently, neutralizing antibodies against sclerostin were tested successfully for the treatment of osteoporosis in rodents. However, sclerostin is not the only signal involved in mechanotransduction. Therefore we investigated whether treatment with sclerostin antibodies (ScAb) can be improved by the addition of mechanical loading in a mouse model for postmenopausal osteoporosis. Forty 15-week-old C57BL/6 mice were subjected to bilateral ovarectomy. Treatment with ScAb (100 mg/kg, i.v. weekly) respective vehicle and mechanical loading of the 6th caudal vertebra of 8 or 0 N were started 5 weeks later for 4 weeks. Cyclic loading was applied via steel pins inserted in the 5th and 7th caudal vertebra (10 Hz, 3000 cycles, three times/week). The loaded vertebra was scanned by in vivo micro-CT (vivaCT 40, Scanoco Medical AG, Brüttisellen, Switzerland) at week 15, 20, 22, and 24. Static as well as dynamic parameters were evaluated. While the controls showed continuous bone loss, treatment with ScAb (0 N) as well as loading increased trabecular bone volumes fraction (BV/TV) by 13%. The combination of loading and ScAb led to a further increase by 28%. The increase in BV/TV was caused by thickening of trabecular while loss in trabecular number could not be prevented by any treatment. The combined treatment increased bone formation rate by 100% as compared to ScAb (0 N) alone and by 200% as compared to untreated and unloaded (0 N) mice while bone resorption rate was significantly reduced by 50% as compared to ScAb (0 N) and by 75% as compared to untreated (0 N) mice.

Our results show that mechanical loading is able to increase bone volume independently of sclerostin antibody treatment.

DOl: 10.1530/boneabs.1.OC4.5

OC4.6

Periostin synergizes with osteocytes β-catenin to mediate the adaptive skeletal response to loading

Nicolas Bonnet & Serge Ferrari
Division of Bone Diseases, Geneva University Hospital and Faculty of Medicine, Geneva, Switzerland.

Mechanical stimulation triggers periostin (Postn) expression in the peristeme and osteocytes (Oc), which downregulates Sost and activates β-catenin signaling. Hence the cortical bone response to loading is abolished in Postn−/− mice. Here we investigated the role of Oc β-catenin and its interaction with Postn on the bone biomechanical response. Postn−/− were bred with Oc-Ctnn−/− mice to generate Postn−/−;Oc-Ctnn−/− and Postn+/−;Oc-Ctnn−/− double heterozygotes, Postn+/−;Oc-Ctnn−/− double heterozygotes, Postn+/−;Oc-Ctnn+/− and their WT littermate. In vivo cyclic axial compression (40 cycles, 1500 peak microstrain, 7 min, 3 days/week) was applied to the left tibia for 2 weeks, while the non-loaded tibia served as paired control. Postn+/−;Oc-Ctnn+/−, Postn−/−;Oc-Ctnn+/− and double KO mice sustained a high rate of spontaneous fractures and death, and were therefore not subjected to mechanical stimulation. Postn−/−;Oc-Ctnn−/− were not different from WT, whereas Postn+/−;Oc-Ctnn−/− have significantly lower femoral BMD, BV/TV, CIBV, and CtTh (−8, −28, −62, and −5.8%, respectively, P<0.05). Double heterozygous mice were similar to Postn−/−;Oc-Ctnn−/−, indicating a predominant influence of Oc β-catenin on bone mass and structure. Following axial compression, tibial BMD gain was similar in Postn−/−;Oc-Ctnn−/−, Postn−/−;Oc-Ctnn+/−, and WT mice (+11.2±0.6 mg/cm²), indicating that neither Postn nor Ctnn haplinsufficiency impaired the biomechanical response. In lower loaded vertebra (10 Hz, 3000 cycles, three times/week), the loaded vertebra was scanned by in vivo micro-CT (vivaCT 40, Scanoco Medical AG, Brüttisellen, Switzerland) at week 15, 20, 22, and 24. Static as well as dynamic parameters were evaluated. While the controls showed continuous bone loss, treatment with ScAb (0 N) as well as loading increased trabecular bone volume fraction (BV/TV) by 13%. The combination of loading and ScAb led to a further increase by 28%. The increase in BV/TV was caused by thickening of trabecular while loss in trabecular number could not be prevented by any treatment. The combined treatment increased bone formation rate by 100% as compared to ScAb (0 N) alone and by 200% as compared to untreated and unloaded (0 N) mice while bone resorption rate was significantly reduced by 50% as compared to ScAb (0 N) and by 75% as compared to untreated (0 N) mice.

Our results show that mechanical loading is able to increase bone volume independently of sclerostin antibody treatment.

DOl: 10.1530/boneabs.1.OC4.5
and CBV increased 12% to 17% in the stimulated vs non-stimulated tibia of Postn/−/−;Oc-Ctnn/−/−, Postn/−/−;Oc-Ctnn/−/+ and Postn/−/+;Oc-Ctnn/−/− (all P < 0.05), but not in Postn/−/+;Oc-Ctnn nor in Postn/−/+;Oc-Ctnn/−/−. BMD increased with loading independently of the genotype. In conclusions, β-catenin haploinsufficiency in osteocytes affects post-natal bone remodelling but not the modelling induced by axial compression. However, the concomitant loss of one β-catenin and one periostin allele impairs the cortex biomechanical response, which implies that periostin and β-catenin expression in osteocytes synergize to mediate skeletal adaptation to loading.

DOI: 10.1530/boneabs.1.LOC4.6

Treatment of osteoporosis

OC5.1

A three-year randomized sham-controlled trial of low magnitude mechanical stimulation in an elderly sample: the ‘VIBES’ trial

Douglas Kiel1, Marian Hannan1, Emily Sisson3, Mary Bouxsein1, Bruce Barton3, Dawn Dewkett1, Jay Magaziner3, Sheryl Zimmerman1, Elizabeth Shane1, Elizabeth Teng Leary8, Danette Carroll1, Brett Allaire1, Thomas Lang6 & Clinton Rubin10

1Harvard Medical School, Institute for Aging Research Hebrew Senior Life, Boston, Massachusetts, USA; 2Boston University School of Public Health Data Coordinating Center, Boston, Massachusetts, USA; 3Harvard Medical School, Center for Advanced Orthopaedic Studies, Beth Israel Deaconess Medical Center, Boston, Massachusetts, USA; 4University of Massachusetts Medical School, Worcester, Massachusetts, USA; 5Division of Gerontology, Department of Epidemiology and Preventive Medicine, University of Maryland, Baltimore, Maryland, USA; 6Program on Aging, Disability and Long Term Care, Cecil C. Sheps Center for Health Services Research, University of North Carolina, Chapel Hill, North Carolina, USA; 7Columbia University College of Physicians and Surgeons, New York, New York, USA; 8Pacific Biomarkers, Seattle, Washington, USA; 9Department of Radiology, University of California, San Francisco, California, USA; 10Biomedical Engineering, SUNY, Stony Brook, New York, USA.

Non-pharmacologic approaches to preserve or increase BMD include whole body vibration (WBV). A meta-analysis and one-year randomized trial concluded that WBV has no effect on BMD in older women; however, previous trials were relatively brief and did not include a sham control group. Therefore, we conducted the Vibration to Improve Bone in Elderly Subjects (‘VIBES’) trial, a randomized, sham-controlled trial of 10 min of daily WBV (0.3 g at 30 Hz) in seniors recruited from 16 independent living communities around Boston, Massachusetts, USA. We randomized 174 men and women (89 active, 85 sham) with T-scores —1 to −2.5 who were not taking bone active drugs and had no diseases affecting the skeleton (mean age 82.8 ± 7 years, range 65–102). The trial was originally planned for 2 years, but was extended for a third year. Participants received calcium 1000 mg and vitamin D 800 IU. Shared trial was originally planned for 2 years, but was extended for a third year.

Conclusions

Risk factors at the start of treatment including BMI and BMD predicted fracture risk on treatment. Accounting for incident fractures and changes in BMD during treatment may also improve the fracture risk prediction while on treatment with denosumab.

DOI: 10.1530/boneabs.1.OC5.2

Effect of blosozumab on bone mineral density: results of a phase 2 study of postmenopausal women with low bone mineral density

Charles Besson1, Deborah Robins2, Robert Recker3, Jahangir Alam1, Alan Y Chiang1, Bruce Mitlak1, Adrien Sipos1 & Leijun Hu1

1Eli Lilly and Company, Indianapolis, Indiana, USA; 2Eli Lilly and Company, Indianapolis, Indiana, USA; 3Department of Radiology, University of California, San Francisco, California, USA.

Effect of blosozumab on bone mineral density: results of a phase 2 study of postmenopausal women with low bone mineral density. Administration of antibodies that neutralize sclerostin has been demonstrated to increase BMD. We report the key findings of a Phase 2 study of the human sclerostin antibody, blosozumab (bmab).

Methods

Study GSDB was a randomized, parallel-design, double-blind placebo-controlled study, designed to assess the dose-response relationship of bmab in postmenopausal women with low bone mineral density (BMD; lumbar spine (LS) T-score, −3.5 to −2.0). Participants were randomized to one of three subcutaneous (SC) bmab treatment regimens (180 mg every 2 weeks (Q2W); 180 mg every 4 weeks (Q4W), and 270 mg Q2W) or placebo for 52 weeks. In a study addendum, additional participants were randomized to bmab 270 mg SC every 12 weeks (Q12W) or placebo. Response was assessed as change from baseline in LS BMD, measured by dual energy X-ray absorptiometry (Table 1).

Secondary outcomes included evaluation of overall safety of bmab.

Results

Overall, 154 postmenopausal women were enrolled (mean baseline age 65 years, LS T-score: 2.76). BMD findings are tabulated (P < 0.001 bmab vs placebo in all cases). The frequency of adverse events was similar across treatment groups. Mild to moderate injection site reactions were more common with bmab.

Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Placebo (n=27)</th>
<th>270 mg Q12W (n=26)</th>
<th>180 mg Q4W (n=31)</th>
<th>180 mg Q2W (n=30)</th>
<th>270 mg Q2W (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Least square mean percent change in LS BMD from baseline</td>
<td></td>
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</tr>
<tr>
<td>12 weeks</td>
<td>−0.92</td>
<td>5.02</td>
<td>3.73</td>
<td>6.18</td>
<td>7.14</td>
</tr>
<tr>
<td>24 weeks</td>
<td>−0.77</td>
<td>6.08</td>
<td>6.32</td>
<td>10.70</td>
<td>12.38</td>
</tr>
<tr>
<td>52 weeks</td>
<td>−1.52</td>
<td>6.72</td>
<td>8.39</td>
<td>14.86</td>
<td>17.75</td>
</tr>
</tbody>
</table>

Background

There are no models for estimating risk of fracture in patients taking treatments for osteoporosis. Knowing a patient’s risk of fracture during treatment may help make future treatment decisions; therefore, the development of on-treatment fracture risk models is needed.

Methods

To assess on-treatment fracture risk, the analysis included subjects who received denosumab (DMAb) 60 mg Q6 every 6 months for at least 1 year in either FREEDOM or its study extension through 6 years, missed no more than one dose, and had complete data on clinical risk factors. Baseline risk factors examined included BMD, T-score, parental hip fracture status, and sCTX. To test the value of assessments during follow-up, we analyzed time-dependent risk factors updated at each year included age, incident vertebral fracture (VFx) and nonvertebral fracture (NVfx), BMD changes and years of exposure to DMB. A continuation ratio model was used for new or worsening VFx and a Cox regression model was used for NVfx.

Results

The baseline model for VFx included BMI and baseline spine and total hip (TH) T-scores; the time-dependent model added percent change in TH BMD, history of VFx as well as NVfx, and exposure to DMB during follow-up. The baseline model for NVfx included BMI, baseline TH T-score, parental hip fracture status, and baseline sCTX; the time-dependent model added history of VFx and NVfx and exposure to DMB during follow-up. Areas under receiver-operating curves indicated better predictive value for models including on-treatment risk factors compared to just the baseline models (0.66 vs. 0.61 for VFx and 0.62 vs. 0.58 for NVfx).

Conclusions

Risk factors at the start of treatment including BMI and BMD predicted fracture risk on treatment. Accounting for incident fractures and changes in TH BMD during treatment may also improve the fracture risk prediction while on treatment with denosumab.

DOI: 10.1530/boneabs.1.OC5.3

Fracture risk factors during treatment with denosumab

Steven Cummings4, Amy Feng5, Dennis Black6, Rachel Wagnman7, Matt Austin5, Andrea Wang9, Mona Walilime6, Lucy Wu6, Lily Lui1 & Eric Vittinghoff5

4CPMC Research Institute, San Francisco Coordinating Center, San Francisco, California, USA; 5Angen, Inc., Thousand Oaks, California, USA; 6University of California, San Francisco, California, USA.

Fracture risk factors during treatment with denosumab

Placebo (n=37) | bmab 270 mg Q12W (n=26) | bmab 180 mg Q4W (n=31) | bmab 180 mg Q2W (n=30) | bmab 270 mg Q2W (n=30)
<table>
<thead>
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</tr>
</thead>
<tbody>
<tr>
<td>12 weeks</td>
<td>−0.92</td>
<td>5.02</td>
<td>3.73</td>
<td>6.18</td>
</tr>
<tr>
<td>24 weeks</td>
<td>−0.77</td>
<td>6.08</td>
<td>6.32</td>
<td>10.70</td>
</tr>
<tr>
<td>52 weeks</td>
<td>−1.52</td>
<td>6.72</td>
<td>8.39</td>
<td>14.86</td>
</tr>
</tbody>
</table>

Bone Abstracts (2013) Vol 1
Romosozumab is an investigational bone-forming agent that inhibits sclerostin. Recent data demonstrated that it stimulated bone formation, decreased bone resorption, and led to rapid and substantial increases in areal bone mineral density (BMD; McClung, *Bone Miner Res* 27 (S1) S8–S9, 2012). In a Phase 1b, randomized, double-blind, placebo-controlled, multiple dose study, we studied the effects of romosozumab administered for 3 months and follow-up of 3 months after the last dose (month 6), in a group of 16 men (12 romosozumab, 4 placebo) and 32 women (24 romosozumab, 8 placebo). Quantitative computed tomography (QCT) was obtained at L1-2 in 24 subjects on romosozumab and 9 subjects on placebo and high resolution QCT (HRQCT) at T12 in a subset of 11 subjects on romosozumab and 3 subjects on placebo. The analyses pooled all romosozumab doses (1 mg/kg every 2 weeks, 2 mg/kg every 2 weeks, 2 mg/kg every 4 weeks, and 3 mg/kg every 4 weeks). Linear finite element modeling of bone stiffness was performed on both QCT and HRQCT data. Repeated measures mixed models were calculated and results expressed as least-square means ± S.E.M. Compared with placebo, the romosozumab group showed improvements at both months 3 and 6 for trabecular BMD by QCT and HRQCT (all P < 0.01), HRQCT-based density-weighted cortical thickness (dwCort.Th; P < 0.001), and HRQCT-based stiffness (P < 0.05). Three months following the last romosozumab dose, there were further improvements in HRQCT-based trabecular BMD and dwCort.Th (all P < 0.05). The improvement in HRQCT-based stiffness with romosozumab administration from baseline was 29.9 ± 6.7 and 34.0 ± 6.7% at months 3 and 6 respectively; placebo subject had changes of 0.9 ± 12.8 and 2.7 ± 12.8% respectively. In conclusion, romosozumab administered for 3 months resulted in very rapid and large increases in trabecular and cortical bone and bone stiffness, which continued to accrue in the 3 months following the last dose of romosozumab.

**Conclusion**

BMBM treatment resulted in a significant increase in LS BMD at all time points and with all doses and was generally well tolerated. These data support its continued clinical study as a potential therapeutic agent for the treatment of osteoporosis in postmenopausal women.

**DOI:** 10.1530/boneabs.1.OCS.5

### OC5.4

**Effects of romosozumab administration on trabecular and cortical bone assessed with quantitative computed tomography and finite element analysis**

C Graef1,2, G Campbell3, J Peña3, D Padhi3, A Grossman3, S Chang3, C Libanati1 & C-C Güler2

1GSI, Darmstadt, Germany; 2Sektion Biomedizinische Bildgebung, Klinik für Radiologie, UKSH, Kiel, Germany; 3Agenus, Inc., Thousand Oaks, California, USA.

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A randomized, placebo-controlled phase 2 study evaluated the safety and efficacy of BA058, an analog of hPTHrP: 12-month extension data from a phase 2 clinical trial in postmenopausal women with osteoporosis. 221 patients were randomized to receive BA058 20, 40, and 80 μg, placebo or teriparatide (TP) 20 μg, by daily s.c. injection. 184 (83%) patients completed an initial 24 weeks of treatment. The mean percent change in lumbar spine BMD at 24 weeks was 1.6% with placebo, 6.7% with BA058 80 μg, and 5.5% with TP. A marked increase in total hip BMD was also seen, where the change was 0.4% with placebo, 2.6% with BA058 80 μg, and 0.5% with TP. 55 of the 69 eligible patients received an additional 24 weeks of treatment. Lumbar spine BMD continued to increase, with a change at 48 weeks of 0.7% with placebo, 12.9% with BA058 80 μg, and 8.6% with TP. Gains in hip BMD were also seen, with a mean change for the total hip 0.7% with placebo, 2.7% with BA058 80 μg, and 1.3% with TP, and at the femoral neck 1.0% with placebo, 4.1% with BA058 80 μg, and 2.2% with TP. BA058 was generally well tolerated with treatment-related TEAEs reported in 66 (30%) of 221 patients during the initial 24 weeks of treatment and 16 (29%) of 55 patients during the extension, with similar proportions across treatment groups. Nine patients (4%) discontinued due to an adverse event; seven during the initial 24 weeks and two during the extension. SAEs were reported in four patients, none were treatment related. In conclusion, treatment with BA058 80 μg resulted in marked spine and hip BMD gains over 48 weeks. BA058 was well tolerated, with safety events comparable to placebo. The safety and efficacy data supported advancement of BA058 80 μg into an ongoing phase 3 fracture prevention study.

**DOI:** 10.1530/boneabs.1.OCS.5

### OC5.6

**Testosterone replacement has a substantial benefit on bone mass, fracture incidence, libido, and sexual activities in male cardiac transplant patients: a 5-year randomized prospective controlled trial**

Doris Wagner1, Guenther Premer1, Harald Dobing1, Hans Peter Dinaz2, Thomas Pieber1, Stefan Pilz1, Andreas Tomashitz1, Karin Arnerin1 & Astrid Fahrlein-Pannmer2

1Division of Transplantation Surgery, Department of Surgery, Graz, Austria; 2Division of Endocrinology and Metabolism, Department of Internal Medicine, Graz, Austria. 3Division of Cardiology, Department of Internal Medicine, Graz, Austria.

Hypogonadism is common in cardiac transplant (CTX) patients and exerts negative effects on bone but also on libido and quality of life. We investigated whether testosterone replacement therapy (TRT) has any positive effects on bone mass, fracture incidence, and quality of sex life when administered in addition to ibandronate (IBN) in hypogonadal CTX recipients. 52 male patients entered the study and received IBN (quarterly 2 μg i.v.), 60% of the patients were hypogonadal and were randomized to receive an additional testosterone therapy or IBN treatment only. At baseline, hypogonadal patients had considerably lower Z-score values at the femoral neck (−1.54 vs 0.15 ± s.d.) and total hip (−1.34 vs 0.01 ± s.d.) and more prevalent vertebral fractures (63 vs 14%, P < 0.0003) when compared to patients with normal gonadal function. After 5 years of IBN, BMD (bone mineral density) had increased in all patients; however, hypogonadal patients with additional TRT showed a significantly higher increase (femoral neck from 12.4 to 16.4%, total hip region from 10.2 to 14.7%, total hip from 9.2% after 1 year to 12.4% after 5 years of therapy; all P < 0.001) when compared to eugonadal patients and unreplacement hypogonadal patients. Fracture incidence was significantly lower in patients receiving TRT (P < 0.001) compared to only IBN treated patients. Baseline, 77% of the hypogonadal patients indicated a loss of libido and an average of seven annual sexual activities (27% of eugonadal men, P < 0.005 with 15 sexual activities P < 0.005). Patients with TRT reported an increase in sexual activities after 1 year (29 ± 8; P < 0.0001) and 5 years (25 ± 9; P < 0.005). No changes in sexual behavior were reported by the other groups. Hypogonadism has a deleterious effect on bone health in transplant patients. IBN therapy increases BMD in CTX patients on immunosuppressive treatment independently of gonadal status. Hypogonadal patients benefit from additional TRT over 5 years with respect to bone mass, fracture rate as well as quality of life. This is the first study that showed IBN in combination with TRT as a safe and well tolerated treatment in CTX patients with osteoporosis.

**DOI:** 10.1530/boneabs.1.OCS.6

### OC6.1

**Npp1 is a key regulator of skeletal and soft tissue mineralisation**

Mark Hajjawi1, Vicky MacRae2, Carmen Huesa3, Jose Luis Millan3, Blandine Poulet1, Timothy Arnett1 & Isabel Orriss1

1Dick School of Veterinary Studies, Edinburgh, UK; 2Sanford-Burnham Medical Research Institute, La Jolla, California, USA.

Ecto-nucleotide pyrophosphatase/phosphodiesterases (NPPs) hydrolyse nucleotide triphosphates to the corresponding nucleotide monophosphate and the mineralisation inhibitor, pyrophosphate (PPi). This investigation examined the role of NPP1 in bone and soft tissue mineralisation in a mouse model lacking NPP1 (Enpp1−/−). At physiological pH 7.35, cultured Enpp1−/− calvarial osteoblasts displayed ≥70% increase in bone mineralisation compared to wild types. Acidosis (pH 6.9), a well-known mineralisation inhibitor, completely
abolished bone mineralisation in wild-type cells but only decreased mineralisation ~30% in 
\textit{Enpp1}^{-/-} osteoblasts. Differentiating and mature \textit{Enpp1}^{-/-} osteoblasts showed ≥70% reduction in constitutive release of ATP, a key NPP1 substrate; this was accompanied by a ~20% increase in total intracellular ATP levels. Fluid flow increased ATP release less than eightfold in wild-type osteoblasts; this response was impaired by ~60% in \textit{Enpp1}^{-/-} cells. Previous studies demonstrated significant changes in the bone structure of \textit{Enpp1}^{-/-} mice. Here, we used microCT (0.5 \mu m) to examine cortical bone changes in detail. Cortical bone volume was increased 28% in 22-week \textit{Enpp1}^{-/-} mice, whilst cortical porosity was reduced 30 and 60% at 15 and 22 weeks respectively. This was accompanied by ~13% decrease in pore diameter and ≤38% increase in inter-pore distance. However, cortical thickness was ≥33% lower in 15 and 22 weeks \textit{Enpp1}^{-/-} mice; thus, their bones were thinner but denser and less porous. We noted that the number of viable osteocytes isolated from the long bones of \textit{Enpp1}^{-/-} mice was decreased ≤50%. These animals also display ectopic joint calcification; in the knee this was accompanied by 30, 15 and 15% reductions in epiphyseal trabecular bone volume, thickness and number, respectively; tibial subchondral bone was reduced ≤17%. MicroCT and histological analysis of soft tissues revealed for the first time calcification of the whisker follicles, ear pinna and hang of \textit{Enpp1}^{-/-} mice. Together, these data highlight the key role of NPP1 in regulating calcification of both skeletal and soft tissues.

DOI: 10.1530/boneabs.1.OC6.1

\section*{OC6.3} \textbf{Collagen XV as a bone matrix organizer}

David Vicente\textsuperscript{1}, Mikko Finnilä\textsuperscript{2}, Valerio Izzii\textsuperscript{1}, Jarkko Koivunen\textsuperscript{1} & Taina Pihlajaniemi\textsuperscript{1}

\textsuperscript{1}Department of Anatomy and Cell Biology, University of Oulu, Oulu, Finland; \textsuperscript{2}Department of Anatomy and Cell Biology, University of Oulu, Oulu, Finland.

Collagen XV is a secreted proteoglycan localized in the outermost layer of the basement membrane and in the fibrillar matrix. Previously, the collagen XV gene (COL15A1) has been linked to osteogenic differentiation, being identified mainly in mature osteoblasts forming new bone tissue or lining bone trabeculae. Our previous data on collagen XV knockout fetuses reports subtle skeletal changes. The aim of this study was to analyse skeletal changes in adult mice lacking collagen XV. To this end, we compared a control group of C57BL/6 male mice (n=8) with littermates lacking Coll15a1 (n=8). Formalin fixed left tibias and femurs were scanned by micro-computed tomography with 6.7 \mu m pixel size. Right hind limbs were tested for mechanical properties in three point bending and axial loading of the femoral neck. Lack of Coll15a1 decreased the trabecular bone volume fraction by 44 and 60%, mainly due to 40 and 58% decreases in the trabecular number in tibias and femurs respectively. Additionally, the distance between trabeculae was increased by 26% in the femurs. There were no changes in cortical bone morphometric parameters but increased mechanical strength was observed in the tibias and the femurs as well as in the femoral neck. Confocal laser scanning microscopy of rhodamin 6G stained osteocytic networks revealed less organized cortical bone in the Coll15a1 knockout mice. Our results suggest a novel role for collagen XV as a matrix organizer during osteoblastic bone formation.

DOI: 10.1530/boneabs.1.OC6.3

\section*{OC6.4} \textbf{Inhibition of PTH-induced vasorelaxation modulates its anabolic action}

Stephanie Gohin\textsuperscript{1,2}, Chantal Chenu\textsuperscript{1}, Andrew Pitsillides\textsuperscript{3}, Timothy Arnett\textsuperscript{2} & Massimo Marenzana\textsuperscript{1,4}

\textsuperscript{1}Imperial College London, London, UK; \textsuperscript{2}University College London, London, UK; \textsuperscript{3}Royal Veterinary College, London, UK; \textsuperscript{4}University of Oxford, Oxford, UK.

The relationship between bone formation and blood flow is unclear. Recently, PTH was reported to activate production of nitric oxide (NO), a potent vasorelaxing agent, in endothelial cells and we and others have confirmed a strong vasorelaxing action of PTH \textit{in vivo}. Here, we tested the hypothesis that a potent NO synthase inhibitor (L-NAME: NG-nitro-L-arginine methyl ester) may alter the effect of intermittent PTH (iPTH) on bone architecture by blocking its vasodilatory effect. Four groups of male BALB/c mice (n=8 per group) were daily injected subcutaneously for 28 days PBS, PTH (1-34) alone (80 \mu g/kg per day), PTH plus L-NAME (30 \mu g/kg per day, i.p.) or L-NAME alone. Hind limb perfusion was measured by laser Doppler imaging. Bone architecture in the femur was imaged by micro-CT \textit{ex vivo}. PTH increased lower limb blood flow by ≥30% within 10 min of injection, an effect that was sustained over the 20 min recording period, compared to placebo (P<0.001). Co-treatment with L-NAME abolished the action of PTH but L-NAME alone had no effect. These acute effects were not attenuated over 28 days repetition. No chronic effects of iPTH or L-NAME were evident when blood flow was monitored 24 h after the last injection.

As expected, iPTH increased femoral cortical thickness (+17%; P<0.001) and trabecular thickness in the secondary spongiosa of the distal femoral metaphysis (+26%; P<0.001). Co-treatment with L-NAME decreased trabecular bone volume (P<0.01) by reducing trabecular number and increasing structural model index, compared to PTH alone. In conclusion, PTH induced robust, acute increases in limb blood flow that were blocked by L-NAME. The anabolic action of iPTH was also blocked by L-NAME in the trabecular but not in the cortical compartment of the femur. These results suggest that the bone anabolic action of PTH could involve in part NO-mediated vasorelaxation.

DOI: 10.1530/boneabs.1.OC6.4

Bone Abstracts (2013) Vol 1
OC6.5

A protective role for FGF23 in local defence against disrupted arterial wall integrity?
Dongxing Zhu1, Neil Mackenzie1, Jose Luis Millan2, Colin Farquharson1 & Vicky MacRae1
1The Roslin Institute and Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Easter Bush, Roslin, Midlothian EH25 9RG, Scotland, UK; 2Sanford Children’s Health Research Center, Sanford-Burnham Medical Research Institute, La Jolla, California 92037, USA.

Increasing interest is focusing on the role of the FGF-23/Klotho axis in mediating vascular calcification. However, the underpinning mechanisms have yet to be fully elucidated. Murine VSMCs were cultured in calcifying medium for a 21-day period. FGF-23 mRNA expression was significantly up-regulated by 7 days (1.63-fold; \( P < 0.001 \)), with a concomitant increase in protein expression. mRNA and protein expression of both FGFR1 and Klotho were confirmed. Increased FGF-23 and Klotho protein expression was also observed in the calcified media of Enpp1K/K mouse aortic tissue. Reduced calcium deposition was observed in calcifying VSMCs cultured with recombinant FGF-23 (10 ng/ml; 28.1% decrease; \( P < 0.01 \)). Calcifying VSMCs treated with PD173074, an FGFR1 inhibitor, showed significantly increased calcification (50 nM; 87.8% increase; \( P < 0.001 \)). FGF-23 exposure induced phosphorylation of ERK1/2. Treatment with FGF-23 in combination with PD98059, an ERK1/2 inhibitor, significantly increased VSMC calcification (10 mM; 41.3% increase; \( P < 0.01 \)). FGF-23 may represent a novel therapeutic strategy for inhibiting vascular calcification.

DOI: 10.1530/boneabs.1.OC6.5

OC6.6

An emerging role of phospho1 in the regulation of energy metabolism
Karla Oldknow1, Nik Morton’s Morton3, Manisha Yadav5, Sophie Rajoanah3, Carmen Huesa1, Lutz Bunge2, Mathieu Ferron4, Gerard Karsenty4, Vicky MacRae1, Jose Luis Milan5 & Colin Farquharson1
1The Roslin Institute, Edinburgh, UK; 2SRUC, Edinburgh, UK; 3Queen’s Medical Research Institute, Edinburgh, UK; 4Columbia University, New York, New York, USA; 5Sanford Children’s Health Research Center, San Diego, California, USA.

Genetic approaches to bone physiology utilizing judicious gain and loss of function models have identified bone as an endocrine organ, being involved in the regulation of energy metabolism and reproduction. Recent advances expand our understanding and identify a new and unconventional role of bone beyond its classical functions. PHOSPHO1 is a bone specific phosphatase with a recognised role in bone mineralisation, but our present studies have now identified a novel role in bone mineralisation, but our present studies have now identified a novel role in energy homeostasis. An initial microarray screen identified Exp, encoding the phosphatase OST-PTP, to be highly expressed by Phospho1−/− osteoblasts. This was confirmed by RT-qPCR (20-fold increase; \( P < 0.05 \)) whereas Exp expression was significantly decreased in PHOSPHO1 overexpressing osteoblasts (\( P < 0.001 \)). Unexpectedly, no change was noted in serum levels of uncarboxylated (Glu) and under-carboxylated (Glu13) osteocalcin. Nevertheless, 120 day-old Phospho1−/− mice were hypoglycaemic (\( P < 0.05 \)) and showed significantly improved glucose (\( P < 0.05 \)) and insulin tolerance (\( P < 0.05 \)) compared to wild-type mice. These observations were consistent with smaller subcutaneous, mesenteric and epididymal fat deposits noted in Phospho1−/− mice (\( P < 0.001 \)), confirmed by MRI analysis which showed substantial differences in body composition. Metabolic and phenotypic changes were conserved following a chronic 12 weeks high-fat diet challenge, suggesting Phospho1−/− mice are protected from obesity. Ambulatory activity was unchanged in the Phospho1−/− mice and not the cause of increased energy requirements. Histological analysis of target tissues of Phospho1−/− mice revealed; smaller epididymal adipocytes, decreased fat content and increased mitochondria number in brown fat and decreased islet number in the pancreas (\( P < 0.05 \)). MRI indicated a fatty liver in Phospho1−/− mice. Significantly, PHOSPHO1 expression (mRNA and protein) was specific to bone with negligible levels recorded in liver, pancreas, muscle and fat, suggestive of a bone driven phenotype. Our findings indicate a novel role of PHOSPHO1 in the regulation of energy status in an osteocalcin independent manner with yet unidentified mechanisms.

DOI: 10.1530/boneabs.1.OC6.6
New Investigator Workshops
NIW1
Genomics and proteomics as emerging technologies in bone research
André G Uitterlinden¹,²,³
¹Department of Internal Medicine, Erasmus MC, Rotterdam, The Netherlands; ²Department of Clinical Chemistry, Erasmus MC, Rotterdam, The Netherlands; ³Department of Epidemiology, Erasmus MC, Rotterdam, The Netherlands.

The quantum leaps in scientific progress have frequently come from technological innovations, which can be referred to as the technology push. In the life-sciences this has been exemplified by the emergence of all kinds of ‘omics’ technologies reflecting the capacity to analyse complete and complex molecular mixtures in a hypothesis-free approach, also known as ‘fishing expeditions’ by more sceptical fellow scientists. Such approaches have been developed for DNA, RNA, and protein molecules and the Human Genome Project has been the flagship project to highlight the successful use of such technologies. As a result many human disease areas, including bone disease, have applied these technologies to progress biological understanding of disease mechanisms.

Driven by technological progress and concomitant shifts in research culture, gene discovery in complex diseases and traits has intensified in the past decade and led to some spectacular findings as a result of sequencing of human pedigrees with segregating bone diseases and genome-wide association studies (GWAS). GWAS build upon i) human genetic variation, ii) genotyping technology, iii) Bio-banks, and iv) collaboration in consortia. I will discuss progress in this field, based on using cohort studies and consortia. Similar but more recent developments have taken place in the fields of RNA expression profiling and measures of DNA methylation, as examples of genomics technologies together with proteomics and metabolomics by mass spectrometry methods. The latest developments include the application of Next Generation Sequencing technologies to analyse DNA sequence, RNA composition, and DNA methylation.

DOI: 10.1530/boneabs.1.NIW1

NIW2
How to manage your research time and team
Eric Hesse
Molecular Skeletal Biology Laboratory (MSB-Lab), Department of Trauma, Hand and Reconstructive Surgery, University Medical Center Hamburg-Eppendorf, Hamburg-Eppendorf, Germany.

Being efficient and productive in research is frequently linked to a structured organization of the available research time. This applies to any individual scientist including PhD students, Postdocs, or PIs. In particular PIs but also to some extend more senior Postdocs have a responsibility for more junior scientists and in the case of PIs even of an entire research team. This does not only require overseeing and organizing his own time but also the time and workload of others to ensure the smooth and successful career progression of an individual fellow scientists but also of the productivity of an entire lab. In addition to organizing a whole lab, developing research projects, acquiring funding, and writing scientific articles, PIs quite often handle a considerable amount of teaching responsibilities and, in the case of physician scientists, are also involved in clinical patient care. All of these tasks are highly demanding, complex, and time-consuming. It is therefore of great importance to develop personal concepts to structure and accomplish this great variety of challenges. These tasks apply in general to all levels of investigators including PhD students, Postdoc’s, and PI’s. This New Investigator Seminar therefore intends to provide the audience with the personal experience of two recently appointed PIs who run a research team, one more advanced senior Postdoc, and a PhD student. In the context of brief presentations the speakers will give insights into their current research life and will touch upon the critical aspects mentioned above, including coping with teaching load and organizing protected time to see patients in the clinics. Following the brief statements, an interactive podium discussion will follow to debate different point of views and to learn from each other in a non-intimidating atmosphere. Coming from different countries, the presenters will discuss specifics of different scientific systems while focusing on more common challenges. It is the goal of this New Investigator Seminar to provide the attendees with concepts on how to organize their time to be productive and efficient in their career. In addition, participants will learn different ways to combine basic science, clinical duties, and teaching responsibilities. Furthermore, we will have an in-depth discussion of approaches to successfully meet the challenges of running an entire research group.

DOI: 10.1530/boneabs.1.NIW2

NIW3
Getting started as a post-doc
Martina Rauner
Dresden, Germany.

The first step to getting started as a post-doc is finding an appealing post-doc position. Be proactive and begin the search and application process early. One of the most critical aspects to think about is the research area. It should be something that excites you and something where you can imagine to work in for the next couple of years. Once you have decided on a research area, you can start thinking about choosing an adviser. The adviser has great power to help build a career so it is advisable to meet the professor in person to find out whether both of your expectations are compatible. Also, you can talk to current and former post-docs who have worked with that investigator to obtain insider experiences on the quality of mentoring and established lab structures. Most post-docs find their positions through personal contacts. Talk to advisers, friends, and contacts from professional meetings. However, since you may wish to apply to more than one position, vacant positions may also be found at university websites or science journals. Finally, take your time to prepare a well-written and nicely organized application and allow for enough time to obtain letter of references and other official documents.

After finding a post-doc position and getting settled possibly in a new country, organize your thoughts on the project in such a way that you can set specific, achievable short-term and long-term aims. To be clear on what experiments you plan, a good knowledge of the scientific background is required. So, before you start planning the experiments, first get acquainted with your (new) research field. Try to think in figures right from the start and be focused on which experiments to perform to obtain the desired results. In research, you are required to have a great deal of resilience. If experiments do not work out the first time, do not frustrate and try again. Sometimes, it is necessary to broaden your horizon and try other things. Also talking to other scientists may help in overcoming particular challenges or lead to new collaborations for future projects. Finally, maintaining a healthy work/life balance will allow you to work more efficiently and keep you mentally and physically healthy. In conclusion, to make your time as a post-doc successful and enjoyable, set aims, work in an organized fashion, and maintain a good work/life balance.

DOI: 10.1530/boneabs.1.NIW3
New Investigator Seminar
NI1  Sclerostin/MEPE axis in OA: lessons from long bone development
Katherine Staines, Blandine Poulet, Colin Farquharson & Andrew Pitsillides

see PP27.

DOI: 10.1530/boneabs.1.PP27

NI2  A GWAS in an extreme high bone mass population shows excess signal from genes associated with BMD in the normal population
Celia L Gregson, Paul J Leo Leo, Graeme R Clark, George Davey Smith, Matthew A Brown, Jon H Tobias & Emma L Duncan Duncan

see PP31.

DOI: 10.1530/boneabs.1.PP31

NI3  Bisphosphonate influence on bone quality at molecular level: study of human jaw bone sequesters by Raman microspectroscopy
Cécile Olejnik, Guillaume Falfayrac, Alexandrine During, Marie-Hélène Vieillard, Jean Michel Maes, Bernard Cortet & Guillaume Penel

see PP38.

DOI: 10.1530/boneabs.1.PP38

NI4  New PI3Kα-specific inhibitor, BYL719: therapeutic interest in osteosarcoma
Bérengère Gobin, Marc Baud’huin, Céline Charrier, Soizic Hervouet, Frédéric Lezot, Frédéric Blanchard & Dominique Heymann

see PP139.

DOI: 10.1530/boneabs.1.PP139

NI5  Identification of a small molecule kinase inhibitor that enhances osteoblast differentiation of human skeletal (mesenchymal) stem cells through regulation of TGFβ signaling
Majken Storm Siersbaek, Abbas Jafari, Walid Zaher, Li Chen & Moustapha Kassem

see PP175.

DOI: 10.1530/boneabs.1.PP175

NI6  Depletion of the autophagy adaptor OPTN leads to increased osteoclast formation, fusion and survival as well as increased NF-κB activation in vitro
Rami Obaid, Sachin Wani, Stuart Ralston & Omar Albagha

see PP230.

DOI: 10.1530/boneabs.1.PP230

NI7  Phenotypic dissection of bone mineral density facilitates the identification of skeletal site specificity on the genetic regulation of bone
John P Kemp, Carolina Medina-Gomez, Karol Estrada, Denise Heppe, Carola Zillikens, Nicholas Timpson, Beate Pourcain, Susan Ring, Albert Hofman, Vincent W Jaddoe, George Davey Smith, André G Uitterlinden, Jonathan H Tobias, Fernando Rivadeneira & David M Evans

see PP282.

DOI: 10.1530/boneabs.1.PP282

NI8  Detection of autoantibodies to osteoprotegerin in patients with rheumatoid arthritis and their association with disease activity
Barbara Hauser, Philip Riches, Tamara Gilchrist, Jim P Wilson, William D Fraser & Stuart H Ralston

see PP383.

DOI: 10.1530/boneabs.1.PP383
Oral Posters
Clinical

OP1
Does vitamin D status impact on hip fracture incidence?: evidence of fracture variation with latitude and season in Sweden
Eugene McCloskey, Helena Johansson, Anders Oden & John Kanis
see PP384.
DOI: 10.1530/boneabs.1.PP384

OP2
Meta-analysis of the effects of vitamin D supplements on bone mineral density in adults
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Clinical case posters

PP1
Ten years follow up after prenatal transplantation of fetal mesenchymal stem cell in a patient with severe osteogenesis imperfecta
Cecilia Götherström1, Katarina Le Blanc1, Eva Aström2, Jahan Talsim1, Gail E Graham3, Uwe Ewald4 & Magnus Westgren5
1Karolinska Institutet, Stockholm, Sweden; 2Karolinska University Hospital, Stockholm, Sweden; 3Uppsala University Hospital, Uppsala, Sweden; 4Children’s Hospital of Eastern Ontario, Ottawa, Ontario, Canada.

Background
Treatment with multipotent mesenchymal stromal cells (MSC) has the potential to ameliorate mesodermal disorders.

Objective
To treat severe osteogenesis imperfecta (OI) with fetal MSC.

Methods
Ten years ago, we treated a fetus with OI type III (COL1A2: c.3008G>A, p.Gly1003Asp) in utero with fetal HLA-mismatched MSC. The procedure was uncomplicated. At the age of 4 months i.v. pamidronate treatment was started due to new vertebral compression fractures. Donor cells (range 0.1–16.4%) were detected in the bone at 9 months of age. At 8 years of age soon after a surgery, the patient was re-transplanted with 2.8×10^5/kg cells and the effect evaluated.

Results
At 10 years of age, 2 years after the combined surgery and re-transplantation, the patient’s ability to walk has improved. She takes dance classes and participates in modified indoor hockey. Over the last 2 years, her linear growth has improved from −6.5 to −6.0 SD. Since birth, 12 fractures and 11 vertebral compression fractures have been confirmed. She has developed scoliosis treated with a brace.

Conclusion
Our findings suggest that transplantation of allogeneic fetal MSC in OI is safe and re-transplantation is feasible. It is not possible from this single case to conclude on beneficial effects of MSC in OI, but the natural history of this severe form of OI is one of early morbidity and an infant with the same mutation who did not receive MSC treatment succumbed at 5 months of age despite postnatal bisphosphonate therapy.

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PP2
Osteonecrosis of the jaw in a patient with rheumatoid arthritis treated with an oral aminobisphosphonate: a clinical case report
Lorena Longato1, Loredana Cavalli2, Gemma Marcucci1, Alessia Metozzi2, Francesca Giusti2, Maria Luisa Brandi2 & Prisco Piscitelli3
1Local Health Authority ASL, Biella, Italy; 2Department of Internal Medicine, University of Florence, Florence, Italy; 3Euro Mediterranean Biomedical Scientific Institute (ISBEM), Brindisi, Italy.

Osteonecrosis of the jaw (ONJ) has been recently described after i.v. administration of amino-bisphosphonates and – less frequently – in association with the use of oral bisphosphonates. Bisphosphonate-related osteonecrosis of the jaw (BRONJ) may affect mandible bone (65%), maxilla bone (26%) and rarely (9%) both sites simultaneously. Although causality may never be proven, emerging experimental data have established a strong association between monthly i.v. bisphosphonate administration and ONJ. Current level of evidence does not fully support a cause and effect relationship between the use of oral BPs and ONJ. In this paper, we report a clinical case of BRONJ in a 73 years old woman affected by rheumatoid arthritis (RA) and periodontitis, after 3 years of treatment with alendronate 70 mg once a week, plus daily calcium and vitamin D. The patient developed a tooth abscess at the lower jaw, accompanied by increased inflammatory markers, that never returned to normal range despite antibiotic therapy, inducing deterioration of joint synovium. The worsening of joint status after the onset of ONJ was reflected by the progressive increase in the number of swollen (SJ) and tender (TJ) joints, by the deterioration of the score DAS 28 (which passed from 5.46 to 7.07), pain (with VAS increasing from 60 to 90), and by a progressively impaired quality of life, as reported using the HAQ score (from 1.25 to 2.5). The patient was switched to antifracture therapy with strontium ranelate and the osteonecrosis was successfully treated with antibiotics, surgical curettage and local ultrasonics.

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PP3
The possibility rule of new mutations in juvenile Paget’s disease (A rare case of mild JPD)
Judith Donath1, Gabor Speer2, Janos Kosa2, Peter Lakatos2 & Gyula Póor1
1National Institute of Rheumatology and Physiotherapy, Budapest, Hungary; 2First Department of Internal Medicine, Semmelweis University, Budapest, Hungary.

Background
Juvenile Paget’s disease (JPD) is a rare autosomal-recessive condition. The disease is typically diagnosed in infants or young children and characterized by a generalized increased in bone turnover, bone pain, skeletal deformity and increased risk of pathological fractures. In our knowledge, inactivating mutations in the TNFRSF11B gene, which encodes osteoprotegerin, cause JPD, yet. There are no randomized controlled trials which to offer the optimal form of the disease management. We summarize the result from the literature and describe a women presented characteristic features of JPD.

Methods
A 30-year-old women presented with both femur and tibia deformity and bone pain. She had pathological fracture when she was 10 years old. 5 mg zoledronic acid infusion was given. Serum alkaline phosphatase (ALP) level, radiology, bone scintigraphy, densitometry were monitored. Genetic markers were evaluated by PCR method.

Results
After zoledronic acid infusion bone pain and ALP level decreased, the densitometry increased. Genetically, after the target genes and regions selection, we found two mutations of genes CSF1 and DCSTAMP.

Conclusions
We conclude that intravenous zoledronic acid therapy are effective for supressing bone turnover and improving symptoms in JPD but the long-term effects on clinical outcomes are unclear.

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PP4
The impact of calcimimetic treatment on bone turnover in a renal patient with high turnover hyperparathyroid bone disease
Barbara Murray1, Sinead Kinsella2, Rory McQuillan3 & Alan Watson4
1Metabolism Laboratory, St Vincent’s University Hospital, Dublin, Ireland; 2Department of Nephrology, St Vincent’s University Hospital, Dublin, Ireland.

Introduction
The availability of bone turnover markers (BTMs) that are kidney independent has facilitated the monitoring of bone turnover in renal patients with hyperparathyroid bone disease. The effects of Cinacalcet on BTMs and on the relationship between parathyroid hormone (PTH) and BTMs were studied.

Methods
The formation marker procollagen type 1 N propeptide (P1NP) and resorption marker tartrate resistant acid phosphatase 5b (TRACP5b) were measured before and at 14 time points during Cinacalcet treatment at doses of 30, 60, 90 and 150 mg/day over 20 months.

Results
P1NP increased from 196.7 to 361.2 μg/l at 6 months and 389.6 μg/l at 12 months when PTH was 576.7, 195.2 and 98.9 ng/l. PINP decreased at each subsequent time point as PTH declined, reaching 89.5 μg/l when PTH was 24.7 ng/l. TRACP5b decreased from 10.3 to 7.16 U/l at 6 months, 5.19 U/l at 12 months and 3.27 U/l at 20 months. There was significant negative correlation between PTH and P1NP (r = −0.69; P < 0.05) when PTH > 90.0 ng/l but no significant correlation when PTH < 60.0 ng/l. Significant positive correlation was found between PTH and TRACP5b (r = 0.7679; P < 0.001) at all levels of PTH.

Conclusion
Increasing doses of Cinacalcet over a period of 20 months reduced PTH levels by 96%. Bone formation increased by 50% when PTH and TRACP5b (r = −0.69) were 96%. Bone formation increased by 50% when PTH was 576.7, 195.2 and 98.9 ng/l. P1NP decreased at each subsequent time point as PTH declined, reaching 89.5 μg/l when PTH was 24.7 ng/l.

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PP5 Late onset autosomal dominant hypophosphatemic rickets; confirmation of the diagnosis with genomic analysis

Symeon Tournis1, Ioannis Statthopoulos1,2, Kalliopi Lampropoulou-Adamidou1,2, Theodora Koromila3, Nikolaos Chatzistamatiou3, Michael Drogaris3, Christos Zafeiriis1, Konstantinos Makris1, Helen Markov1, Nikolaos Papaioannou1, Panagoula Kollia1 & Gazi Gazi1
1Laboratory for the Research of the Musculoskeletal System 'Theodoros Garofalidis', KAT Hospital, University of Athens, Athens, Greece; 2Third Orthopaedic Department, KAT Hospital, University of Athens, Athens, Greece; 3Laboratory of Human Genetics, Department of Biology, University of Athens, Athens, Greece; 4Rheumatology Department, KAT Hospital, Athens, Greece; 5Biochemistry Department, KAT Hospital, Athens, Greece.

Introduction
Autosomal dominant hypophosphatemic rickets (ADHR) is a rare form of inherited isolated renal phosphate wasting with two distinct clinical phenotypes; early-onset and late-onset. Late-onset ADHR is characterized by normal phosphate levels and growth during childhood, followed by osteomalacia with bone pain, pseudofractures and weakness in adolescence or adulthood, but with no lower extremity deformities. Most of the late-onset ADHR patients are women and pregnancy seems to be a precipitating event, while a number of patients may spontaneously resolve the phosphate wasting defect.

Case report
A 38-year-old female was referred to our department due to delayed union of a transverse cervical fracture of the left femur, severe hypophosphatemia, generalized bone pain and proximal muscle weakness. Past history revealed two distinct episodes of diffuse musculoskeletal pain following her pregnancies that resolved spontaneously. Family history was negative and her two children had normal phosphate levels. Radiology investigation revealed looser zones on pubic rami and right ischial ramus and diffuse osteopenia with biconcave deformation of lumbar vertebrae. Laboratory investigation revealed severe hypophosphatemia, phosphaturia, normal calcium, iPTH and 25(OH)D levels, while calcitriol levels were inappropriately normal. She was treated with phosphate salts and calcium and pregnancy seems to be a precipitating event, while a number of patients may spontaneously resolve the phosphate wasting defect.

PP7 Severe pregnancy- and lactation-associated osteoporosis: teriparatide treatment

Kalliopi Lampropoulou-Adamidou, Christos Kosmidis, Ioannis P Statthopoulos, Nikolaos A Papaioannou & George Trovas
Laboratory for the Research of Musculoskeletal System ‘Th. Garofalidis’, KAT Hospital, Athens, Greece.

Introduction
Pregnancy- and lactation-associated osteoporosis (PLO) is an uncommon disease. The majority of cases are seen in the third trimester or early post-partum in the primigravid women and the prominent clinical feature of PLO is the severe and prolonged back pain and height loss. To date the prevalence and the aetiology of this disorder are unclear and there are no guidelines for its treatment.

Case report
We report the outcomes of teriparatide (TRP) treatment in a woman suffering from severe PLO with six fragility vertebral fractures, severe back pain very low BMD and low levels of vitamin D.

Results
Breast-feeding was terminated (2 months after delivery) and treatment was started with calcium 500 mg/day, vitamin D, 2,200 IU/day and TRP 20 µg/day. Shortly after the initiation of TRP treatment, the back pain gradually decreased. Thirteen months later, the patient was almost free of back pain. There was no new clinical vertebral fracture. Her laboratory tests were all normal. BMD increased by 24.4% at the lumbar spine, 9.9 and 4.6% at the left and the right total hip and 12.6 and 7.8% at the left and right femur neck, respectively.

Conclusion
Women with PLO may suffer from fragility vertebral fracture(s), often multiple, which cause severe and disabling back pain and kyphosis in the third trimester of pregnancy or in the early post-partum. Treatment with TRP which stimulates bone formation, simultaneously with weaning, calcium and vitamin D supplementation, increases considerably BMD, improves severe back pain and quality of life, and prevents further occurrence of vertebral fractures, making TRP a helpful tool in restoring bone strength in PLO patients.

PP6 Diagnosis of fibrous dysplasia with DNA tests

Ioannis Statthopoulos1,2, Alexia Balanka1, Christos Baltas4, Kalliopi Lampropoulou-Adamidou1,2, Theodora Koromila3, Panagoula Kollia1, Symeon Tournis1, Nikolaos Papaioannou1 & Aikaterini Katsarla1
1Laboratory for the Research of the Musculoskeletal System ‘Theodoros Garofalidis’, KAT Hospital, University of Athens, Athens, Greece; 2Third Orthopaedic Department, KAT Hospital, University of Athens, Athens, Greece; 3Computed Tomography Department, General Hospital ‘Asklepieio Voulas’, Athens, Greece; 4Radiology Imaging Department, General Hospital of Athens ‘G. Genimatas’, Athens, Greece; 5Laboratory of Human Genetics, Department of Genetics and Biotechnology, Faculty of Biology, University of Athens, Athens, Greece.

Introduction
Fibrous dysplasia (FD) of bone is a benign, non-inheritable disease characterized by bone pain, bone deformities and fractures. Its prevalence is ~1 in 30,000 individuals and diagnosis is based on the clinical and radiologic findings and is confirmed by biopsy. Yet, in some cases biopsy is not applicable.

Case report
A young woman presented to our outpatient clinic with a history of pain localized at the distal half of the left tibia that had begun 8 years ago and appeared occasionally thereafter. The patient was otherwise healthy. Based on the clinical and imaging findings the predominant diagnosis was that of polyostotic FD. The patient denied a confirmatory bone biopsy, so genomic analysis offered an alternative approach, since FD has a demonstrated association with somatic mutations at codon 201 of the α subunit of G protein (Gαs), encoded by the GNAS gene.

Results
The R201C mutation was detected which was confirmatory for the diagnosis of FD.

PP8 Phenotypic change in a patient with hypophosphatasia with the onset of renal failure

Tim Cundy1, Toshimi Michigami2, Kanako Tachikawa2, Michael Dray1 & John Collins1
1Department of Medicine, University of Auckland, Auckland, New Zealand; 2Department of Bone and Mineral Research, Osaka General Medical Center, Osaka, Japan.

Hypophosphatasia is a recessively inherited disorder with a wide phenotypic manifestation ranging from lethality in neonates to asymptomatic in adults. The severity of the phenotype is largely determined by the nature of the ALPL mutations. We describe a previously asymptomatic adult whose phenotype dramatically changed after he developed renal failure. A 50-year-old man was diagnosed with IgA nephropathy. At age 52 (eGFR 50 ml/min) he suffered his first metatarsal fracture. A DXA scan showed osteopenia, and he was prescribed alendronate. His renal failure progressed and he began dialysis (CAPD) at age 55. Prior to and after starting CAPD he suffered multiple non-traumatic fractures.
Fibrodysplasia ossificans progressiva.

No effective medical treatment is available. Surgical treatment is almost always avoiding contact sports and surgical/dental procedures. Corticosteroids, etidronate, aluminium. Genetic analysis showed compound heterozygosity for missense mutations in ALPL (T117H and G438S). Expression plasmids for the mutant ALPs fused to green fluorescent protein were transfected into COS7 cells, and the cell lysates were harvested to assay enzymatic activity. The T117H mutant had almost no enzymatic activity, but the G438S mutant retained similar activity to wild-type ALP. Six months treatment with teriparatide produced an increase in ALP activity and histological improvement in bone, but significant side effects. After the restoration of renal function by transplantation there was complete symptomatic and histological resolution. It is probable that as the patient developed renal failure, phosphate retention inhibited his residual ALP enzyme activity, resulting in a marked clinical deterioration – an interesting example of a reversible genotype-environment interaction affecting phenotype.

PP9
Fibrodysplasia ossificans progressiva
Firuzan Altun1, Ozer Burnaz1, Levent Özgönenc1,2 & Nil Çağlar1,2
1Kocaeli Derince Education and Research Hospital, Kocaeli, Turkey; 2Istanbul Education and Research Hospital, Istanbul, Turkey.

Fibrodysplasia ossificans progressiva (FOP) or myositis ossificans progressiva is a hereditary mesodermal tissue characterized by progressive ossification of striated muscle, tendon, ligament, fasciae, aponeurose and occasionally skin. A single common heterozygous mutation has been identified in the cytoplasmic domain of activin receptor IA/activin-like kinase 2 (ACVR1/ALK2). FOP is very rare with a worldwide prevalence of ~1 case in 2 million individuals. Diagnosis is based on clinical observations and radiological findings. There is often a significant delay between the onset of the disease and its diagnosis because it may be confused with infection, bruising or tumor. Disease is frequently seen in adolescents and young adults with male predominance. Treatment consists of supportive care, genetic counselling and education regarding the importance of avoiding contact sports and surgical/dental procedures. Corticosteroids, etidronate, radiotherapy and surgery have been used with limited efficacy. Etidronate has been used to prevent recurrence of ectopic ossification after removal of bone. No effective medical treatment is available. Surgical treatment is almost always contraindicated, since new heterotopic ossification can develop. We report a 33 years old man with fibrodysplasia ossificans progressiva and review literature about FOP in light of this case.

Key words: Fibrodysplasia ossificans progressiva.

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PP10
Hajdu–Cheney syndrome: report of two cases in a family
Georgina Terroso1, Miguel Bernardes1, Abelia Aleixo1, Pedro Madureira1, Romana Vieira1,2, Rita Fonseca1, Diana Goncalves1 & Lucia Costa1
1Centro Hospitalar Sao Joao, Porto, Portugal; 2Hospital do Funchal, Funchal, Portugal.

Objectives
To describe two familiar cases of Hajdu–Cheney syndrome, a rare genetic disorder associated with skeletal dysplasia, craniofacial abnormalities, short stature,acro-osteolysis and osteoporosis.

Materials and methods
A 51-year-old woman (case 1) presented in our outpatient clinic with pseudo-clubbing of some fingers and toes. She was short (139 cm) and thin (34 kg). She had facial and cranial abnormalities: thin lips, long philtrum, full cheeks, micrognathia, short neck, bushy eyebrows and coarse hair. Upon palpation, open skull sutures were noted. Her 21-year-old daughter (case 2) was also observed and showed similar facial and cranial abnormalities with short stature (141 cm) and low weight (31 kg).

Results
Imaging in case 1 revealed: radiographs withacro-osteolysis of some distal phalanges in fingers and toes, persistence of skull sutures and enlargement of the sella turcica. Bone densitometry with dual-energy X-ray absorptiometry (Lunar Expert): T-score of ~4 in lumbar spine (L1–L4) and a T-score of ~2 in total hip and femoral neck. Blood and urinary test results revealed high β-crosslaps and low vitamin D levels, without further abnormalities. Investigation in case 2 revealed: radiographs with persistence of skull sutures and enlargement of the sella turcica. Bone densitometry with dual-energy X-ray absorptiometry (Lunar Expert): T-score of ~2.3 in lumbar spine (L1–L4) and a T-score of ~1.1 in total hip and femoral neck. Blood and urinary tests revealed high beta-crosslaps and very low vitamin D levels, without further abnormalities.

Conclusion
Based on clinical, radiologic and laboratory findings, Hajdu–Cheney syndrome was diagnosed in both cases. Recently, it was found that mutations in the NOTCH2 gene are responsible for the syndrome. The majority of the reported cases are sporadic although a genetic background with an autosomal dominant pattern of transmission has been reported. Our cases further support the syndrome’s inheritable pattern.

DOI: 10.1530/boneabs.1.PP10

PP11
Gorham disease; a case with severe cervical spine involvement
Georgina Terroso1, Andre Rodrigues Pinho2, Manuel Santos Carvalho2, Joana Freitas2, Francisco Serdoura2 & Vitorino Veludo1,2
1Rheumatology Department of Centro Hospitalar Sao Joao, Porto, Portugal; 2Orthopaedics and Traumatology Department of Centro Hospitalar Sao Joao, Porto, Portugal.

Introduction
Gorham disease (GD), also known as Gorham–Stout syndrome, massive osteolysis or disappearing bone disease, is a very rare disease characterized by spontaneous and progressive osteolysis of one or more bones. Its prognosis is highly variable and unpredictable, ranging from minimal disability to death, due to involvement of vital structures, such as the vertebral column and rib cage. Osteoclasts hyperactivity has been suggested as potential pathogenetic mechanism for GD but lymphangiomatous vessel proliferation may be the responsible for bone osteolysis as well as soft tissue involvement.

Description of methods
A 26-year-old man was observed for cervical pain. Loss of lordosis and limited range of movement were noted at the cervical spine.

Results
Cervical X-rays showed vertebral lysis and instability. Laboratory blood exams didn’t show any abnormalities. Dissectomy and anterior arthrodesis were performed, complicated by easy bleeding and neurological symptoms (left C6 radiculopathy) immediately afterwards. Re-intervention was required. Histological examination revealed a hemangiomatous lesion.

Three months later, X-rays showed C3–C4 subluxation which required surgical correction. Imaging (X-ray and IRM) evolution since the symptoms started revealed progressive osteolysis with disappearance of posterior vertebral structures.

Conclusion
Cervical GD diagnosis was made based on clinical, imaging and histopathological abnormalities. There is no recognized effective treatment for this disorder. Surgery, radiotherapy, therapy with biphosphonates or interferon-α2b have been tried. In this case, the patient remained in stable remission after surgical management. The report of such a rare disease and in an uncommon site should be kept in mind in the differential diagnosis of osteolysis of unknown cause.

DOI: 10.1530/boneabs.1.PP11

PP12
Severe osteoporosis associated with Hajdu–Cheney syndrome: follow-up after 2 years of teriparatide therapy
Georgina Terroso1, Miguel Bernardes1, Abelia Aleixo1, Romana Vieira1,2, Pedro Madureira1, Rita Fonseca1, Diana Goncalves1 & Lucia Costa1
1Centro Hospitalar Sao Joao, Porto, Portugal; 2Hospital do Funchal, Funchal, Portugal.

Objectives
To describe the response to treatment with teriparatide for osteoporosis associated with Hajdu–Cheney syndrome after a follow-up 2 years.

Materials and methods
A 51-year-old woman presented in our outpatient clinic with pseudo-clubbing of some fingers and toes. She was short (139 cm) and thin (34 kg). She also had some

Bone Abstracts (2013) Vol 1
Arthritis and other joint diseases: translational and clinical
PP13
Clinical and histomorphometrical assessment of bone quality in hip osteoarthritis and osteoporosis
Maurizio Peola, Cecilia Rao, Monica Celi, Elena Gasbarra & Umberto Tarantino
University of Tor Vergata, Rome, Italy.

Osteoarthritis (OA) and osteoporosis (OP) are two diseases characterized by the alteration of bone quality, that affect mainly elderly people reducing their quality of life. Although an inverse relationship between has been shown by some studies, other reports supported the co-existence of these pathologies. In this study we combined clinical and structural features to clarify the relationship between OA and OP.

Among all the patients who underwent a total hip Arthroplasty in our Hospital we selected 80 patients, divided into four groups according to BMD values and diagnosis, femoral neck fractures (n=20, mean age 79.7 ± OA (n=60: 20 patients with normal BMD, 20 patients with osteopenic BMD and 20 patients with osteoporotic BMD; mean age 68.4 years).

We performed an X-ray of the hip to assess the OA severity through the Kellgren–Lawrence scale and we used HHS to evaluate the functionality of the hip and the clinical severity of OA.

During surgery, an osteotomy of the femoral head was performed and the samples were used for histomorphometry through Bio Quant software.

Histomorphometrical analysis showed that bone volume fraction was significantly lower in subjects with femoral neck fracture (19.98 ± 4.72%) than subjects with non-osteopenic OA (31.19 ± 5.47%); P < 0.01) or osteoporotic OA (28.45 ± 5.77%; P < 0.01), respectively. No difference between subjects with OP fractures and those with combined OA and OP (23.58 ± 4.47%) was detected.

Our data supports evidence indicating impaired bone quality in patients with OA and the absence of the protective effect against OP. The worst bone quality in patients with the lowest HHS and the most surface macroscopic alterations suggests that severe OA can be related to OP especially in older patients. It could be useful to determine the presence of a condition of Poor Bone Quality in patients with severe OA who need surgery, to make an adequate pharmacological and surgical approach.

DOI: 10.1530/boneabs.1.PP13

PP14
Prophylaxis of gout flare with colchicine and vitamin C
Simeon Monov1, Daniela Monova2 & Rasho Rashkov1
1Medical University, Clinic of Rheumatology, Sofia, Bulgaria; 2Medical Institute, MVR, Sofia, Bulgaria.

Background
The incidence and prevalence of gout have markedly increased over the last few decades in keeping with the rise in prevalence of obesity and metabolic syndrome.

The management of gout in patients with associated metabolic syndrome and comorbid illnesses such as renal impairment was difficult because of limited treatment options. Recent efficacy and safety data favour lower over higher doses of colchicine, and oral corticosteroids over non-steroidal anti-inflammatory drugs for patients with acute gout. Colchicine is a tricyclic alkaloid that interrupts multiple inflammatory response pathways. Its principal mechanism of action in gout is thought to be inhibition of cytoskeletal microtubule polymerization, an important process in neutrophil functioning.

Objectives
This article discuss the prophylaxis of chronic gout with colchicine therapy and supplementation with vitamin C.

Methods
We aimed to evaluate the effect of regular colchicine treatment in patients with gout. Ninety six patients (84 males and 12 females, 57.6 ± 19.8 years) with gout (mean duration 5.4 ± 1.3 years, average number of attacks over the past year 4.56 ± 1.8, mean duration of last attack 5.74 ± 2.58 days) were included in the study. These patients were on colchicine (0.5 mg daily, per os) and vitamin C (500 mg every other 5 days, per os) treatment were studied again no earlier than 6 months.

Results
We found in 6-month observation period gouty attacks in only 21 patients with mean duration of 4.23 ± 1.2 days. Ten patients stopped treatment after 3 months because of side effects occur – diarrhea, nausea. There were no any other clinical or laboratory changes.

Conclusions
Administration of colchicine in low doses with vitamin C markedly reduces the gout attacks.

Keywords
Colchicine, vitamin C, gout attacks.

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PP15
Cartilage intermediate layer protein is produced in synovial membrane of osteoarthritic joint and upregulated in osteoarthritic associated fibrosis
Irina Kerna1, Kalle Kissand2, Ann Tammi3 & Agu Tammi1
1Department of Internal Medicine, University of Tartu, Tartu, Estonia; 2Department of Immunology, University of Tartu, Tartu, Estonia; 3Department of Sports Medicine and Rehabilitation, University of Tartu, Tartu, Estonia.

Introduction
Cartilage intermediate layer protein (CILP) is a promising marker of osteoarthritis (OA). CILP is an extracellular matrix glycoprotein, which is produced by cartilage chondrocytes. Still, there are data that CILP could be found also in other tissues. We aimed to investigate the expression level of CILP mRNA in the synovial membrane and evaluated the associations of CILP expression with traits of radiographic knee OA (rKOA) and features of histological synovitis.

Methods
The synovial biopsy samples were harvested during arthroscopy from 44 subjects with chronic knee complaints. The rKOA features (the presence of osteophytes, joint space narrowing (JSN)) were evaluated on plain radiographs. Different histological features of synovitis (number of synovial lining cells, lymphocyte infiltration, fibrosis, hyperaemia, fibrin deposits, and perivascular oedema) were graded 0–3 in synovial biopsies. Expression of CILP mRNA in synovial samples was measured by the TaqMan gene expression assay.

Results
The early rKOA (grade I) was found in 29 subjects and late rKOA (grade II–III) in ten subjects. The CILP mRNA expression was observed in 96% of synovial samples. The downregulation of CILP mRNA in synovial membrane was observed in patients with late stage of JSN, than compared to subjects without radiographic changes (P=0.006). The analysis of histological synovitis features revealed that CILP mRNA is overexpressed in fibrotic samples and correlated with severity of synovial fibrosis (P=0.31, P=0.026). Additionally, the negative correlation with thickness of synovial lining (P=−0.4, P=0.003) and CILP mRNA was observed.

Conclusion
• The production of CILP seems to be not restricted to cartilage. The presence of CILP mRNA in synovial tissue suggests its possible production in synovium.
• Upregulation of CILP mRNA in fibrotic synovial tissue could suggest the involvement of CILP in OA-associated remodelling of the synovial membrane.
• Synovial production of CILP mRNA seems to be downregulated in late radiographic stage of knee OA.

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PP16

U-C2C in Estonian early knee OA cohort: progressive and non-progressive cases
Agu Tammi, Ann Tammi, Jaanika Kumm, Marek Vija & Mare Lintrop
University of Tartu, Tartu, Estonia.

Biomarkers are required to detect early OA for intervention and to monitor disease progression. A collagen type II neoepitope C2C was developed for these purposes. The aims of the study to test: i) the biomarker’s ability to differentiate osteo-arthritis (OA) patients with and without structural changes and ii) possible contribution of progression of the OA.

Material and methods
We investigated 159 knee OA patients aged 36-62 (mean 50) years. For 112 patients the progression of the knee OA during the past 3 years was available. Standardised radiographs of the tibiofemoral (TF) and patello-femoral (PF) joints were assessed. Radiographic progression was defined as: i) the presence of osteophytes and/or joint space narrowing (JSN) in subjects with no previous radiographic OA or ii) an increase in the grade of them.

The immunoassay used was C2C-HUSA (IBEX, Canada) that measures the C2C neoepitope fragments present in human urine samples.

Results
The most frequent radiographic finding was osteophytosis in the TF compartment. A significant correlation (r = 0.460, P < 0.0001) between output of uC2C and TF grades of OA was found. There was a highly significant difference in uC2C between the groups with TF grade 0 and grade 2 (or 3). UC2C excretion was significantly higher in patients with progressive OA (P < 0.0001). A large part of the variability of knee OA was describable by clinical risk factors, i.e. age, gender, and overweight. In case uC2C was added into models, description of variability of osteophytosis in the TF joint improved the model from 15 to 24%, and in the PF joint – from 4 to 23%.

Conclusions
Presence of osteophytosis, whether isolated or in combination with the JSN form, played a crucial role in this series. UC2C excretion was significantly higher in patients with OA in comparison of cases without clinical changes. Many cases with progressive OA had increased output of uC2C.

A substantial impact of uC2C was observed in the models of osteophytosis.

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PP17

Bone is the main target of activation of Canonical Wnt pathway in osteoarthritis
Thomas Funck-Brentano1,2, Wafa Bouaziz1,2, Valerie Geofferoy3, Didier Hannouche1,2, Caroline Marty4,5, Eric Hay1,2 & Martine Cohen-Solal1,2
1INSERM UMR-606, Paris, France; 2Université Paris-Diderot, Sorbonne Paris Cité, Paris, France; 3Department of Rheumatology, Lariboisière Hospital, Paris, France.

Objective
Wnt/β-catenin pathway is a main regulator of bone remodeling, but might be inhibited in cartilage in osteoarthritis (OA). We here investigated the effect of mechanical loading in Wnt activation and the expression of Wnt antagonists in the joint tissues.

Methods
Topgal mice were used. Mice underwent partial meniscectomy (Mx) and sacrificed at 4, 6, or 9 weeks. Dissected knees were scanned by microCT and then prepared for cryosectioning to quantify Wnt activity by X-gal staining and the expression of Wnt antagonists such as Dkk-1, sclerostin, and sFRP-3.

Results
At baseline, Wnt activation was mainly located in osteocytes in subchondral bone and mostly absent in articular cartilage. In subchondral bone, osteocytes displayed a decrease in Wnt activity at week 4 (Mx/sham knees compared to baseline: 0.50 ± 0.08, P = 0.034), and then an increase at weeks 6 and 9 (1.63 ± 0.43, P = 0.004 and 2.33 ± 0.82, P = 0.009 respectively). This activity paralleled the changes in BV/TV. Wnt activity was found also in the endocortical surface of growing osteophytes and in the perichondrium. The activation of Wnt was low in articular chondrocytes during the development of OA, but increased in focal cartilage lesions. In late stages, Wnt activation remained predominant in subchondral bone, osteophytes and synovium of Mx-knee. Moreover, the expression of Dkk-1 markedly decreased in chondrocytes of the superficial layers of cartilage after partial meniscectomy compared to the sham-operated mice in which Dkk-1 was highly expressed. Sclerostin and sFRP-3 were expressed only in calcified cartilage and increased with the loss of cartilage in OA.

Conclusion
The canonical Wnt signaling pathway is mainly activated in the surrounding tissues in particular in subchondral bone and osteophytes. Therefore, modulators of Wnt activity might have different impact in joint tissues in OA.

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PP18

Milk fat globule-epidermal growth factor factor 8 is a critical determinant of bone mass and alters the course of inflammation in arthritis
Kathrin Sinningen1, Sylvie Thiele1, Sylvia Grossklaus2, Mark Udey3, Lorenz C Hofbauer4, Triantafyllos Chavakis5,6 & Martina Rauner7
1Division of Endocrinology, Diabetes, and Bone Diseases, Department of Medicine III, Technical University, Dresden, Germany; 2Division of Vascular Inflammation, Diabetes and Kidney, Department of Medicine III, Technical University, Dresden, Germany; 3Dermatology Branch, Center for Cancer Research, National Cancer Institute, NIH, Bethesda, Maryland, USA; 4DFG Research Center for Regenerative Therapies, Dresden, Germany.

Milk fat globule-epidermal growth factor factor 8 (MFG-E8) is a glycoprotein that controls the engulfment of apoptotic cells and exerts anti-inflammatory effects. It has been implicated in the pathogenesis of several diseases, but its role in the bone microenvironment is still unknown. Here we tested the hypothesis that MFG-E8 also regulates bone metabolism and the development of arthritis.

MFG-E8 expression was detected in mouse bones and primary murine osteoblasts and osteoclasts. MFG-E8 expression levels in osteoblasts increased with cellular differentiation and reached a maximum after 14 days (3.4-fold). In osteoclasts, MFG-E8 expression increased up to 20-fold in mature osteoclasts. To elucidate whether MFG-E8 affects bone remodeling, we analyzed the bones from 6 weeks old MFG-E8-knockout (MFG-E8-KO) and wild-type (WT) mice. The trabecular bone mineral density at the lumbar spine in MFG-E8-KO mice was reduced by 10% (P < 0.01) compared to WT mice. Serum levels of the bone formation marker P1NP were decreased by 57% (P < 0.01) in MFG-E8-KO mice as were the mRNA levels of several osteoblast markers (Runx2, 50%; alkaline phosphatase, 60%; and osteocalcin, 75%). In contrast, bone marrow macrophages from MFG-E8-KO mice differentiated more effectively into osteoclasts compared to wild-type cells, producing threefold more osteoclasts.

To further determine whether MFG-E8 also plays a role in inflammatory arthritis, we subjected MFG-E8-KO and WT mice to the K/BxN serum transfer arthritis model and monitored signs of inflammation for 26 days. In the early arthritic phase, paws of WT and MFG-E8-KO mice showed similar signs of inflammation. However, by day 16 paws of MFG-E8-KO mice remained inflamed for a longer period of time compared to WT mice as reflected by a 15% increase in the paw thickness (P < 0.01) and a 2°C higher paw temperature (P < 0.05). Thus, these data indicate that MFG-E8 controls bone metabolism and inflammation in arthritis, and may represent a novel mediator of osteoimmunology.

DOI: 10.1530/boneabs.1.PP18

PP19

Vitamin K2 administration is associated with decreased disease activity in patients with rheumatoid arthritis
Kosuke Ebina1, Tokimitsu Morimoto1, Kenmi Shi1, Shoichi Kaneshiro1, Kota Koizumi2, Makoto Hirao3, Jun Hashimoto4, Hideki Yoshikawa1
1Department of Orthopaedics, Graduate School of Medicine, Osaka University, Osaka, Japan; 2Department of Orthopaedics, Osaka Minami Medical Center, National Hospital Organization, Osaka, Japan; 3Department of Rheumatology, Osaka Minami Medical Center, National Hospital Organization, Osaka, Japan.

Objectives
Recent studies have demonstrated that vitamin K2 (VitK2) induces apoptosis of not only osteoclasts but also rheumatoid arthritis (RA) synovial cells in vitro, while
VitK2 may have the potential to improve disease activity besides osteoporosis.

Conclusions

VitK2 treatment were 46.2%.

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treated with VitK2 showed significant decreases in serum CRP (1.1–0.6 mg/dl; P<0.001), matrix metalloproteinase-3 (MMP-3) (220.4 vs 127.6 ng/ml; P<0.001), and disease activity score assessing 28 joints with CRP (DAS28-CRP) (2.9 vs 2.3; P<0.05). There was no significant difference in age, duration of disease, BMI, rheumatoid factor positivity, Steinbrocker’s stage, and treated dose of methotrexate between two groups, while VitK2-treated group showed lower doses of prednisolone treatment than the VitK2-naïve group (3.4 vs 1.7 mg/day; P<0.001). In the longitudinal study, patients who were additionally treated with VitK2 showed significant decreases in serum CRP (1.1–0.6 mg/dl; P<0.001), MMP-3 (160.1–125.0 ng/ml; P<0.05), and DAS28-CRP (3.1–2.4; P<0.001) after 3 months. Patients who showed good or moderate response (improvement in DAS28-CRP of >0.6 and a final DAS28-CRP of ≥4.1) to VitK2 treatment were 46.2%.

Conclusions

VitK2 may have the potential to improve disease activity besides osteoporosis of RA.

DOI: 10.1530/boneabs.1.PP20

PP20

Immunological profile of 110 rheumatoid arthritis

Kawtar Nassar, Saadia Janani, Wafaa Rachidi & Ouafaa Mkinsi

Rheumatology, Casablanca, Montserrat.

Introduction

Rheumatoid arthritis is the most common inflammatory arthritis. It is also an autoimmune disease. Its immunological profile is typical and often correlated with clinical presentation. If acticorps anti-CCP and rheumatoid factor are part of the diagnostic criteria. Anti-nuclear antibody are found in 15–40%.

Objectives

Studying the immunological profile in 110 patients treated for rheumatoid arthritis.

Materials and methods

Study of 110 cases of RA (ARA and EULAR criteria) collected in the rheumatology department. The study of immunological profile, including rheumatoid factor (RF), anti-CCP antibody (ACPA) and antinuclear antibodies (ANA) were analyzed in all patients. Rheumatoid factor positive when >1/64 by agglutinin or 20 IU by ELISA method. AAN positif when >1/80. and ACPA considered significantly positif when exceed 50 IU.

Results

110 patients were included. The mean age was 51 years. Patients were female predominantly (87.2%). Mean duration of rhumatisme arthritis was 8 years. Regarding the immunological profile, all patients had rheumatoid factor and antinuclear antibody, except two respectively. The antibody anti CCP were performed in 70% of patients. ANA were positive in 26% of cases. Rheumatoid factor positive in 73% of cases, and anti CCP Acts which were found in 77 cases were positives in 74% of patients. Among the patients with three parameters were made, 77 cases, all were positive in 11 cases (14.3%) and anti CCP Ab and rheumatoid factor positive in 32 cases (41.5%).

Conclusion

Our results were comparable to the literature. Rheumatoid arthritis is an inflammatory arthritis tropic genetic and immunological important. No only immunological parameters for the diagnostic criteria are present, but also anti-nuclear antibody.

DOI: 10.1530/boneabs.1.PP19

PP21

Monosodium urate crystals inhibit tenocyte viability and function: implications for periarticular involvement in chronic gout

Ashika Chhana1, Karen Callon1, Michael Dray2, Bregina Pool1, Dorit Naot1, Greg Gamble1, Brendan Coleman1, Fiona McQueen1, Jillian Cornish1 & Nicola Dalbeth1

1University of Auckland, Auckland, New Zealand; 2Waikato Hospital, Hamilton, New Zealand, 3Middlemore Hospital, Auckland, New Zealand.

Background

In patients with gout, urate deposition has been observed both adjacent to and within tendons, suggesting that monosodium urate monohydrate (MSU) crystals are likely to be in direct contact with tenocytes, the stromal cells of tendons. The aim of this study was to determine the effects of MSU crystals on tenocyte viability and function.

Methods

Cultures of primary rat tenocytes were prepared from Wistar rat tails. Primary human tenocytes were prepared from patients undergoing orthopedic surgery. MTT assays were used to assess tenocyte viability following culture with MSU crystals and flow cytometry was used to determine changes in the levels of apoptosis. Real-time PCR was used to determine changes in gene expression and Sirius red staining to detect changes in collagen deposition in tenocytes cultured with MSU crystals.

Results

MSU crystals reduced viability in a dose-dependent manner in both primary rat and human tenocytes. Differing MSU crystal lengths and increased serum levels in cultures did not alter this effect. Soluble uric acid did not reduce cell viability. Flow cytometry showed that MSU crystals rapidly induced cell death, but apoptosis levels remained unchanged. Culture with MSU crystals reduced mRNA expression of collagen types 1 and 3; and tenocytic markers, including tenomodulin, scleraxis and tenasin-C. Collagen deposition was inhibited in tenocytes cultured with MSU crystals in a dose dependent manner. In joint samples from patients with chronic gout, MSU crystals were identified within the tendon, adjacent to and invading into tendon, and at the enthesis.

Conclusion

These data indicate that MSU crystals directly interact with tenocytes to reduce cell viability and function. These interactions may contribute to tendon damage in patients with chronic gout.

DOI: 10.1530/boneabs.1.PP21
PP22
Generalized long term bone loss in early rheumatoid arthritis in the biologic treatment era: a 10-year prospective observational study
Anne Prøven1,2*, Knut Helgetveit1,2 & Glenn Haugeberg1,2
1 Martina Hansens Hospital, Bærum, Norway; 2 Hospital of Southern Norway Trust, Kristiansand, Norway.

Background
Several short-term studies have been performed in rheumatoid arthritis (RA) reporting a rapid rate of generalized bone loss. Aggressive anti-inflammatory treatment with biologic disease modifying anti-rheumatic drugs (DMARDs) has been shown to reduce the rate of bone loss. There is a lack of long term follow-up studies.

Objectives
To study 10-year changes in generalized bone loss in early RA patients in the biologic treatment era.

Methods
Between 1999 and 2001, 93 RA patients fulfilling the ACR RA criteria (disease duration <1 year) were included in a long term observational study. Demographic and clinical data was collected. Bone mineral density (BMD) measurements at lumbar spine and hip (femoral neck and total hip) were performed using the same dual energy x-ray absorptiometry (DXA) equipment Lunar Prodigy (General Electric) at baseline and after 2, 5, and 10 years.

Results
Patient characteristics at baseline: mean (S.D.) age 50.4 (13.6) years, females 61.7%, RF +62.8%, CCP +60.6%, and erosive 21%. Baseline disease characteristics: swollen, 28 joints 8.4; tender, 28 joints 9.7, MHAQ 0.68; ESR 29.2 mm/h; and CRP 28.9 mg/dL. Ever use of prednisolone 73.4%, synthetic DMARDs 93.6% and biologic DMARDs 53.2%. Rate of bone loss at 2, 5 and 10 years is shown in the table. At femoral neck and total hip bone loss was linear with average annual bone loss of 0.64% at femoral neck and 0.49% at total hip.

Table 1

<table>
<thead>
<tr>
<th>Bone Measurement</th>
<th>2 years</th>
<th>5 years</th>
<th>10 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Femoral neck</td>
<td>-2.00</td>
<td>-3.97</td>
<td>-6.43</td>
</tr>
<tr>
<td>Total hip</td>
<td>-1.92</td>
<td>-2.90</td>
<td>-4.88</td>
</tr>
<tr>
<td>Spine L1–4</td>
<td>-0.85</td>
<td>-0.07</td>
<td>-0.19</td>
</tr>
<tr>
<td>Spine L2–4</td>
<td>-1.46</td>
<td>-0.83</td>
<td>-1.06</td>
</tr>
</tbody>
</table>

Conclusions
Our data indicate that aggressive anti inflammatory treatment protects against bone loss at spine whereas bone loss continues at the hip. However interestingly the rate of bone loss is at the same rate as reported in the general population.

Reference

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PP23
Ultrasound carotid plaque morphology in rheumatoid arthritis women without previous cardiovascular events
Alice Castro1, Diana Carmona-Fernandes2, Maria José Santos3, Luis Mendes-Pedro2, Helena Canhaõ & Joao Euriço Fonseca4
1 Hospital de Santa Maria, CHLN, Lisboa, Portugal; 2 Unidade de Investigação em Reumatologia, Instituto de Medicina Molecular, Lisboa, Portugal; 3 Hospital Gascia de Orta, Almada, Portugal; 4 Hospital de Santa Maria, CHLN, Lisboa, Portugal; 5 Hospital de Santa Maria, CHLN, Lisboa, Portugal.

Introduction
In rheumatoid arthritis (RA) patients subclinical atherosclerosis and cardiovascular events (CV) occur more frequently and at younger ages than in the general population. Previous data suggest that heterogeneous plaques on USA are more unstable and frequently contain a higher amount of lipids and which make them hypoechoic and had higher potential for embolization and thrombosis. The aim of our work was to estimate the prevalence and the ultrasound morphology of carotid plaques in a cohort of RA patients without previous CV events.

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The evidence for the effectiveness of early rehabilitation of patients with rheumatoid arthritis (RA) is scanty. The aim of the study is to evaluate the efficiency of rehabilitation program for patients with early RA.

Methods
34 study group patients with early RA underwent 6-month rehabilitation (hospital stage: 15-min local air cryotherapy (−60 °C, Crojet Air C600) for hand, knee or ankle joints, 45-min therapeutic exercises under the supervision of a trainer, 45-min occupational therapy (joint protection strategies, use of assistive devices), ten sessions, education program (four daily 90-min studies) and outpatient stage: 45-min exercises three times a week, functional wrist and knee orthoses, customized foot insoles). Twenty-six patients received only drug therapy and ten patients underwent rehabilitation. The control group (25 patients) was treated with symptomatic and disease-modifying drugs. The rehabilitation program included: education, physical exercises, occupational therapy, orthoses, and cryotherapy, physical exercises, occupational therapy, orthoses, and therapeutic education.

Conclusion
The rehabilitation program reduces diseases activity, improves functional ability, and quality of life in patients with early RA.

DOI: 10.1530/boneabs.1.PP25

Rehabilitation of patients with early rheumatoid arthritis, including cryotherapy, physical exercises, occupational therapy, orthoses, and therapeutic education
Evgeniya Orlова1, Dmitry Karateev1, Andrey Kochetkov2 & Lev Denisov1
1Research Institute of Rheumatology Under the Russian Academy of Medical Sciences, Moscow, Russia; 2Central Rehabilitation Hospital of Federal Medical Biological Agency, Moscow, Russia.

Introduction
The effectiveness of early rehabilitation of patients with rheumatoid arthritis (RA) is scantly. The aim of the study is to evaluate the efficiency of rehabilitation program for patients with early RA.

Methods
34 study group patients with early RA underwent 6-month rehabilitation (hospital stage: 15-min local air cryotherapy (−60 °C, Crojet Air C600) for hand, knee or ankle joints, 45-min therapeutic exercises under the supervision of a trainer, 45-min occupational therapy (joint protection strategies, use of assistive devices), ten sessions, education program (four daily 90-min studies) and outpatient stage: 45-min exercises three times a week, functional wrist and knee orthoses, customized foot insoles). Twenty-six patients received only drug therapy (control). Tender and swollen joint count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), joint pain on 100-mm VAS, DAS28, HAQ, RAPID3, grip strength of a more affected hand enhanced by 44.9%, of a less affected – by 75.8% (P<0.01), pain – by 70.4% (P<0.01), DAS28 – by 31.9% (1.38±0.2, P<0.05), HAQ – by 75.8% (0.97±0.56, P<0.01), RAPID3 – by 40.1% (5.98±0.92, P<0.01). The grip strength of a more affected hand enhanced by 44.9%, of a less affected – by 31.3% (P<0.05). The average extension power of a weaker knee increased by 81.6%, of a less affected – by 70.2% (P<0.01). The average flexion power of a more affected ankle joint elevated by 81.6%, of a less affected – by 70.2% (P<0.01). The changes in the control group were less pronounced, which determined statistically significant differences between the groups in most indicators.

Conclusion
The rehabilitation program reduces diseases activity, improves functional ability, mobility, activity, quality of life in patients with early RA.

DOI: 10.1530/boneabs.1.PP26

The femoral neck fractures in patients with rheumatoid arthritis
Marina Podvorotova & Irina Dudykina
Research Institute of Rheumatology, Moscow, Russia.

The femoral neck fractures (FNF) are one of the most common non-traumatic fractures in elderly people. Frequently the patients with fractures of this localization need surgical treatment and long-term rehabilitation. It’s widely known that FNF in patients with rheumatoid arthritis (RA) occur more often than in the population.

Background
To determine the frequency of FNF in patients with RA and to characterize the patients with RA and with FNF at the time of the fracture occurrence.

Methods
254 women aged 40–75 years old with RA in clinical database of Institute Rheumatology (Moscow) were included in the study. It was examined anamnesis, bone mineral density (BMD) for all patients.

Results
Ten patients (3.9%) from 254 had FNF. The mean age of the patients at time of fracture was 50.5±16.0 years (from 22 to 67 years). All fractures occurred after the diagnosis of RA. The mean duration of RA at the time of fractures was 12.9±7.4 years. 70% of the fractures occurred during treatment with glucocorticoids (GCs; the mean duration of GC therapy was 8.3±5.8 years). Three patients with FNF have never taken GCs. In 30% of the cases the fractures were before menopause. Seven patients had familial or tubular osteoporosis. It was found three cases of nephropathy (two cases of them were amyloidosis). Four women with FNF have normal values BMD or osteopenia. Osteoporosis in femoral neck was diagnosed in five cases only, and osteoporosis in lumbar area – in 4 of 10 cases.

Conclusions
The FNF in patients with RA occur in younger age than in the population, frequently before menopause, on the background of long duration of RA and GC treatment. These cases associate with more severe course disease with complications. The FNF in patients with RA are not always associated with osteoporosis and can occur with normal BMD values.

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Sclerostin/MEPE axis in OA: lessons from long bone development
Katherine Staines1, Blandine Poulet1, Colin Farquharson1 & Andrew Pitsillides1
1The Royal Veterinary College, London, UK; 2Roslin Institute and R(D)SVS, The University of Edinburgh, Edinburgh, UK; 3University College London, London, UK.

The re-initiation of developmental processes in osteoarthritis (OA) has emerged with similarities to endochondral ossification; responsible for long bone development. We aimed to establish the role of the Wnt inhibitor, sclerostin in endochondral ossification, and its relationship with MEPE, a calcification inhibitor with potential downstream functions. Knee joints from male Str/ort (spontaneous OA) and age-matched CBA control mice were analysed at 8, 18, and 40+ weeks of age (before, early and late OA). Subchondral bone (SB)-thickening was measured by microCT. Joints were scored for OA hallmarks and related to immunohistochemical (IHC) sclerostin/MEPE expression. We have previously established MEPE as an inhibitor of endochondral ossification however the role of sclerostin is unknown. Thus embryonic and postnatal growth-plates were analysed for sclerostin by IHC. Chondrogenic ATDC5 cells were cultured in mineralizing conditions and examined for sclerostin protein by western blotting. Our results reveal enhanced sclerostin expression at the osteochondral interface, and enhanced MEPE expression in the articular cartilage (AC) in unaffected regions of the Str/ort mouse joint. At advanced stages of OA, site-specific suppression of sclerostin and MEPE expression is observed in regions of SB-thickening (analysed by microCT scanning) and where AC integrity is compromised. Strong expression of sclerostin and MEPE are observed in ossified ligaments, menisci, and emerging osteophytes; all increased with disease severity. Osteophytes form through endochondral ossification thus we examined localization of sclerostin during endochondral bone growth. Interestingly, sclerostin expression is observed in embryonic proliferating and calcifying hypertrophic chondrocytes, however this is lost in all postnatal chondrocytes. Our results also show increased sclerostin expression in ATDC5 cells, consistent with increasing calcification. Our data suggest sclerostin and MEPE are pivotal in restricting the endochondral processes observed in osteophyte formation and OA pathology. Further investigation into their underpinning mechanisms will identify whether their targeted delivery can protect against OA pathology.

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The glutamate receptor antagonist NBQX alleviates inflammation, pathology and gait abnormalities in rat antigen induced arthritis
Cleo Bonnet, Anwen Williams, Sophie Gilbert, Ann Harvey, Bronwen Evans & Deborah Mason
1Cardiff University, Cardiff, UK.

Objectives
Synovial fluid glutamate concentrations increase in various arthritides. Activation of kainic acid (KA) and AMPA glutamate receptors (GlurAs) increase interleukin 6 (IL6) release and cause arthritic pain respectively. GluR antagonists represent potential peripheral treatments for inflammatory arthritis and inflammatory...
mechanisms that contribute to osteoarthritis (OA). We hypothesised that AMPA and KA GluRs are expressed in arthritic joint tissues and that peripheral administration of NBQX (AMPA/KA Glur antagonist), would attenuate joint pathology in antigen-induced arthritis (AIA) in vivo.

Methods

Synovial inflammation and joint degradation were related to GluR immunohistochemistry in matched synovium and tibial plateaux from OA patients. NBQX was applied to three primary human osteoblast cell lines and mineralisation assessed. NBQX was injected intra-articularly into the affected knees of AIA rats at the time of arthritis induction. Knee swelling and gait patterns of AIA (n = 15), AIA + NBQX (n = 15), and naive rats (n = 6) were measured over 21 days. On day 21, histological tissues were taken for QRT-PCR, X-ray, magnetic resonance imaging (MRI), histology, and GluR immunohistochemistry.

Results

NBQX prevented mineralization in all cell lines. Human OA tissues showed extensive degradation and synovial inflammation with abundant GluR immunostaining (AIA) and reduction of GluR after NBQX treatment. NBQX treatment significantly reduced knee swelling (P < 0.001, days 1–21), gait abnormalities (days 1–3), end-stage cartilage destruction (P < 0.05), synovial inflammation (P < 0.001), meniscal IL6 and whole joint catherpsin K mRNA expression (P < 0.05). X-ray and MRI revealed a smoother articular surface; fewer bone erosions and less inflammation after NBQX treatment.

Discussion

AMPA/KA GluRs are abundantly expressed in human OA joints accompanied by synovial inflammation, and in a model of inflammatory arthritis. The attenuation of inflammation, pathology and pain in vivo, by intra-articular NBQX treatment, shows promise as a new disease-modifying drug for inflammatory- and osteoarthritits.

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PP29

A deletion mutation of the gene PSMB8, the cellular inhibitor of PKR and PERK, results in a degenerative joint phenotype in mice

Sophie Gilbert1, Mari Nowell1, Cleo Bonnet1, Warren Ladiges1,2, John Morton1,2, Vic Dunca1 & Debbie Mason1
1Cardiff University, Cardiff, UK, 2University of Washington, Seattle, USA.

Objective

The protein kinases, PKR, and PERK have been implicated in pro-inflammatory cytokine-mediated cartilage degradation in vitro and endolysosomal reticulum stress-induced arthritis respectively. The objective of this study was to establish whether loss of PSMB8, an inhibitor of PKR and PERK, results in a degenerative joint phenotype in vivo.

Methods

Sections of knee joints from PSMB8-null and wild-type mice aged 12–13, 18, and 23–25 months were stained with Toluidine blue, joints scored using the OA.RSI system for degenerative changes and subchondral bone areas measured. In addition, bone changes were assessed by radiology of hind limbs. To determine the presence of ER stress, immunohistochemistry was carried out using antibodies to phosphorylated PERK and GADD153.

Results

PSMB8-null mice demonstrated significantly higher total OA.RSI scores in the medial femoral condyle (P = 0.016) as well as significant remodelling of the bone (P = 0.013). In addition, medial tibial plateau bone area was increased in younger (P = 0.033), but significantly lower in older (P = 0.02), null mice. Bone area and cartilage damage within the lateral tibial plateau of null mice were reduced. A severe phenotype was observed in a subset of null mice with complete loss of the articular cartilage from the medial compartment and heterotrophic chondro-osseous tissue formation in the capsule surrounding the medial meniscus. Although, phosphorylated PERK and GADD153 were detected in both null and wild-type mice, the loss of PSMB8 resulted in more extensive staining throughout the joint.

Conclusions

This is the first demonstration of a critical role for PSMB8 in maintaining joint integrity, implicating PKR and PERK in the pathogenesis of joint degeneration in vivo. Remodelling of the medial compartment suggests that mechanical load within the joint may precipitate degenerative changes. Thus PKR/PERK may be influenced by mechanical as well as inflammatory signals important in osteoarthritis.

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PP30

Changes and comparison of bone metabolism, bone mineral density, MRI in early rheumatoid arthritis

Diana Vershyhyna1, Varejí Ryzhyk1, Olena Mikhailchenko1,2, Iryna Golovatch1,2, Peter Dudíj1, Igor Semyen1,2 & Oliha Shevchuk1
1National Medical University, Ivano-Frankivsk, Ukraine; 2Clinical Hospital Feofania, Kyiv, Ukraine.

The problem of early diagnosis of rheumatoid arthritis remains an important area of research in rheumatology. We investigated changes in bone metabolism, bone mineral density in early rheumatoid arthritis (ERA) up to 2.5 months. Data were compared with changes in the MRI study of the dominant hand.

We observed 24 patients with ERA, the average age = 33.6 ± 5.7 years. The men were 6 (25%), women – 18 (75%). Bone mineral density (BMD) was determined by DSA «Challenger» (DMS, France). Measurements of urinary pyridololine (PYD) and deoxypyridinoline (DPD) and osteocalcin (OC) were performed. MRI of the dominant hand was performed in 24 patients, fulfilling the American College of Rheumatology criteria for RA. The MRI protocol consisted of fat-suppressed T2, and plain and contrast enhanced T1-weighted sequences. Assessment of bone marrow edema, synovitis and bone erosions was performed by the OMERACT RA MRI scoring system. Clinical assessment was evaluated using the disease activity score for 28 joint indices (DAS-28).

We found a significant increase in PYD and DPD and no changes of the OC level in the ERA patient’s. The severity of inflammation and the number of bone inflamed joints by DAS28 score was associated with increased excretion of PYD and DPD, but not to the level of OC. Decrease BMD in the wrist and radius was observed in all patients. BMD correlated with markers of formation and resorption, as well as higher levels of disease activity on DAS28. MRI of the dominant hand in patients with ERA in scoring (quantitative) assessment identified by OMERACT synovitis (all patients), bone marrow edema (all patients) and erosions (16 patients – 66.7%). Identification of synovitis and erosions on MRI correlated with high disease activity, decreased BMD. The appearance of bone erosions was associated with a slight decrease in OC and increased excretion of PYD and DPD.

These data emphasize the early increase in the activity of bone resorption markers with the absence of reducing the activity of bone formation markers in early rheumatoid arthritis. Changes in bone metabolism with an increased resorption correlate with disease activity and detection of erosions on MRI.

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Bone biomechanics and quality

PP31

A GWAS in an extreme high bone mass population shows excess signal from genes associated with BMD in the normal population

Celia L Gregory1, Paul J Leo Leo1, Graeme R Clark2, George Davey Smith1, Matthew A Brown1, Jon H Tobias1, & Emma L Duncan3
1Musculoskeletal Research Unit, University of Bristol, Bristol, UK; 2Diamantina Institute, University of Queensland, St Lucia, Queensland, Australia; 3MRC CAiTe Unit, Department of Social and Community Based Medicine, University of Bristol, Bristol, UK; 4Diamantina Institute, Royal Brisbane and Women’s Hospital, University of Queensland, Queensland, St Lucia, Australia.

Extreme high bone mass (HBM) may be monogenic (e.g. due to mutations in SOST or LRPS) or polygenic, due to variants in the same genes determining bone mineral density (BMD) as found in the general population. We aimed to determine the genetic cause underlying HBM in an extreme HBM population.

258 unexplained HBM cases (defined as L1 Z-score ≥ 1.5) and total hip Z-score ≥ 1.2, or total hip Z-score ≥ 1.5, and L1 Z-score ≥ 1.2) were recruited from 15 UK centres, by screening 335, 115 DXA scans 1. Individuals with established SOST and LRPS mutations were excluded by Sanger sequencing (n = 3). We performed a GWAS for HBM, genotyping 240 HBM cases using Infinium OmniExpress-12v1.0 DNA analysis beadchips and clustering using GenomeStudio software (Illumina). Controls constituted two previously genotyped populations: i) unselected (n = 967, 1958 British Birth Cohort) and ii) ethnically-matched low BMD (n = 900, Anglo-Australasian Osteoporosis Genetics Consortium® (AOGC) post-menopausal women with BMD Z-scores ≥ 4.0 to < 1.5). Samples were assessed for cryptic relatedness, excess heterozygosity/missingness. SNPs with MAF < 1%, and/or not in HWE were removed, leaving 181, 323 SNPs. The dataset was imputed using the 1000 Genomes Project; SNPs with r² threshold ≥ 0.8 were retained. SNPs were tested for association with BMD using PLINK, assessed separately for each control group. Results demonstrated over-representation of associations with BMD loci identified from the normal population (Figures 1 (HBM vs WTCCC) and 2 (HBM vs low AOGC)). Over-representation was greater when HBM was
We have reported femoral osteopenia in short term-vitamin D restricted rats without deterioration in tibial cortical bone volume (CBV), geometry or strength. This study aimed to establish the effect of extended vitamin D deficiency in aged rat tibial volume and strength. Female Sprague-Dawley rats (9 m, n=6/group) were fed a diet containing varying vitamin D3 (D) levels (0, 2, 12, and 20 IU/day) with either low (0.1%, LCa) or high (1%, HCa) dietary calcium for 6 m. At 15 m blood was taken for 25 hydroxyvitamin D (25D) and 1,25 hydroxyvitamin D (1,25D). PTH and Ca analyses and tibiae and femora retrieved for bone analyses. 3D micro-CT scans (SkyScan 1174) were used to determine CBV, mid-shaft sagittal cortical thickness (Chsag) and metaphyseal BV/TV. Tibial peak load was determined by three-point bending (Test Resources 800LE4). 25D and 1,25D were determined by RIA (IDS) and PTH by IRMA (Immutopics). Group serum 25D levels ranged from 22 (±2.9) to 161 (±38.8) nmol/l and serum calcium levels from 2.5 (±0.05) to 3.2 (±0.2) mmol/l. Circulating 25D was a determinant of BV/TV (r²=0.23, P<0.001) and CBV (r²=0.22, P<0.01). In multiple linear regression neither serum Ca, PTH nor 1,25D were determinants of bone volume when 25D was accounted for. Dynamic histomorphometry indicated that high dietary Ca reduced bone turnover only in animals with circulating 25D levels above 85 nmol/l with the greatest reduction achieved in the 20 IU/day group (20D) (BFR (µm²/mm² per day): LCa20D 33.9 (3.4) vs HCa20D 21.8 (2.3), P<0.05). Tibial peak load was related to Chsag (r²=0.39, P<0.001). Thus, optimisation of bone volume and strength requires the combination of high dietary Ca intake and circulating 25D above 85 nmol/l. However, our previous demonstration that high dietary Ca is required to maximise circulating 25D levels2,3 in combination with the present findings suggest that the mechanism for vitamin D-optimisation of bone is not mediated via a calcemic effect.


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PP32

Vitamin D levels of >85 nmol/l in the presence of adequate dietary Ca minimise bone turnover and improve bone strength

Peter O’Loughlin1,2, Alice Lee1, Paul Anderson1, Roland Steck2, Mark Forwood1, Rebecca Sawyer2 & Howard Morris1,3

1IMVS Pathology, Adelaide, South Australia, Australia; 2University of Adelaide, Adelaide, South Australia, Australia; 3University of South Australia, Adelaide, South Australia, Australia; 4Queensland University of Technology, Brisbane, Queensland, Australia; 5Griffith University, Gold Coast, Queensland, Australia.

We have reported femoral osteopenia in short term-vitamin D restricted rats without deterioration in tibial cortical bone volume (CBV), geometry or strength. This study aimed to establish the effect of extended vitamin D deficiency in aged rat tibial volume and strength. Female Sprague-Dawley rats (9 m, n=6/group) were fed a diet containing varying vitamin D3 (D) levels (0, 2, 12, and 20 IU/day) with either low (0.1%, LCa) or high (1%, HCa) dietary calcium for 6 m. At 15 m blood was taken for 25 hydroxyvitamin D (25D) and 1,25 hydroxyvitamin D (1,25D). PTH and Ca analyses and tibiae and femora retrieved for bone analyses. 3D micro-CT scans (SkyScan 1174) were used to determine CBV, mid-shaft sagittal cortical thickness (Chsag) and metaphyseal BV/TV. Tibial peak load was determined by three-point bending (Test Resources 800LE4). 25D and 1,25D were determined by RIA (IDS) and PTH by IRMA (Immutopics). Group serum 25D levels ranged from 22 (±2.9) to 161 (±38.8) nmol/l and serum calcium levels from 2.5 (±0.05) to 3.2 (±0.2) mmol/l. Circulating 25D was a determinant of BV/TV (r²=0.23, P<0.001) and CBV (r²=0.22, P<0.01). In multiple linear regression neither serum Ca, PTH nor 1,25D were determinants of bone volume when 25D was accounted for. Dynamic histomorphometry indicated that high dietary Ca reduced bone turnover only in animals with circulating 25D levels above 85 nmol/l with the greatest reduction achieved in the 20 IU/day group (20D) (BFR (µm²/mm² per day): LCa20D 33.9 (3.4) vs HCa20D 21.8 (2.3), P<0.05). Tibial peak load was related to Chsag (r²=0.39, P<0.001). Thus, optimisation of bone volume and strength requires the combination of high dietary Ca intake and circulating 25D above 85 nmol/l. However, our previous demonstration that high dietary Ca is required to maximise circulating 25D levels2,3 in combination with the present findings suggest that the mechanism for vitamin D-optimisation of bone is not mediated via a calcemic effect.


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PP33

Influence of the organic matrix of mineralized tissues on their dynamic mechanical properties assessed by scanning acoustic microscopy

Stéphane Blouin1, Stephan Puchegger2, Klaus Klaushofer1, Paul Roschger1 & Peter Fratzl3

1Ludwig Boltzmann Institute of Osteology at the Hanusch Hospital of WGKK and AUVA Trauma Centre Meidling, 1st Medical Department Hanusch Hospital, Vienna, Austria; 2Faculty of Physics, University of Vienna, Dynamics of Condensed Systems, Vienna, Austria; 3Department of Biomaterials, Max Planck Institute of Colloids and Interfaces, Postdam, Germany.

Mineralized tissues like bone, articular calcified cartilage or mineralized turkey leg tendon (MTLT) are build by a composite of hydroxyapatite nano-particles and organic matrix. In bone and MTLT the matrix is formed by collagen type-I, but in contrast to bone in MTLT the collagen is uniaxial orientated, while in cartilage the matrix consists of collagen type-II and proteoglycans. Composition/orientation differences were investigated by a new scanning acoustic microscopy method (SAM-TOF). Time-of-flight differences of ultrasound pulses obtained from human femoral head and distal MTLT samples with known thickness (30 microns) were determined with 0.125 ns time resolution to obtain sound velocities maps with 2 µm pixel resolution using a 330 MHz lens (Kibero GmbH). The velocity maps were combined with calcium content maps obtained by quantitative backscattered electron imaging to extract dynamic elastic moduli (E) maps.

Bone was found to require a lower mass density (−4.3%) than cartilage to achieve similar velocity (range 3700–4300 m/s) or elastic modulus (range 22–30 GPa), which is qualitatively in line with nanoindentation results. In circumferential compartment of MTLT, an axial/transversal velocity ratio of 1.13 and E ratio of 1.28 and in the interstitial compartment 1.16 and 1.32 ratios, respectively, were found. This anisotropy is clearly due to the preferred orientation of collagen. However, the higher E in cartilage-bone and lower ratio in MTLT compared with what is typically measured with (quasi-)static mechanical test such as uniaxial tension or nanoindentation could indicate an influence of relaxation processes. These first results suggest that TOF scanning acoustic microscopy may be able not only to provide mechanical maps of mineralized tissues but to extend our understanding of the mechanical properties of bone and cartilage to the region of high loading rates, which may be highly relevant for the fracture resistance under an impact, e.g., during a fall.

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PP34

Obesity induced by a sucrose-rich diet promotes deficits in bone mineralization and microarchitecture

Bruna Bifle1, Maria Tereza Nunes2, Antonio Augusto Carvalho1, Vilma Colli1, R. Bornelles1, Ana Claudia Nakamune1, Pedro Florindo1 & Mario Jefferson Louzada2
1 São Paulo State University – UNESP, Araçatuba, São Paulo, Brazil; 2University of São Paulo – USP, São Paulo, São Paulo, Brazil.

In order to examine metabolic and biophysical parameters arising from obesity, male rats were given drinking 30% sucrose (p/v) for 8 weeks. During the experimental period, animals in the control group (C) consumed higher amounts of food and water, but the body mass was smaller than the group receiving sucrose (S). In this group, the caloric load given to the animals for eight weeks resulted in increased energy consumption, in glycemia and in plasma leptin and abdominal fat. However, did not alter the plasma concentration of insulin. The analysis of bone showed smaller bone density for the S group considering the comparison between their initial and final values. Moreover, the amount of bone mineral material was lower in animals in that group. Complementing these data, was found in group S smaller trabecular bone volume as percentage of tissue volume (BV/TV) and trabecular thickness (Tb.Th) resulting in increase intertrabecular space in the group that ingested 30% sucrose. Therefore, obesity induced by means of a sucrose-rich diet had a negative influence of bone mineralization and microarchitecture.

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PP35

The effect of fluoride on the DEXA score and material properties of ex vivo emu tibiae

Sidney Omelon1, Kevin Nhan1, Gillian Reil-Schachter2, Nicolas Lacroux2, Malaika Miles-Rossov1 & Fabio Variola2
1University of Ottawa, Ottawa, Ontario, Canada; 2Queen’s University, Kingston, Ontario, Canada.

Bone is a tough composite material, comprised of a compliant collagenous matrix, brittle apatite mineral crystals and a suite of non-collagenous proteins (NCPs). Bone material properties depend on these components, and their interactions at the mineral-collagen interface. ‘Bone mineral density’ (BMD), a parameter used to predict fracture risk, is routinely quantified by DEXA. Previous studies used ex vivo emu tibiae as a model to test the effect of organic component quality on BMD and material properties. Endocortical infusion with 1 M KOH caused no change in BMD, but reduced the three-point bending failure stress, and increased the failure strain. These changes were attributed to in situ collagen degradation, and possibly denatured NCPs, which could weaken the mineral-collagen interface. In this study, the ex vivo emu tibia model was used to test the effects of weakening the interface between bone mineral and collagen. It was assumed that the electrostatic attraction between positively charged bone minerals and electronegative domains of some NCPs is the primary chemical bond between the inorganic and organic components of bone. It was hypothesized that small, electronegative fluoride ions (F-) could migrate to the positively charged apatite surface, and disrupt this electrostatic bond. Endocortical F-infusion should not affect BMD, and would be expected to increase the post-yield material properties in 3-point bending, as yielding occurs at the peristomial border. It was not exposed to F. Emu tibiae were endocortically infused with 0.05, 0.1, or 1 M NaF at neutral pH for two weeks. The BMD, elastic modulus, yield stress/strain, ultimate stress, and failure stress showed no statistical difference. However, increased post-yield strain and failure strain, coupled with a decreased endocortical hardness of the F-treated bones, suggest a role for mineral-collagen interface strength that is not detected by DEXA.

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PP36

Regional heterogeneity of trabecular bone microdamage density in association with trabecular microarchitecture and bone resorption in whole human lumbar vertebrae

Vincent T Carpentier1,2, Helen Tsangari1, Nick L Fazzalari1 & Julia S Kuliwaba3,4
1Bone and Joint Research Laboratory, Directorate of Surgical Pathology, South Australia Pathology and Hanson Institute, Adelaide, South Australia, Australia; 2Department of Oral Medicine, Infection and Immunity, Harvard School of Dental Medicine, Boston, Massachusetts, USA; 3Discipline of Anatomy and Pathology, School of Medical Sciences, The University of Adelaide, Adelaide, South Australia, Australia; 4Bone and Joint Research Laboratory, Adelaide Centre for Spinal Research, South Australia Pathology and Royal Adelaide Hospital, Adelaide, South Australia, Australia.

Study aim

Vertebral strength is determined by bone size, shape, bone mineral density, microarchitecture, and bone material properties. Despite its importance to vertebral mechanics, no studies have reported on the variation of bone microdamage present in the human vertebra. Thus, the aim of this study was to assess regional changes in trabecular bone microdamage in association with bone microarchitecture and resorption in whole human lumbar vertebrae.

Methods

L2 vertebrae were obtained from 12 human cadaveric spines (six males, aged 53–82 years; six females, aged 56–87 years). Parasagittal slices cut from each vertebral body were en bloc-stained in basic fuchsin, cut into nine sectors, and resin embedded. Histomorphometric assessment of trabecular bone microarchitecture, in vivo bone microdamage, and extent of bone resorption was undertaken.

Results

Data analysis revealed few differences for the nine sectors and no differences for the antero-posterior axis were observed. For the cranio-caudal axis, the mid-vertebral region had the lowest bone volume fraction (P < 0.03), trabecular number (P < 0.02), and highest trabecular separation (P < 0.03). Microcrack density parameters were highest in the mid-vertebral region (P < 0.04) and lowest in the caudal region (P < 0.01). Diffuse microdamage was minimal or absent. Bone resorption was highest in the cranial region (P < 0.03).

Conclusions

For the cranio-caudal axis of the L2 human vertebra, the mid-vertebral region may be biomechanically compromised due to reduced bone volume and microarchitectural changes being accompanied by an increased microcrack burden. The increased bone resorption found in the cranial region may be an adaptive response to intervertebral disc degeneration. The implications of these observations are being further investigated with comparison to available biomechanical and intervertebral disc grading data.

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PP37

Microarchitectural decay and microdamage accumulation in vertebral trabecular bone: a comparative analysis of the iliac crest, proximal femur, and vertebral body in the aged postmenopausal skeleton

Vincent T Carpentier1,2, Dzenita Muratovic1,3, Ian H Parkinson1,3, Nick L Fazzalari1 & Julia S Kuliwaba3,4
1Bone and Joint Research Laboratory, Directorate of Surgical Pathology, South Australia Pathology and Hanson Institute, Adelaide, South Australia, Australia; 2Department of Oral Medicine, Infection and Immunity, Harvard School of Dental Medicine, Boston, Massachusetts, USA; 3Discipline of Anatomy and Pathology, School of Medical Sciences, The University of Adelaide, Adelaide, South Australia, Australia; 4Bone and Joint Research Laboratory, Adelaide Centre for Spinal Research, South Australia Pathology and Royal Adelaide Hospital, Adelaide, South Australia, Australia.

Study aim

The general assumption that changes in bone microstructure and material properties at the iliac crest are representative of skeletal sites that are susceptible to osteoporotic fracture has not yet been addressed. Therefore, our study aim was to perform a comparative analysis of bone microarchitecture, accumulated microdamage and osteocyte morphology between the iliac crest, proximal femur and vertebral body.

Methods

Trabecular bone cores were obtained from the iliac crest, proximal femur (intertrochanteric region) and T12 vertebral body (central region) from seven postmenopausal female cadavers, aged 70–98 years (mean 79 ± 2.9 years), with no history of disease/medication that may have affected bone turnover. All bone cores were micro-CT imaged for 3D microarchitecture, then divided lengthwise for histomorphometric assessment of microdamage and osteocyte morphology.

Results

BV/TV, Tb.N, and DA were lower for vertebral bone compared to iliac crest (P < 0.05). Other architectural parameters were not different between sites. Microcrack, crack surface and diffuse damage density were higher in vertebral vs iliac crest and proximal femur bone (P < 0.01). Crack lengths were similar between vertebral and femoral bone, with both sites higher vs iliac crest (P < 0.05). Osteocyte, empty lacunar and total lacunar densities were not different.
PP38
Bisphosphonate influence on bone quality at molecular level: study of human jaw bone sequestrates by Raman microspectroscopy
Cécile Olejnik1,2, Guillaume Falgayrac1, Alexandre During1, Marie-Hélène Vieillard1,2, Jean Michel Maes3, Bernard Cortet1,3 & Guillaume Penel1,2
1EA 4490 Physiopathologie des Maladies Osseuses Inflammatoires, Lille, Nord Pas de Calais, France; 2Service d’Odontologie, Centre Abel Caumartin, CHRU de Lille, Lille, Nord Pas de Calais, France; 3Service de Rhumatologie, Hôpital Roger Salangan, CHRU de Lille, Lille, Nord Pas de Calais, France; 4Service de Chirurgie Maxillo Faciale et Stomatologie, Hôpital Roger Salengro, CHRU de Lille, Lille, Nord Pas de Calais, France.

Bisphosphonates (BP) are used as anti-resorptive drugs in benign (osteooporosis) and malignant (myeloma, bone metastasis) bone diseases. Their high affinity for bioapatites allows prolonged storage within bone. However information about molecular impact of BP on bone quality are missing. Better understanding of BP properties to optimize their clinical use is needed. The aim of this study was thus to investigate human bone physicochemical changes upon BP uptake.

Methods
Bone sequestrates obtained from 24 patients (42–94 years old) suffering from BP-related osteonecrosis of the jaw were used and split into two groups: low-dose (BPnlow group, n=8) and high-dose (BPnhigh group, n=16) therapies, respectively for benign and malignant bone diseases. The control group (CTL, n=24, 64–93 years old) was composed of cadaver mandibular samples. Raman microspectroscopy measured mineral/organic ratio, carbonate/phosphate ratio, crystallinity degree, and mineral and collagen matures. Chemometric discriminant method was used to isolate spectral features of each group.

Results
In the BPnhigh group, mineralization, mineral and collagen matures were increased significantly by 15, 43 and 57% respectively, compared to the CTL group (P<0.01). In contrast, crystallinity was lowered by 2.3% in the BPnhigh group compared to the CTL group. Chemometric distinguished the CTL group as characterized by organic components (amides and collagen) from BP groups, indicating a greater mineralization with BP. In addition, the v1 phosphate band shifted between CTL and BP groups, suggesting changes in apatitic crystal organization by BP.

Conclusion
This study highlights the modifications of bone quality in mandibular bone during BP treatment at a molecular level. These changes occur in both mineral and organic compartments of bone.

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PP39
Glucose-dependent insulinoctotropic polypeptide receptor deletion results in a reduced bone strength and quality
Aleksandra Mieczkowska1, Nigel Irwin1, Peter R Flatt2, Daniel Chappard1 & Guillaume Mahié1
1LUNAM Université, Angers, France; 2University of Ulster, Coleraine, UK.

Objectives
Glucose-dependent insulinoctotropic polypeptide (GIP) is secreted by intestinal K-cells into the blood supply in response to nutrient ingestion and absorption. Although osteoblasts and osteoclasts express the GIP receptor (GIPR), the main action of the GIP/GIPR pathway in bone physiology and bone quality is unknown. The aim of the present study was to investigate bone quality in a mouse model of GIPR deficiency.

Methods
Eleven 16 weeks old GIPR knock-out male mice, with a deletion of the first six exons of the GIPR gene, were age- and sex-matched with 12 wild-type (WT) mice for this study. Resistance to fracture was studied by three-point bending in femur, whilst cortical microarchitectures were determined by high resolution microCT and quantitative X-ray imaging. Intrinsic material properties were investigated by nanoindentation. In addition, bone mineral and collagen properties were assessed by quantitative backscattered electron imaging (qBEI) and Fourier-transformed infrared microscopy (FTIRM). Non-parametric Mann-Whitney U test was used to compare differences between groups.

Results
As compared with control mice, GIPR KO animals presented a reduction in bone strength as evidenced by significant decreases in ultimate load (~11%) and absorbed energy (~28%). Cortical microarchitecture was also affected by the lack of a functional GIPR as demonstrated by significant reductions in cortical thickness (~20%) and cross-sectional moment of inertia (~18%). These microarchitectural modifications were accompanied by alterations of intrinsic material properties. Indeed maximal load and hardness as assessed by nanoindentation on hydrated bone were significantly reduced by 13 and 16% respectively. Furthermore, bone mineral density distribution was also decreased by 12% and the ratio of mature/immature collagen cross-links was reduced by 16%.

Conclusion
The inactivation of the GIP/GIPR pathway resulted in marked alterations of cortical microarchitecture, bone matrix properties and bone strength. Overall, these data support a fundamental role of the GIP/GIPR pathway in bone physiology.

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PP40
Prediction of vertebral body stiffness in patients with multiple myeloma using qCT-based finite element models
Gracene Campbell1, Christian Graeff1, Sarah Giraven1, Felix Thomsen1, Jaime Pena1, A Wulff1, A Günther1, Claus C Glüer1 & Jan Börggreffe1,2
1Christian-Albrechts Universität zu Kiel, Kiel, Germany; 2Universitätsklinikum Köln, Köln, Germany; 3GSI Helmholtzzentrum für Schwerionenforschung GmbH, Darmstadt, Germany.

Multiple myeloma (MM) is associated with lytic bone destruction leading to high fracture incidence in the vertebrae. Accurate assessment of fracture risk is required for physicians to determine the necessity for surgery. This risk is currently determined by examining lesion size or number; however, this method does not consider the biomechanical attributes of the bone. Finite element (FE) modelling can simulate mechanical loading on vertebral bodies, and estimate mechanical integrity, potentially giving a more reliable prediction of fracture risk. Vertebral quantitative computed tomography (qCT) scans were evaluated in 60 MM patients, 30 with fracture and 30 without. From the images, in-house software was used to generate linear FE models that consisted of tetrahedral elements with transverse isotropic material properties. Element stiffness was calculated from local bone mineral density (BMD) values. Uniaxial compression was simulated to a deformation of 1 mm and the apparent level stiffness determined. t-Tests were used to compare stiffness between fracture and non-fracture groups, and standardized odds ratios (normalized to S.D.) and 95% CIs were calculated. Using structural and mineral data from previous work, correlations between stiffness and bone volume ratio (B/TV), trabecular BMD (tBMD), trabecular separation (ThSp), and cortical BMD (cBMD) were determined.

Vertebral body stiffness in patients with fracture was significantly lower than those without (16.3 ± 23.4 kN/mm, P = 0.012), and the age-adjusted logistic regression revealed an OR/S.D. of 3.57 (1.17–10.84). Significant correlations between stiffness and tBMD (R=0.6639, P<0.001), Tb.Sp (R = −0.5255, P<0.001) and cBMD (R=0.52, P<0.001) were observed.

Reduced vertebral stiffness was associated with fracture prevalence, indicating that this is a potential parameter for the assessment of fracture risk in patients with MM. Both trabecular (tBMD, Th.Sp) and cortical (cBMD) parameters significantly correlated with stiffness. Future work will involve the development of nonlinear FE models in order to predict vertebral strength.

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PP42

Bone quality in young thalassaemic patients

Alberto Argentiero1, Nadia Agnello1, Cosimo Neglia1, Giovanna Chitano1, Alessandra Della Rosa1, Giovanni Quarta1, Frisco Piscitelli & Alessandro Distanti1

1Euro Mediterranean Biomedical Scientific Institute, ISBEM, Brindisi, Puglia, Italy; 2Local Health Authority, ASL, Brindisi, Puglia, Italy.

Background

Osteoporosis is a leading cause of morbidity in patients affected by β-thalassaemia major (TM) and intermediate thalassaemia (TI). Appropriate supportive care and identification of long-term sequelae of therapy are important in thalassaemic patients. As low bone mineral quality (BMQ) in patients can be considered a marker of possible degeneration to osteopenia and osteoporosis in adulthood, we evaluated bone features in a young population followed at ‘A. Perrino’ Hospital in Brindisi.

Methods

Fifty-five thalassaemic patients (29 males, 26 females; aged 18–45 years) were analyzed during 2012 and compared vs a matched control population (55 healthy adults: 24 males, 31 females; aged 18–46 years). Seven patients were affected by TI while the rest was affected by TM. BMQ was assessed by quantitative ultrasound (QUS) technique at the phalanx level. The main values of phalangeal QUS are the amplitude-dependent speed of sound (AD-SoS, m/s) and the bone transmission time (BTT, µs) and the bone transmission time (BTT, µs).

Results

QUS values were significantly lower in cases than in controls (AD-SoS: 2119.4±53.9 and BTT: 1.75±0.3 µs in controls; AD-SoS: 2031±75.2 and BTT: 1.43±0.3 µs in cases). AD-SoS was negatively associated with BMI (r=-0.36, P=0.0067 in controls; r=-0.37, P=0.0054 in cases), while BTT was correlated with gender in both cases (P<0.01) and controls (P<0.0001), showing lower values in females.

Conclusion

Our results suggest that bone quality in thalassaemic young patients is influenced by many factors that were not present in control subjects, such as iron chelation therapy, delayed sexual maturation, GH deficiency, parathyroid dysfunction, hypothyroidism, and liver diseases.

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PP43

Protective effect of polyphenols from Aronia melanocarpa berries against cadmium-induced weakening of the femur biomechanical properties in rats

Małgorzata Małysza, Alicja Roszczenko & Joanna Rogalska

Department of Toxicology, Medical University of Białystok, Białystok, Poland.

Bone damage is one of the main unfavourable health effects of chronic exposure to cadmium (Cd). This heavy metal stimulates osteoclastic bone resorption and inhibits osteoblastic bone formation resulting in decreased bone mineralization and as a result weakening of the bone biomechanical properties. Recently, using a rat model of chronic human exposure to cadmium, we have revealed that even low exposure to this metal may increase bone vulnerability to fracture. Taking into account that polyphenol compounds have been known to have beneficial impact on bone metabolism and strength we have undertaken the study aimed to investigate whether these compounds are capable of improving the bone strength properties under chronic exposure to cadmium corresponding to low and moderate human exposure. For this purpose, biomechanical properties (yield strength, fracture strength, tension, stiffness, and young modulus) of the femur of the female Wistar rats administered as the only drinking fluid 0.1% water extract of polyphenols from the berries of Aronia melanocarpa or/and cadmium in diet (1 and 5 mg/kg) for 17 months were determined. The bone was subjected to a three-point bending test performed with the use of universal testing machine (Zwick; Z2.5TS, Germany). The low (1 mg Cd/kg) and moderate (5 mg Cd/kg) chronic exposure to cadmium to a similar extent weakened the femur biomechanical properties making them more vulnerable to fracture. The administration of polyphenolic compounds under the exposure to 1 and 5 mg Cd/kg importantly improved the bone biomechanical properties. The results of the present study allow for the conclusion that consumption of polyphenolic compounds from the berries of Aronia melanocarpa may decrease the risk of bone fractures under chronic exposure to cadmium.

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PP44

2-Oxoglutaric acid protects against side effects of maximal therapeutic doses of dexamethasone in piglets skeleton

Piotr Dobrowolski1, Ewa Tomaszewska2, Paulina Kurłąk1 & Stefan Pierzyński1,4

1Maria Curie-Sklodowska University, Lublin, Poland; 2University of Life Sciences in Lublin, Lublin, Poland; 3Lund University, Lund, Sweden; 4Institute of Agricultural Medicine in Lublin, Lublin, Poland.

Synthetic glucocorticoids such as dexamethasone (Dex) are widely used for treatment of premature infants with chronic lung disease or respiratory distress syndrome or in allergic conditions such as asthma. Adverse effect of these treatments is glucocorticoids-induced osteoporosis. On the other hand there are functional foods, for instance 2-oxoglutaric acid (2-Ox) a precursor of hydrocortisone the prevailing amino acid in bone collagen, which reduce the risk of osteoporosis. The aim of this study was to determine whether 2-Ox can prevent bone changes caused by maximal therapeutic doses of dexamethasone. 24 male and 24 female piglets, were divided in two groups: the control group (DEX; male n=12 and female n=12) piglets injected intramuscularly with Dex (1 mg/kg BW daily) and the experimental group (2-Ox; male n=12 and female n=12) piglets receiving Dex at the same manner as control group and 2-Ox administered orally (0.4 mg/kg BW daily). The study lasted for 35 days. At the end of study piglets were euthanized and left femora, humerus and two ribs (6th-7th) were isolated, weighed and measured, mechanical properties, geometry, bone mineral density (BMD), bone mineral content (BMC), histomorphometry parameters were determined. Serum bone alkaline phosphatase (BAP), osteocalcin (OC), GH, leptin, and IGF1 concentrations were determined. Piglets receiving 2-Ox had significantly heavier, denser and stronger bones in both sexes as well as the higher concentration of GH. Only some geometry parameters and leptin as well BAP concentration was higher in piglets receiving Dex alone. 2-Ox almost fully abolished the effects of maximal therapeutic dose of Dex which influenced bones, hence can be advised as a protective substance along with glucocorticoids therapies.

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PP45
Analysis of the microarchitecture of the human femoral head using micro-computertomography
Hyungmin Ji, Ye-Yeon Won1 & Ye-Soo Park2
1Ajou University Hospital, Suwon, Republic of Korea; 2Hanyang University School of Medicine, Gyunggi-do, Republic of Korea.

Purpose
The purpose of this study was to scan femoral heads from cadaveric donors and investigate the microarchitecture within each femoral head comprehensively.

Material and methods
Ten proximal femora was harvested from eight human cadaveric donors and these specimens were scanned using micro-computed tomography. Reconstructed batches of images were aligned along the main trabecular direction (MTD). The upper hemisphere of each femoral head was included in the analysis. Femoral neck area was designated as 12 o'clock and 12 identical 30-degree arcs around a same center were assigned in each image. Each volume of interest was sub-divided into proximal and distal segment. Morphometric parameters were obtained in each reconstructed 3D volume of interest (VOI).

Results
In proximal segments structure model index (SMI), trabecular number (Tb. N), trabecular separation (Tb. Sp), and degree of anisotropy (DA) were statistically different among VOIs. Bone volume fracture (BV/TV), SMI, Tb. N, Tb. Sp, DA, and connectivity density (Conn. D) were differed among VOIs in distal segments. In 90–120° area, which is located in posterior area BV-TV was highest and SMI was lowest. In 150–210° and 330–60° area DA was higher than other areas and increased in proximal segment. In 210–300° area trabecular thickness and number tended to be increased only in distal segments. In 0–60° Conn. D was higher in proximal segments.

Conclusion
When the microarchitecture within human femoral head was analysis along the MTD, morphometric parameters were distinctively different among VOIs. The findings are assumed to be mainly due to the morphology and orientation of the primary compressive trabeculae.

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PP46
Low magnitude vibration signals attenuate the rapid bone mass induced by lipopolysaccharide
In Sook Kim, Taiyung Cho1, Beomseok Lee1 & Soon Jung Hwang1,2
1Dental Research Institute, Seoul National University, Seoul, Republic of Korea; 2Department of Oral and Maxillofacial Surgery, Brain Korea 21 2nd Program for Cranio-Maxillofacial Life Science, School of Dentistry, Seoul National University, Seoul, Republic of Korea.

Introduction
Low-magnitude, high-frequency (LMHF) mechanical stimuli lead to enhance bone formation and decrease resorption. This study aimed to investigate the effect of vibration on the bone loss induced by inflammatory cytokine, lipopolysaccharide (LPS).

Methods and designs
Balb-C mice were administered to LPS (5 mg/kg) with two i.p. injections on days 0 and 4, while sham control group was injected with 400 μl of water for injection without LPS. Animals were sacrificed at days 7 (n=15) and 14 (n=13) after second injection of LPS. Vibration (0.4 g, 45 Hz) was exposed to LPS-injected group next day after second injection for 10 min/day for 4 days (n=10), and then sacrificed for micro-computed tomography (micro-CT) analysis of bone mass. Bone chips extracted from tibia (n=6) was examined for the change of gene expression using real time RT-PCR.

Results
Micro-CT-based evaluation showed that LPS injection led to significant decrease of bone volume (BV) at days 7 after injection, while there was little change in BV at 14 days post-injection. Bone loss was apparent in tibia region rather than other skeletal sites such as femur or calvarium, with significant decrease by 26% in BV, and by 35% in bone mineral density (BMD). Vibratory stimulation after LPS injection led to the increase of both BV and BMD by 18 and 24.5%, respectively, compared to those of non-vibrated, LPS-injected group, which corresponds to ~80% in sham control. Real time RT-PCR using bone chips extracted from tibia revealed the increased expression of type I collagen and osteopontin genes in vibrated group more than non-vibrated, LPS-injected groups.

Conclusions
These findings exhibited that systematic injection of inflammatory cytokine, LPS induced the significant loss of BV and BMD in tibia. Rapid bone loss induced by LPS was efficiently suppressed by LMHF vibration.

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PP47
Osteoalterations in condylar head after directional change of functional loading in rabbit mandibular condyle
Soon Jung Hwang1,2, Hoon Joo Yang1, Taiyung Cho1, Ji Hye Oh1 & In Sook Kim2
1Department of Oral and Maxillofacial Surgery, Brain Korea 21 2nd Program for Cranio-Maxillofacial Life Science, School of Dentistry, Seoul National University, Seoul, Republic of Korea; 2Dental research Institute, Seoul National University, Seoul, Republic of Korea.

Purpose
The purpose of this study was to investigate the bony change of mandibular condyle when the originally less-loaded or unloaded surface was subjected to functional loading by the newly designed animal experiment, and to evaluate whether this experiment is adequate for the animal model of condylar resorption due to mechanical loading.

Methods
Twelve adult male New Zealand white rabbits were used. Unilateral oblique vertical body osteotomy (UOVBO) was performed on the right side of the mandible. The proximal segment was rotated counterclockwise by 1 mm (group I, n=6) or 3 mm (group II, n=6). The rabbits were sacrificed four weeks postoperatively, and osseous changes of condyles were analyzed using micro-computed tomography and histological evaluation. The comparison was performed between condyles on the right and left (control) sides. Since the left condyle might be affected by the operation on the right side, the results were also compared with the healthy control (n=2, no operation).

Results
The CCWR of the proximal segment after UOVBO led to osteoporotic change of condyle including significantly reduced bone volume, decreased bone mineral density, thin trabecular thickness, small trabecular number and wide trabecular separation (P<0.05 for all parameters), with thinning of condylar cartilage and reduced density of cartilaginous cells compared with the left condyle. However, these changes were not affected by the amount of CCWR of the proximal segment. There was no significant difference between the left condyle and healthy control.

Conclusion
The osteoporotic change of condyle occurred with the CCWR of the proximal segment. We suggest that 1 mm-CCWR of the proximal segment is an adequate animal model to observe bone and cartilage alterations after directional change of functional loading, not an animal model for condylar resorption due to mechanical loading.

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PP48
Nano-structural signs of the cortical bone fragility: atomic force microscopy study in the femoral neck of elderly hip fracture patients and healthy aged controls
Petar Milovanovic1, Zlatko Rakoczevic2, Jelena Potocnik2, Danijela Djonic1, Vladimir Zivkovic1, Slobodan Nikolic1 & Marija Djurs1
1Laboratory for Anthropology, School of Medicine, Institute of Anatomy, University of Belgrade, Belgrade, Serbia; 2Laboratory for Atomic Physics, Institute of Nuclear Sciences Vinca, University of Belgrade, Belgrade, Serbia.

The purpose of this study was to investigate the bony change of mandibular condyle when the originally less-loaded or unloaded surface was subjected to functional loading by the newly designed animal experiment, and to evaluate whether this experiment is adequate for the animal model of condylar resorption due to mechanical loading.

The osteoporotic change of condyle occurred with the CCWR of the proximal segment. We suggest that 1 mm-CCWR of the proximal segment is an adequate animal model to observe bone and cartilage alterations after directional change of functional loading, not an animal model for condylar resorption due to mechanical loading.

Apart from analyses of well-known correlates of age-related hip fracture risk, such as low BMD, impaired external geometry and deteriorated micro-architecture, there is increasing interest to elucidate nano-structural determinants of fracture risk at the bone mineralized matrix level. In this study we analyzed cortical bone specimens of the femoral neck region in five elderly women who sustained hip fracture and in four healthy women of corresponding age. Atomic force microscopy was performed at external cortical surface providing simultaneously topographical data and phase composition of the examined bone specimens, as well as measures of nano-structural roughness and surface complexity.

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Simultaneous acquisition of 3D topography data and phase composition revealed granular organization of surface mineral phase. The results showed that distribution of grain size was skewed to larger grains in hip fracture cases with mean grain size 65.22±41.21 nm, whereas control cases displayed significantly smaller grains (36.75±18.49 nm, P<0.0001). In contrast to the control group with unimodal grain size distribution, data deconvolution showed two distinct peaks in the fracture group reflecting two groups of mineral grains (peak positions: 36 and 87 nm; both occupying similar areas under the curve). Roughness analysis showed lower surface fractal dimension in fracture cases (1.40 vs 1.56) indicating lower and/or slower surface mineral deposition processes which might suggest a decreased periosteal apposition in patients who would suffer from hip fracture. Based on previous observations that large-grained materials are accompanied by decreased mechanical properties in comparison with fine-grained fabrics, the findings of larger grains in fracture group offer additional explanation for decreased strength of the cortical bone. These results contribute to the understanding of nano-structural basis of age-related bone fragility.

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PP49
Nanomorphological and compositional basis of devitalized tooth fragility
Ksenija Žekic Mihailović1, Petar Milovanović1, Zlatko Rakovec2, Sonja Askrabic3, Jelena Potocnik3, Miroslav Popovic & Marija Djuric1
1Laboratory for Anthropology, School of Medicine, Institute of Anatomy, University of Belgrade, 4/2 Dr Subotica, Belgrade, Serbia; 2Laboratory of Atomic Physics, University of Belgrade, INS Vinca, 12–14 Mike Alasa, Belgrade, Serbia; 3Institute of Physics, Center for Solid State Physics and New Materials, University of Belgrade, 118 Pregrevica, Belgrade, Serbia; 4Faculty of Physics, University of Belgrade, 12-16 Stadentski Trg, Belgrade, Serbia.

Tooth fracture is considered as a major problem in dentistry. As it is commonly observed in dental practice, one of the main factors that lead to increased tooth fragility is its devitalization. However, there is no definite mechanistic explanation for such phenomenon. We hypothesize that the possible response to this matter lies in the changes that occur in dentin due to altered microenvironment after endodontic procedure. Therefore, in this study we analyzed the structural and compositional differences between vital and devitalized dentine. Atomic force microscopic imaging (AFM), and micro-Raman spectroscopy were performed on 16 dentine specimens, eight taken from vital teeth and eight taken from teeth that underwent root canal treatment at least 2 years before extraction and had no infection in root canals. All teeth were upper premolars. The mean size of mineral grains, showed by AFM topography images, was larger in devitalized than in healthy dentine in the same age category. AFM phase shifts in devitalized cases revealed altered mechanical characteristics and suggested differences in composition of material between devitalized teeth and healthy controls. Micro-Raman analyses showed that in devitalized teeth, apart from hydroxyapatite, mineral contained significant amounts of apatite phases with lower calcium content: octacalcium phosphate, dicalcium phosphate dihydrate and tricalcium phosphate. Differences between vital and devitalized dentine bring new insight into basis of devitalized tooth fragility. Larger mineral grains could account for decreased mechanical strength in devitalized teeth. Moreover, calcium-phosphate phases with lower Ca content have lower material strength, and the presence of these phases in devitalized teeth may explain their increased fragility.

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PP50
Micro-morphological properties of osteons reveal changes in cortical bone stability during aging, osteoporosis and bisphosphonate treatment in women
Andreas Bernhardt, Petar Milovanovic, Michael Hahn, Daniela Djoric, Matthias Krause, Stefan Breer, Klaus Pueschel, Elizabeth A Zimmermann, Marija Djoric, Michael Amling & Bjorn Busse
1Department of Osteology and Biomechanics, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; 2Laboratory for Anthropology, School of Medicine, Institute of Anatomy, University of Belgrade, Belgrade, Serbia; 3Department of Forensic Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; 4Lawrence Berkeley National Laboratory, University of California, Berkeley, California, USA.

Bone remodeling is the key process in bone structural reorganization, and its alterations lead to changes in bone mechanical strength. Since osteons reflect different bone remodeling patterns, we hypothesize that the femoral cortex of females with miscellaneous age, disease, and treatment conditions will display distinct osteonal morphology and osteocyte lacunar numbers along with different mechanical properties. The specimens used in this study were collected at autopsy from 35 female donors (young group, n=6, age: 32±8 years; aged group, n=10, age 79±9 years; osteoporosis group, n=10, age of 81±4.9 years; bisphosphonate group, n=9, age 81±7 years). Von Kossa modified stained femoral proximal diaphyseal sections were evaluated for osteonal morphometric parameters and osteocyte lacunar data. Geometrical indices of osteonal cross-sections were calculated to assess the mechanical stability of individual osteons, in terms of their resistance to compression, bending, and buckling. The morphological assessment of osteons and quantification of their osteocyte lacunae revealed significant differences between the young, aged, osteoporosis, and bisphosphonate-treated groups. Calculated osteonal geometric indices provided estimates of the osteons’ resistance to compression, bending and buckling, showing that fracture susceptibility can be already deduced from individual osteons characteristics. In particular, the osteons in the bisphosphonate-treated group presented a spike in two parameters reflecting fracture strength of the cortical bone. The data derived from osteons (as the basic structural units of the cortical bone) in different skeletal conditions can be employed to highlight structural factors contributing to the fracture susceptibility of different groups of individuals.

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PP51
Microstructural adaptation of bone tissue of the facial skeleton to the distribution of occlusal load under physiological conditions
Aleksa Janovic, Petar Milovanovic, Igor Saveljic, Dalibor Nokile, Michael Hahn, Bjorn Busse, Zoran Rakovevic, Nenad Filipovic, Michael Amling & Marija Djuric
1Department of Radiology, School of Dentistry, University of Belgrade, 11 000 Belgrade, Serbia; 2Laboratory for Anthropology, Department of Anatomy, School of Medicine, University of Belgrade, 11 000 Belgrade, Serbia; 3Bioengineering Research and Development Center (BioRRC), Faculty of Engineering, University of Kragujevac, 34 000 Kragujevac, Serbia; 4Department of Osteology and Biomechanics, University Medical Center Hamburg-Eppendorf, D-22529 Hamburg, Germany.

Despite widely accepted classical mechanical theory of buttresses as a load bearing areas in the midfacial skeleton, the data related to its microstructural adaptation on functional demands are scarce. In this study we investigated microstructural features of the different regions in the facial bones in relation to the occlusal load dissipation using a combination of finite element analysis (FEA) and micro-CT analysis (µCT). The FEA was performed on the model of the dry human skull in order to show stress distribution through the midfacial bones during biting. The same skull was used as a source of bone specimens. Cortical (n=25) and cancellous (n=12) bone specimens were detached from the sites of the maxilla and the zygomatic bone, which suffered different stress during FEA. Bone sections were scanned using Scanco Medical µCT 40. Finite element analysis showed an uneven stress distribution through the facial skeleton with the highest stress along the buttresses. There were also differences in the microarchitecture of cancellous bone microarchitecture. Trabecular thickness, number of trabecules, and density of bone volume were higher in the regions subjected to different stress. Cortical bone was found to be thicker, denser, less porous, and with a greater pore diameter in the regions where high stress was noted on FEA. Regions of the midfacial skeleton with different loading history also showed differences in cancellous bone microarchitecture. Trabecular thickness, number of trabecules, and density of bone volume were higher in the regions subjected to different stress. Cortical bone exhibited regional differences in cortical and cancellous bone microarchitecture that could be a consequence of different functional demands under physiological conditions.

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Peripheral quantitative computed tomography (pQCT) is receiving considerable attention in the diagnosis and monitoring of human bone diseases. It is well accepted that lower image resolution compared to micro-computed tomography (micro-CT) affects bone morphometry. With advances in micro-CT evaluation techniques such as sample-specific remodeling simulations or dynamic bone morphometry, there is the potential to also allow the application of such techniques to clinical pQCT scans. Therefore, virtual high-resolution image reconstruction was considered to improve image resolution and with that to allow advanced quantification schemes. We hypothesized that upsampling pQCT images either preserves or enhances bone morphometry.

Accuracy was investigated by downsampling 16 ex vivo human vertebral grayscale scans from 17.4 to 87.0 μm and subsequent upsampling to higher image resolutions (17.4, 34.8, 52.2, and 69.6 μm). The morphometric indices, bone volume fraction, specific bone surface, trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), trabecular number (Tb.N), structural model index, degree of anisotropy, and connectivity density were compared to the lowest and the highest image resolution. Reproducibility was assessed by precision errors of 14 times three repeated cadaveric forearms scanned at 82 μm and virtually reconstructed at 41 μm resolution. Sensitivity was investigated by a clinical study of 100 fractured and 105 non-fractured human forearm pQCT scans.

Regarding accuracy, the scans upscaled from the 87-μm-resolution images deviated maximum 11.1% (Tb.N, 17.4 μm) and 42.3% (Tb.Sp, 17.4 μm) from the original 17.4-μm-resolution images, indicating that bone morphometry could be preserved but not enhanced. The technique was reproducible (1.96–7.88%) and sensitive to changes as in the clinical study, all indices (except Tb.Th) were significantly lower in the fractured group at 41-μm-resolution (P<0.05). These results agreed with the differences at 82-μm-resolution where all indices showed significant differences (P<0.05). We conclude that virtual high-image-resolution reconstruction can be applied to pQCT scans, however, it does not provide more information than the original lower image resolution.

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**PP55**

In *vitro* exposure of rat femur to strontium chloride influences bone material level properties and increases bone strength

Patrick Ammann & René Rizzoli
Division of Bone Diseases, Department of Internal Medicine Specialties, Faculty of Medicine, Geneva University Hospitals, 1211 Geneva, Switzerland.

Bone microarchitecture and material level properties independently contribute to the improvement of bone strength induced by strontium (Sr) ranelate treatment as evaluated by µCT-based finite element analysis. The influence of *in vitro* Sr exposure on material level properties and on bone mechanical properties is unknown.

We investigated whether *in vitro* exposure of rat femurs to Sr is able to modify the bone mechanical properties independently of geometrical changes. One femur was exposed overnight to 1 M SrCl2 solution and the controlateral to 1 M NaCl2 solution. Then three point-bending tests were performed allowing the determination of maximal load, stiffness, and energy as well as post yield behaviors, i.e. post yield load and deformation characterizing plastic phase. Similar protocol was performed using 1 M CaCl2 solution to investigate the specificity of Sr. Bone material properties was evaluated using nanoindentation. The total number of investigated bone samples was 32, significant differences were evaluated by student paired t-test.

The *in vitro* exposure to 1 M SrCl2 solution increased significantly maximal load (+13%), energy (+30%) but not stiffness. In this model, modification of bone mass, geometry, or micro architecture could be excluded since exposure to Sr was performed *in vitro*. Modification of mechanical properties could thus only be attributed to modification of bone material level properties; which were all significantly increased by *in vitro* Sr exposure. Furthermore, parameters characterizing plastic deformation of the femur were markedly improved by Sr exposure: plastic energy (+76%) post yield load (+45%) and post yield deflection (+62%). Interestingly, these results are similar to those obtained by *in vivo* by Sr ranelate treatment. Exposure to CaCl2 did not affect mechanical properties underlying the selectivity of the Sr effect.

These results further support the important role of bone material level properties as a determinant of bone strength.

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significantly altered: like geometry (decreased cortical thickness) and bone material level properties (decreased modulus, hardness and working energy). Parameters of micro-indentation were significantly affected on the same direction: IDI was significantly increased and unloading stiffness and average energy dissipated were significantly decreased. The values of micro-indentation were systematically correlated with the other measurements. The best significant correlations were observed between indentation distances and hardness (nanoindentation) and between average energy dissipated and plastic energy (biomechanics); for all the significant correlation $r^2$ are ranged between 0.5 and 0.327. These observations indicate that parameters of microindentation predict values of material level properties measured by nano-indentation and biomechanics. These observations open large possibilities to investigate in vivo material level properties.

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**PP56**

**Quantitative assessment of bone remodelling and osteophyogenesis in murine osteoarthritis**

Patricia Borges1, Tonia Vincent2 & Massimo Marenzana1,2

1 Imperial College London, London, UK; 2 University of Oxford, Oxford, UK.

Subchondral bone remodelling and osteophyte growth are widely recognised hallmarks of knee osteoarthritis (OA) although their contribution to disease is not fully understood. Murine models, with targeted genetic modifications, have become powerful tools for discovering disease pathophysiology. Our unpublished observations suggest that osteophyte formation is independent of cartilage loss thereby implying potentially independent molecular drivers. We have developed a novel imaging method to automatically quantify osteophyte growth and subchondral bone remodelling in murine OA. OA was induced by surgical destabilization of the medial meniscus (DMM) in the right knee joint. 10-week-old C57Bl/6J mice (n = 6) were operated and sacrificed at 1, 2, 4, 8, 12, and 20 weeks post-surgery. The tibia was imaged by microCT (5 μm/pixel) and analysed by our automated software (Matlab code). Whole epiphyseal volumes were computed from virtually dissected epiphyses (above the growth plate) and any internal porosity was included in the total volume calculation. Automated analysis revealed significant tibial plate thickening from 2 weeks post-surgery, epiphyseal trabecular volume fraction increased and whole epiphyseal volume was expanded from 4 weeks in the DMM group compared with the contralateral. Medial osteophytes were identified by microCT, starting at 4 weeks post-DMM surgery, and confirmed by histology. Osteophyte volume at 4 weeks was $3.3\pm0.3%$ of the whole epiphysis volume in the DMM group, and up to 4% at 20 weeks post-surgery. However, osteophyte growth showed strong correlation with whole epiphysis expansion only at 20 weeks post-DMM, implying that additional shape modelling contributed to the expansion in earlier time points. Our quantitative automated image analysis identified bone changes, including subchondral plate and trabecular sclerosis and osteophyte growth, from early stages in the DMM model of murine OA. This represents a robust and potentially high throughput method for the assessment of bone structural changes in murine OA.

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**PP57**

**Role of receptor activity modifying protein 3 in the response of bone to mechanical loading**

Matthew Livesey, Suruchi Pacharne, Ning Wang, Peter Grabowski, Lang Yang, Gareth Richards & Tim Skerry

Department of Human Metabolism, The Mellanby Centre for Bone Research, The University of Sheffield Medical School, Sheffield, UK.

Adaptive responses of the skeleton to loading changes architecture and physical properties in order to optimise strength for function. However, bone is subjected to many local and circulating osteotropic factors, most acting on G-protein coupled receptors. Receptor activity modifying protein-3 is a single transmembrane domain receptor accessory protein, which aids in trafficking of calcitonin and calcitonin-like receptors to the cell surface and changes ligand selectivity. As RAMP3−/− mice have a high bone mass phenotype, we hypothesised that their bones would respond less to mechanical loading than wild types as they already have a skeleton that is adapted to supra-physiological loads. We applied cyclical dynamic loads to left tibia of RAMP3−/− (n = 8) and WT (n = 7) male mice, using a trapezoidal waveform, with peak compressive loads of 15N, engendering high physiological strain magnitudes at 180 000 microstrain per second on alternate days for two weeks. Right tibiae were internal non-loaded controls. In WT mice, whole bone volume was increased by 18% in the loaded tibia (P = 0.03) when compared to a 15% increase in the RAMP3−/− group (P = 0.05). Loading induced significant changes in cortical bone volume of both groups compared with contra-lateral non-loaded tibiae, but there was no difference between the two groups (WT: 11%, increased cortical volume, $P = 0.0001$ compared with 10% increase in RAMP3−/− mice, P = 0.0168). Analysis of surface properties of bones in the two groups using a reference point micro-indentation device showed that there was no difference in the surface mechanical properties of loaded bones in the two groups (total indentation distance in RAMP3−/− mice: 36 ± 9 μm compared with 30 ± 7 μm in WTs). These results are consistent with an ability of RAMP3 to exert an inhibitory effect on bone formation, not through a change in sensitivity to mechanical loading, but through a receptor mediated endocrine or paracrine response.

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**PP58**

**Diagnostic discrimination of TBS and spine BMD in glucocorticoid-induced and postmenopausal osteoporosis**

Margaret Paggios1, Nicola Peel2 & Richard Eastell1

1 Mellanby Centre for Bone Research, University of Sheffield, Sheffield, South Yorkshire, UK; 2 Sheffield Teaching Hospitals NHS Foundation Trust, Sheffield, South Yorkshire, UK.

Glucocorticoids inhibit osteoblast function and cause an increase in osteoblast and osteocyte apoptosis. Bone remodelling defects occur resulting in an increase in fracture risk that cannot be fully explained by decreases in bone mineral density (BMD). We propose that this may be due to alterations in bone quality. Trabecular bone score (TBS) correlates with 3D bone micro-architectural parameters and can be derived directly from grey-level variations within 2D DXA images. We assessed the ability of BMD, TBS, and BMD + TBS to discriminate between healthy women and i) glucocorticoid-treated women and ii) women with recent fractures. Locally recruited older women (n = 484, ages 55–79 years) had either i) taken prednisolone ≥ 5 mg/day (or equivalent) for > 3 months (n = 64, average dose range 5.0–20.0 mg/day) or ii) sustained a recent fracture of the distal forearm (n = 46), proximal humerus (n = 37), vertebral (n = 30), or proximal femur (n = 28). They were compared to healthy population-based women without prevalent fractures (n = 279). Lumbar spine BMD was measured by DXA (Hologic QDR 4500A) and TBS values were derived following scan image reanalysis using TBS – Clinical Data Analysis software v1.6 (Med-Imaps).

BMD + TBS values were calculated using logistic regression analysis. The discriminatory ability; area under the curve (AUC); of BMD, TBS and BMD + TBS for prevalent fracture or glucocorticoid use was determined using receiver operator characteristic (ROC) analysis. The AUCs for i) BMD and TBS; ii) BMD and BM + TBS; and iii) TBS and BM + TBS were compared using pairwise comparisons of ROC curves (P < 0.05).

**Table 1** Discriminatory ability of BMD, TBS, and BMD + TBS for prevalent fracture or glucocorticoid use.

<table>
<thead>
<tr>
<th>Study group</th>
<th>BMD</th>
<th>TBS</th>
<th>BMD + TBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC 95% CI</td>
<td>AUC 95% CI</td>
<td>AUC 95% CI</td>
<td>AUC 95% CI</td>
</tr>
<tr>
<td>Glucocorticoids</td>
<td>0.572</td>
<td>0.491–0.653</td>
<td>0.721*</td>
</tr>
<tr>
<td>Forearm fracture</td>
<td>0.641</td>
<td>0.567–0.715</td>
<td>0.621*</td>
</tr>
<tr>
<td>Femur fracture</td>
<td>0.689*</td>
<td>0.602–0.776</td>
<td>0.767*</td>
</tr>
<tr>
<td>Vertebreal fracture</td>
<td>0.876*</td>
<td>0.816–0.935</td>
<td>0.806*</td>
</tr>
<tr>
<td>Hip fracture</td>
<td>0.739*</td>
<td>0.649–0.830</td>
<td>0.696*</td>
</tr>
</tbody>
</table>

*AUC different from 0.5 (P < 0.05)  
*BMD differs between BMD and TBS (P = 0.002).  
**BMD differs between BMD and BM + TBS (P = 0.002).  
***BMC differs between TBS and BM + TBS (P = 0.002).

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Bone development/growth and fracture repair

**PP59**

The effect of mTORC1 on postnatal skeletal development

Mary Matthews1, Andrew Zannettino1, Stephen Fitter2 & Sally Martin1,2

1University of Adelaide, Adelaide, South Australia, Australia; 2SA Pathology, Adelaide, South Australia, Australia.

Mammalian target of rapamycin (mTOR) is a serine–threonine kinase that plays a central role in a number of key cellular pathways that have been previously implicated in bone formation. mTOR mediates these diverse roles by forming two multi-protein complexes, mTORC1 and mTORC2, each of which is defined by unique proteins raptor and rictor respectively.

Studies from our laboratory have previously demonstrated that inhibition of mTORC1 increases the osteoblastic potential of MSCs and increases mineral production while simultaneously inhibiting adipogenic differentiation, suggesting the potential for mTORC1 as a therapeutic target for osteoporosis-related bone disease. To determine the effect of mTORC1 on the formation of the skeleton, we have utilised the Cre-loxP system to generate mice with targeted deletion of *raptor* in pre-osteoblast cells. This was achieved by crossing mice expressing the Cre recombinase under control of the pre-osteoblast specific osteonexin promoter with mice harboring floxed *raptor* genes.

This study examined the *in vivo* effect of osteoblast specific knockout of *raptor* on postnatal skeletal development. Male and female *OB*^raptor−/−* (hom), *OB*^Kapo−/−* (het) and wildtype (WT) littermate controls were harvested at 4, 8 and 12 weeks of age. Histological and *μ*CT analyses were used to assess changes in skeletal development. When compared to WT, hom and het animals display a stunted phenotype with a significant reduction in weight and height at 4, 8 and 12 weeks of age. Analysis of the tibial micro-architecture by *μ*CT indicates a disruption of trabecular bone formation during development in the hom and het animals. Histological analyses show that this is coupled with a decrease in width of the tibial growth plate at 4 weeks. Furthermore, *μ*CT images of the calvaria demonstrate a decrease in mineral thickness and impaired suture formation in both het and hom mice compared to WT at all time points examined. These findings implicate mTORC1 in osteoblast maturation and function in postnatal skeletal development.

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**PP60**

MEK inhibitors in fracture healing and NF1 pseudarthrosis

David Little1,2,3,4, Jud El-Hoss1,4, Mille Kollind1,5, Nikita Des6,7, Michelle McDonald8, Kate Sullivan9, Chris Little10,11 & Aaron Schindele12,13

1The Children’s Hospital at Westmead, Westmead, New South Wales, Australia; 2Royal North Shore Hospital, St Leonards, New South Wales, Australia; 3University of Sydney, Sydney, New South Wales, Australia; 4Garvan Institute, Darlinghurst, New South Wales, Australia.

Neurofibromatosis type 1 (NF1) is a genetic disorder with an incidence of 1/3000. Inactivating mutations in the NF1 gene cause Ras-MEK overstimulation, and predisposes NF1 patients to cancer. A new generation of MEK inhibitors (PD0325901 and AZD6244) are under clinical trials in cancer patients, including NF1 patients. Congenital pseudarthrosis of the tibia is a major complication for NF1 patients, and associates with loss-of-heterozygosity of the NF1 gene. The primary aim of this study is to assess the impact of clinically available MEK inhibitors on bone homeostasis and fracture healing. The secondary aim of this study is to assess whether the MEK inhibitor PD0325901 is able to inhibit Ras-MEK over-activity and thus promote fracture healing in a mouse model of NF1 pseudarthrosis.

C57Bl/6 mice underwent a tibial midshaft fracture, and were treated with PD0325901 or AZD6244 at 10 mg/kg once a day. PD0325901 increased cartilage production while simultaneously inhibiting adipogenic differentiation, multi-protein complexes, mTORC1 and mTORC2, each of which is defined by unique proteins raptor and rictor respectively. Inactivating mutations in the NF1 gene cause Ras-MEK overstimulation, and thus promote fracture healing in a mouse model of NF1 pseudarthrosis. These compounds may be therapeutically beneficial in NF1 pseudarthrosis, where they act synergistically with rhBMP2 to promote bone formation and fracture healing.

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**PP61**

Vascularisation and progenitor cells of primary and secondary ossification centres in the human growth plate

Sonja M Walzer1, Erdal Cetin1, Ruth Grubl-Barabas1, Irene Sulzbacher2, Beate Rüger3, Werner Girsch3, Reinhard Windhager2 & Michael B Fischer2

1Clinic of Orthopaedic Surgery, Medical University Vienna, Vienna, Austria; 2Clinic for Bone Group Serology and Transfusion Medicine, Medical University Vienna, Vienna, Austria; 3Department of Pathology, Medical University Vienna, Vienna, Austria.

The switch from a cartilage template to bone during endochondral ossification of the growth plate requires dynamic and close interaction between the cartilage and the developing vascular structures. Vascular invasion of hypertrophic cartilage, with blood vessels coming from the bone collar, serves to bring in osteoblast- and endothelial precursor cells along with chondroclasts and their precursors into future ossification centres of the growth plate. Potential progenitor cells in different zones of the growth plate and the surrounding encircling fibrochondroossesous structure was investigated. Vascularization of growth plate in ossification centres was studied by immunohistochemistry using markers specific for endothelial cells CD34 and CD31, smooth muscle cells α-SMA, endothelial progenitor cells CD133, CXCR4, VEGFR-2 and mesenchymal progenitor cells CD90 and CD105. Morphometric analysis was performed to quantify RUNX2 and DLX5 hypertrophicochondrocytes, RANK chondro- and osteoclasts, and CD133 progenitor cells in the different zones of the growth plate.

Vascular invasion of primary ossification centres with CD34+ endothelial cells, that did not express the mature endothelial cell marker CD31 yet led to the formation of vessels that lacked abluminal coverage with α-SMA+ smooth muscle cells. In close proximity to the sequestration vessels, single CD133+ cells were found, that seemed to be involved in the formation of the future stem-cell niche rather than in vasculogenesis because they lacked expression of VEGFR-2. Vessels in newly formed bone, in perichondrial groove of Ranvier that harboured CD90/CD110 chondro-progenitors, and in perichondrium were shown to be more developed because they were stabilized by α-SMA+ smooth muscle cells. In conclusion, vascularisation of ossification centres of the growth plate seem to be mediated by the sprouting of newly formed capillaries coming from the bone collar or by intussusceptions rather than by *de novo* vessel formation by endothelial progenitor cells.

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**PP62**

Intermittent administration of parathyroid hormone (1–34) may induce the formation of cementum and bone; a histological study in rats

Daniel Vasconcelos1, Marcelo Marques2, Silvana Barros3, Any Carolina Vasconcelos4, Bruno Benatti5 & Pedro Novaes5

1Federal University of Piauí, Parnaíba, Piauí, Brazil; 2University of Campinas, Piracicaba, São Paulo, Brazil; 3University of North Carolina, Chapel Hill, North Carolina, USA; 4Federal University of Maranhão, São Luiz, Maranhão, Brazil.

The aim of this study was to evaluate the effect of anabolic PTH on periodontal repair and mandibular bone defect in rats. Fenestration defects were created unilaterally of lower first molars in Wistar rats (n = 32), and both periodontal ligament and cementum were removed. Animals were treated 3 times a week and then assigned to four groups (n = 8): i) C14 – placebo administration for 14 days; ii) P14 – PTH administration for 14 days; iii) C21 – placebo administration for 21 days; and iv) P21 – PTH administration for 21 days. Analyzed: I-extension of the initial defect; II-extension of the remaining defect; III-area of the remaining...

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Bone surface in mm² was 0.33

Tissucol

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Financial support

procedures the present results show that neither Gelfoam irrespective the treatment bone volume and surface were kept constant from 4 to 8 control group at 4 and 8 weeks, respectively. There is no statistically significant

control (Kruskal–Wallis test (0.98 and 6.21 G 2.51 in Gelfoam (0.27 in Tissucol w 2.72 and 6.99 in control group, and 0.24

group, 6.53

G 3.33 in control group, respectively. Bone surface in mm² was 0.33±0.05 and 0.27±0.08 in Gelfoam® group, 0.32±0.13 and 0.45±0.27 in Tissucol® group, and 0.24±0.01 and 0.28±0.11 in control group at 4 and 8 weeks, respectively. There is no statistically significant difference in both bone volume and surface among the three groups. Furthermore, irrespective the treatment bone volume and surface were kept constant from 4 to 8 weeks. Despite the potential to act as scaffolds in bone tissue engineering procedures the present results show that neither Gelfoam® nor Tissucol® are capable of stimulating bone formation.

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Abstract withdrawn.

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Bone morphogenetic protein 6 (BMP6) is a member of TGF-β superfamily with a high potential to induce new bone and cartilage. Here we demonstrate that BMP6 compared to the BMP7 paralog has unique biological properties. Previously, we showed that BMP6 is more active at lower amounts then BMP7 because of increased resistance to noggin due to lysine in position 60. Next, we discovered that BMP6 binds to blood coagulum components, which when modified with calcium salt and a fibrin (WBCD) serve as a bio compatible BMP6 carrier for bone fracture repair. We confirmed by dot blot analysis specific binding affinity of BMP6 to components of blood coagulum including fibrinogen-like molecules.

In vivo we proved that low amounts of BMP6 were two orders of magnitude more potent than BMP7 used in current commercial devices. In an animal model of critical size defect of rabbit ulnae we compared WBCD alone, the commercial device containing 1 g bovine collagen and 3.5 mg BMP7 (Ostigraft), and WBCD containing 50 μg BMP6 for 8 weeks, and found that 50 μg BMP6 was more efficacious than 3.5 mg of BMP7. At 8 weeks, critical size ulna defect in rabbits treated with WBCD containing BMP6 fully bridged the bone defect at a significantly accelerated rate than the commercial bone device. Recombinant human GMP produced BMP6 will be clinically investigated in indications for regeneration of the metaphyseal bone fracture repair which could not be achieved by BMP2 and BMP7 based bone devices. Development of the new bone device OSTEOGROW is supported by a seventh framework program (FP7).

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PP67

Hip fracture induces a transient appearance of circulating mesenchymal stem cells
Terhi J Heino1–2, Bettina Sederquist1 & Hannu T Aro2
1Department of Cell Biology and Anatomy, University of Turku, Turku, Finland; 2Orthopaedic Research Unit, University of Turku, Turku, Finland.

We have previously demonstrated the presence of circulating mesenchymal stem cells (MSCs) in fracture patients. However, the exact time for their appearance in circulation remains unknown. Nine patients (five females, four males, age 70-12 years, range 55-89 years) with a fresh femoral neck fracture were recruited in the study, which was approved by the local ethical committee. Bone marrow (BM) sample drawn from the iliac crest of all patients served as the individual MSC reference. Peripheral blood (PB) sample was drawn prior to fracture surgery and subsequently on days 1, 2, 3, 7, 14, and 42, corresponding to the median times of 31, 46, 77, 99, 194, 363, and 1031 h after fracture. Mononuclear cells were successfully isolated from 8/9 BM samples and from 56/57 PB samples by Ficoll gradient centrifugation. Small colonies of plastic-adherent, fibroblast-like cells were found in BM samples of all patients and in PB samples of 6 patients. In PB samples, the earliest time-point for positive cultures of plastic-adherent cells after fracture was 26 h and the latest time-point 434 h. Cultures of these cells were expanded and cells were characterized for proliferation, colony formation and tri-lineage differentiation, as well as surface marker expression. No significant differences were observed between BM and PB derived cells in proliferation, colony formation or osteogenic differentiation. Both were also positive for CD105, CD73 and CD90 and negative for CD14, CD45 and CD34. Majority of PB samples, which were positive for MSCs were obtained within 4 days after fracture. Two patients had cells in two or more samples and four only in a single sample. In conclusion, MSCs appear in peripheral blood mainly within the first four days after fracture but individual variation exists. Catching and identification of MSCs proved to be tedious, making this kind of studies challenging.

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PP68

Long bone phenotypic analyses of a RANK transgenic mouse line
Vanessa Baaroun1, Amélie Couder1, Caroline Marty1, Beatriz Castaneda1, Ariane Berald1 & Vianney Descroix2
1INSERM UMR 972, Paris, France; 2INSERM UMR 606, Paris, France.

Introduction
Bone metastasis pathophysiology is currently defined as a vicious circle. Indeed, tumor cells co-express RANK-L and its receptor, RANK, allowing their own proliferation and peritumoral bone resorption necessary to the lesion expansion. Osteotogenic tumours, which occur within the jaw, seem to present the same pathophysiological characteristics. Thus, studying the impact on bone of RANK over-expression by the osteoclasts would permit a better understanding of those tumoral processes. We will here describe a part of our data, concerning the bone phenotype of a transgenic mice model over-expressing RANK in the monocyte-macrophage lineage.

Description of methods
Forty-one 6 weeks old mice were used for this study; they were analyzed according to their genotype and sex. For each mouse, right femur was used for in vivo analysis of bone phenotype. As we described recently that osteoclasts lacking Dock5, an activator of the GTPase Rac, cannot resorb the calcified hypertrophic cartilage during endochondral ossification and growth of long bones, we hypothesized that RANK over-expression by the osteoclasts would induce structural modifications similar to those observed in Dock5−/− mice. To confirm this hypothesis, we studied the expression of MMP9 and MMP13 in BM of 6 weeks old mice from days E17.5 to P0. Aortic arch vessel samples were collected for in vitro analysis of RANK-L expression. Our results show that the sealing zone is dispensable for osteoclasts to resorb the mineralized hypertrophic cartilage. They further show that osteoclasts deficient for bone resorption are unable to resorb the mineralized hypertrophic cartilage. This study aims at determining if casein would lead to a better bone status than soy in the context of a moderate protein restriction (6% of total energy intake) in growing mice.

PP69

The sealing zone is not required for mineralized cartilage resorption during endochondral ossification and growth of long bone
Heiani Touaiatuhatu1,2, Gaelle Cres1,2 & Anne Blangy1,2
1CNRS UMR 5237 CRBM, Montpellier, France; 2Montpellier University, Montpellier, France.

Introduction
Osteoclasts are the only cells with the capacity to degrade mineralized matrices, such as bone and calcified cartilage. During bone remodeling, osteoclasts secrete proteases to achieve the acidic dissolution of hydroxyapatite to make bone collagen amenable to digestion by the proteases they produce. This requires the sealing zone, a ring of densely packed podosomes that surrounds the ruffled border, which is the secretion apparatus of the bone resoring osteoclasts. Osteoclasts also resorb the calcified hypertrophic cartilage during endochondral ossification and growth of long bones, but the mechanism is poorly characterized. We showed recently that osteoclasts lacking Dock5, an activator of the GTPase Rac, cannot form sealing zones and are unable to resorb the bone in vitro and in vivo, leading to high trabecular BV/TV (JBMR 2011 26 (5) 1099–1100).

Results
Here we analyzed further the development and growth of long bones of Dock5−/− mice between E17.5 and P35. The structure of the growth plate and the expression of MMP9 and MMP13 were normal in Dock5−/− mice, as was the secretion of MMP9, TRAP and CtsK by Dock5−/− osteoclast. The primary spongiosa, where hypertrophic mineralized cartilage is replaced by bone, was also indistinguishable between WT and Dock5−/− animals. They had identical BV/TV and osteoclast numbers from E17.5 to P4. This suggests that hypertrophic cartilage replacement by bone is not affected in Dock5−/− mice. Interestingly, after P7, when bone remodeling starts in the secondary spongiosa, Dock5−/− mice showed higher trabecular BV/TV selectively in the secondary spongiosa, whereas BV/TV remains identical to Dock5+/+ mice in the primary spongiosa. Higher trabecular BV/TV persisted in Dock5−/− mice until P35 and the end of bone growth. Finally, the overall bone length was identical between Dock5−/− and Dock5+/+ mice.

Conclusions
Our results show that the sealing zone is dispensable for osteoclasts to resorb the mineralized hypertrophic cartilage. They further show that osteoclasts deficient for bone resorption are unable to resorb normal endochondral ossification and growth of long bones. For the first time, we demonstrate here that the lack of the sealing zone only affects the bone remodeling activity of the osteoclasts but not their ability to resorb the hypertrophic mineralized cartilage.

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PP70

A casein-based diet leads to a better bone status than a soy protein-based diet during moderate protein restriction in growing mice
Emilien Rouy1,2, Norbert Laroché1, François Blachier1, Daniel Tomé1, Laurence Vico1 & Anne Blais1
1UMR 914 Inra Agro Paris Tech, Paris, France; 2Yoplait France, Boulogne-Billancourt, France; 3U1059 Inserm, Saint Etienne, France.

This study aims at determining if casein would lead to a better bone status than soy in the context of a moderate protein restriction (6% of total energy intake) in growing mice.

Ten-week-old female Balb/C mice were divided in four groups of 15 animals. Two groups received 6% of their energy intake as protein, one as casein and the other as soy protein. The third group was a normal-protein control receiving 20% soy protein. The last group (positive control) was fed the 6% soy protein diet and had a daily injection of PTH 1–34 used as an anabolic agent. After 8 weeks, all animals were sacrificed, blood parameters were measured and L2 vertebrae and femur were analysed by micro-tomography.

The 6% soy mice were smaller and lighter than their casein counterparts because or reduced lean tissue gain. In the femur, the 6% casein and 20% soy diets led to a better bone status than the 6% soy diet as evidenced by higher cortical thickness, bone volume (BV/TV) and trabecular number. The 6% soy mice also had a higher femoral structure model index (SMI), i.e. more plate-like structure conferring more mechanical resistance. Serum analysis showed higher levels of P1NP, IGF1 and CTX in the 6% casein and 20% soy groups than in the 6% soy group. Uterus and spleen weight were smaller in the 6% soy group. PTH daily injection had a beneficial effect on P1NP and on femoral parameters when compared with the 6% soy group, and a beneficial effect on vertebral parameters against all other groups. This study demonstrates that during moderate protein restriction, casein maintains better bone status than soy. The mechanism involved is not known but may partly
PP71

Accuracy errors in longitudinal QCT measurements of cortical thickness bone mineral density and bone mineral content using different segmentation techniques: a simulation study

Bastian Gerner, Oleg Museyko, Dominique Töpfer & Klaus Engelke
University of Erlangen, Erlangen, Germany.

Introduction

The quantification of cortical BMD and thickness in QCT images remains challenging due to the limited spatial resolution of CT scanners. We simulated the impact of longitudinal cortical BMD and thickness changes on accuracy of cortical measurements using three different segmentation algorithms.

Methods

A step function of varying width d (cortical thickness) and height (cortical BMD) and an additional step representing trabecular BMD was convoluted with a Gaussian function of varying full width at half maximum (FWHM) describing the CT scanner resolution and simulating the density distribution within a reconstructed CT image. Used segmentation algorithms: local adaptive 50% thresholds (LA), global thresholds (GT), Levenberg-Marquardt based optimization method (OM)1. Accuracy errors of ΔBMD, Δd and ΔBMC measurements in the CT images were estimated by simulating a 2.5, 5 and 7.5% BMD increase at constant d and a 5 and 10% increase of d at constant BMD.

Results

i) Simulated change in d: with LA and GT increasing accuracy errors in d occur for d<2FWHM and with OM for d<4FWHM. All three algorithms resulted in false cortical BMD increases. ii) Simulated BMD change: all three algorithms showed accuracy errors in cortical BMD for d<2FWHM. LA showed no effects on d. GT overestimated d for d<2FWHM, while OM overestimated d for d<4FWHM. Added noise (20HU, obtained from standard QCT images of the spine) affected particularly OM if the cortex was thin (d<FWHM). In general, BMC errors were smaller than those for BMD.

Conclusion

The investigated algorithms show good results for d>2FWHM. For thinner cortices, each segmentation method affects cortical parameters differently. It is important to measure cortical BMC in addition to BMD.

Reference

1. Treece et al. Medical Image Analysis 2010.

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PP72

Calcium phosphate cement/strontium enhances bone formation in the metaphyseal osteoporotic fracture

Thaqif El Khassawna1, Seemun Ray1, Ulrich Thormann2, Katrin Lips1, Michael Gelinsky3, Matthias Schumacher3, Alexander Claus Langheinrich4, Reinhard Schnettler1,2 & Volker Alt1,2

Objective

Osteoporotic fractures are a growing problem especially in aged societies of industrialized countries. Therefore, a clinical demand for synthetic bone graft substitutes is increasing. Despite the general success CPC showed clinically, development of CPC-based material by adding strontium could improve its suitability to treat osteoporotic fractures.

Methods

Sprague-Dawley rates model of induced osteoporosis via multi-deficiencies diet combined with bilateral ovarectomy were utilized. A 4 mm wedge-shaped metaphyseal osteotomy was introduced in the left femur. CPC and CPC/strontium were compared with an empty defect in their ability to remedy osteoporotic fracture after 6 weeks. Morphological evaluation by means of μCT and histomorphometry on Movat pentachrom staining was performed. TRAP staining was carried out to evaluate catabalism at the fracture site. Samples were not normally distributed, therefore a Kruskal–Wallis Test was used with 0.05 significance cutoff.

Results

μCT evaluation of fracture healing indicated increases in bone mineral density and bone volume in CPC/strontium group compared to CPC healing groups, both were higher than the empty defect group. Histomorphometry confirmed the bone formation results of the μCT analysis. Moreover, CPC group showed significantly higher soft tissue and cartilaginous tissue area than the CPC/strontium one. However, both were significantly lower the empty defect group. CPC/strontium group had a higher osteoclasts count than CPC group, which was in turn higher than the empty defect.

Discussion

CPC/strontium treatment showed an enhanced healing of osteoporotic fractures after 6 weeks than CPC alone. CPC/strontium appears to increase osteoclasts activity, which is needed for degradation of the implant. Moreover, the smaller cartilage fraction seen in CPC/strontium group suggests a positive effect on endochondral ossification. Furthermore, the currently ongoing analysis of bone anabolism and biomechanical competence would indicate strontium’s effects on other aspects of bone healing.

PP73

Feasibility of local CD133+ cell transplantation to avoid non-unions in biological impaired bone healing

Anke Dienelt1,2, Andrea F Sass1, Bernd Preininger2, Katharina Schmidt-Bleek2 & Georg N Duda1
1. University Hospital Carl Gustav Carus, Dresden, Saxony, Germany; 2. Medical Research, University Hospital Carl Gustav Carus Dresden, Dresden, Saxony, Germany.

The clinical orthopaedic problem of delayed healing or non-union after complex fractures affects 5–10% of all patients, especially within the elderly population. Recently several in vitro studies showed that CD133+ cells bare angiogenic capacities and contribute to a better outcome concerning ischemia induced angiogenesis in vivo. A local administration of these specific cells to the fracture gap appears feasible as a new treatment option for biological impaired fracture healing.

We analyzed availability, angiogenic and osteogenic properties of CD133+ cells derived from peripheral blood of healthy young and aged, male and female probands in vitro to answer the question whether cells obtained from aged patients bare the same regenerative potential as cells from young donors. For this purpose flow cytometric measurements, co-cultures with endothelial cells and osteogenic differentiation assays together with mesenchymal stroma cells were performed. The regenerative capacities of CD133+ cells were also investigated in vivo in an aged animal model with biological impaired fracture healing. The experiments confirmed that CD133+ cells contain high angiogenic capacities. We also observed that the quantity of CD133+ cells increases twofold in aged people, making them an even more attractive target for intraoperative transplantation. The positive effect of local CD133+ cell transplantation could also be revealed in vivo by an enhanced bone tissue formation accompanied by a twofold increased bone mineral content. The improved bone regeneration went along with a threefold elevated development of new blood vessels within the fracture site.

Aiming to identify a new source for cells utilisable for cell therapy, we could prove that CD133+ mononuclear cells derived from peripheral blood feature bone regenerative capacities. Thus, an application of these cells to fracture sites is a promising approach for the treatment of impaired fracture healing.

PP74

Metaphyseal fracture healing in a sheep model of low turnover osteoporosis induced by hypothalamic–pituitary disconnection

Ronny Bindl1, Ralf Oheim2, Pia Pogoda1, Frank Timo Beil2, Katharina Gruchenberg1, Sandra Rettemaier1, Tim Wehner1, Enrico Calcia1, Peter Radermacher1, Lutz Claes1, Michael Amling1 & Anita Ignatius1

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We recently established a large animal model of osteoporosis in sheep using hypothalamic–pituitary disconnection (HPD). As central regulation is important for bone metabolism, HPD-sheep developed severe osteoporosis because of low bone turnover. In this study we investigated metaphyseal fracture healing in HPD-sheep. To elucidate potential pathomechanisms, we included a treatment group receiving thyroxine (T4) and 17β-estradiol. Because clinically osteoporotic fractures often occur in the bone metastasis, HPD-sheep and healthy controls received an osteotomy in the distal femoral condyle. Half of the HPD-sheep were systemically treated with T4 and 17β-estradiol during the healing period. Fracture healing was evaluated after 8 weeks using pQCT, μCT and histomorphometrical analysis. Bone mineral density (BMD) and bone volume/total volume (BV/TV) were significantly reduced by 32 and 34%, respectively, in the osteotomy gap of the HPD-sheep compared to healthy sheep. Histomorphometry also revealed a decreased amount of newly formed bone (~30%) and some remaining cartilage in the HPD-group. T4 and 17β-estradiol substitution completely rescued bone healing in the HPD-sheep.

In summary we found disturbed metaphyseal bone healing in a sheep model of low turnover osteoporosis induced by HPD. This was confirmed by a decreased amount of newly formed bone in the osteotomy gap of HPD-sheep compared to healthy sheep. The mechanisms being responsible for the low turnover osteoporosis in the HPD-sheep are not yet fully clarified. In this pilot study we demonstrated that the substitution of the thyroid hormone T4 and the estrogen 17β-estradiol nearly completely abolished the deleterious effect of HPD on fracture healing indicating that the deficiency of these hormones play an important role in the pathomechanisms of disturbed fracture healing in the HPD-sheep.

DOI: 10.1530/boneabs.1.PP74

PP76
The effect of post-natal (childhood) obesity on skeletal development
Efrat Monsonorge-Ornan, Stav Simsa-Maziel, Janna Zareski, Sergey Anpilov & Gili Solomon
The Hebrew University, Jerusalem, Israel.

Childhood obesity is a serious global public health problem, reaching 40% of children in developed countries. While the connection between under-nutrition and growth retardation is well documented, the opposite connection between over-nutrition and bone development was barely studied. Obese children grow faster in height than normal-weighted children, and prospective studies demonstrated an over-presentation of obese children amongst fracture cases. Yet, the cellular and molecular underlying mechanisms to this phenomenon are largely unknown.

We analyzed in depth the effect of childhood obesity on young bone elongation and bone quality. Multiple complementary in-vivo models were utilized to characterize in details the growth-plate phenotype as well as the bone structure and mechanical properties. The various models we used are: pharmaceutical inhibition of leptin signaling (by leptin antagonists) and various types of obesogenic diets such as high fat diet (HFD). We found that obesity in young age affected both bone elongation and bone quality. Furthermore, the type of the diet, distinctly from its obesogenic effect, modified bone development and quality. For instance, while HFD based on poly unsaturated fatty acids impairs bone morphology, omega-3 fatty acids improves it. Our studies demonstrated the involvement of metabolic signals such as adiponectin, leptin and IL-1.We discovered a novel mechanism by which osteocalcin shifts chondrocytes toward glycolytic breakdown of glucose and stimulates their calcification, in a HIF-1α-dependent manner. Based on these findings, we suggest that the metabolic status in obesity and the specific component in the diet affect directly the metabolic state of bone cells, leading to accelerated bone elongation and modified processes of bone formation and resorption. This topic is of tremendous importance for both basic and applicative scientists in the fields of pediatrics, nutrition, endocrinology, bone health and development.

DOI: 10.1530/boneabs.1.PP76

PP75
Interplay of physical and biological cues in the regeneration of critical-sized bone defects
Amaia Cipitria1, Johannes C Reichert1,2, Claudia Lange3, Hanna Scholl1, Manav Mehta1, Wolfgang Wagenermaier4, Paul Zaslansky1, Peter Fratzl1, Dietmar W Hutmacher1 & Georg N Duda1
1Julius Wolff Institute and Center for Musculoskeletal Surgery, Berlin-Brandenburg Center for Regenerative Therapies, Charité – Universitätsmedizin Berlin, Berlin, Germany; 2Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Queensland, Australia; 3Department of Trauma, Hand, Plastic and Reconstructive Surgery, Julius-Maximilians-University, Wuerzburg, Germany; 4Department of Biomaterials, Max Planck Institute of Colloids and Interfaces, Potsdam, Germany.

The transplantation of autologous bone graft represents the ‘gold standard’ treatment for large bone defects, despite the harvesting co-morbidity and limited availability. An alternative scaffold-based approach is presented. Our aim was to investigate what degree structured scaffolds alone, or in combination with biological stimuli, allow guiding tissue regeneration. Scaffolds consisting of medical-grade polycaprolactone and tricalcium phosphate microparticles, combined with 3.5 mg rhBMP-7, were implanted in critical-sized segmental defects (3 cm) in sheep tibia. The results were compared with a non-loaded scaffold, with autograft and with an untreated defect. Torsional testing to failure, microcomputed tomography (mCT), histology, SEM, nanoindentation and small angle X-ray scattering (SAXS) were used to assess the regenerated tissue after 3 and 12 months post surgery. After 12 months, biomechanical and mCT analysis showed significantly greater bone formation and superior strength for the scaffolds loaded with rhBMP-7 compared to the autograft. Scaffolds alone induced significantly lower bone formation than the autograft and rhBMP-7 group. Histological analyses unveiled that the scaffold architecture guides the formation of highly organized fibrous tissue across the defect, which influences the microstructure of newly formed bone. SAXS measurements confirmed the alignment of microparticles in the proximal area of the scaffold and lack of alignment in distant regions. Applied clinically, this scaffold-based approach could overcome autograft-associated limitations. Furthermore, the study proves the structural benefit of the presence of a scaffold for soft and mineralized tissue organization. However, the interplay of physical and biological cues is not yet understood. Ongoing analyses compare the morphology, local mechanical properties, mineral particle thickness and orientation of the rhBMP-7 treated and non-treated scaffolds.

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Sex-related differences of femur properties in silver foxes (Vulpes vulpes)
Marcin Tatara¹, Witold Krupski², Andrzej Jakubczak³, Marek Bienko¹ & Krzysztof Kostro²
¹Department of Animal Physiology, University of Life Sciences in Lublin, Lublin, Poland; ²II Department of Radiology, Medical University of Lublin, Lublin, Poland; ³Department of Biological Bases of Animal Production, University of Life Sciences in Lublin, Lublin, Poland; ⁴Department of Epizootiology and Clinic of Infectious Diseases, University of Life Sciences in Lublin, Lublin, Poland.

Considering limited information available on skeletal system properties in foxes, the aim of this study was to determine morphological, geometrical and densitometric parameters of femur obtained from males and females. The study was performed on 1-year old male (n=5) and female (n=6) silver foxes. Right femur was isolated and its weight and length were measured. Using quantitative computed tomography (QCT) technique and Somatom Emotion Siemens apparatus, bone volume (Bvol) and volumetric bone mineral density of the trabecular (Td) and cortical bone (Cd) were determined. Bone mineral density (BMD) and bone mineral content (BMC) of whole femur were determined using dual-energy X-ray absorptiometry (DEXA) method and Norland Excell Plus Densitometer (Fort Atkinson, WI, USA) equipped with Research Scan software. Geometrical parameters of the bones such as cross-sectional area (A), second moment of inertia (Ix), mean relative wall thickness (MRWT) and cortical index (CI) were determined on the basis of vertical and horizontal diameters (both internal and external) at the midshaft. Statistical comparison of the investigated parameters of femur between males and females was performed with a use of non-paired Student’s t-test and P<0.05 was considered as statistically significant. The obtained results showed significantly higher values of length, weight, Td and Cd of femur in males when compared to these parameters determined in females (P<0.05). Similar mean values of A, Ix, MRWT and CI were stated in male and female silver foxes (P>0.05). In conclusion, this study has shown sex-related differences of femur and tibia in silver foxes. Obtained results indicate that silver foxes may serve as an attractive experimental model for further studies on bone metabolism regulation of mammals in response to physiological, pharmacological, nutritional and toxicological factors, being an alternative model for metabolic response of skeleton to physiological, nutritional, toxicological and pharmacological factors influencing bone tissue metabolism.

Bone metabolism is influenced by serum 25-hydroxyvitamin D in healthy children
Edyta Czekuc-Kryskiewicz¹, Elżbieta Karczemarewicz¹, Maciej Jaworski¹, Justyna Czeh-Kowalska², Anna Gorska², Jerzy Konstantynowicz³, Paweł Pładowski¹, Jarosław Piskorski² & Roman Lorenc¹
¹Department of Radioimmunology, Biochemistry and Experimental Medicine, The Children’s Memorial Health Institute, Warsaw, Poland; ²Neonatal Intensive Care Unit, The Children’s Memorial Health Institute, Warsaw, Poland; ³Department of Family Medicine and Community Nursing, Medical University of Bialystok, Bialystok, Poland; ⁴Department of Pediatrics and Developmental Disorder, Medical University of Bialystok, Bialystok, Poland; ⁵Institute of Physics, University of Zielona Gora, Zielona Gora, Poland.

Introduction
Serum 25(OH)D concentrations for optimal bone metabolism in children is unknown. Only few data exist describing the effects of increasing serum 25(OH)D on bone metabolism markers.

Aim
The aim of the study was to explore the association between serum 25(OH)D and bone metabolism markers in children.

Patients and methods
Serum levels of bone formation (OC, P1NP) and bone resorption (CTX) markers (Cobas e411, Roche Diagnostics) were determined in 161 healthy children (mean age: 9.47±4.94 years; range: 1.92–19.66). Vitamin D status was evaluated by serum levels of 25(OH)D and PTH (Cobas e411; Roche Diagnostics). Bone metabolism markers reference intervals were prepared according to age and gender.

Results
Serum 25(OH)D levels <10 ng/ml were described in 25.0% children, 10–20 ng/ml in 40.8% children and >20 ng/ml in 34.2% cases. Only 12.5% patients have serum 25(OH)D >30 ng/ml. Positive correlations were observed among the three bone metabolism markers (R at range 0.67–0.76, P<0.001). The correlation between serum 25(OH)D and PTH (R = -0.26, P = 0.002) indicate significant negative association between these parameters. Multivariate analysis for predictors of age-adjusted bone metabolism markers showed that serum 25(OH)D was strongly and positively associated with OC, P1NP and CTX in healthy children, explaining 10.3% of the variance in OC (P<0.001), 12.5% in P1NP (P<0.0001), and 16.2% in CTX (P<0.0001). Not significant effect of PTH on bone metabolism was evidenced in our study.

Conclusions
Strong and positive association of serum 25(OH)D with bone formation as well as resorption markers indicates that proper vitamin D status is very important for bone health especially in period of bone mass accrual.

A characterization of an Y1R antagonist as a drug for bone regeneration
Inês Alencastre¹, Catarina Almeida¹, Diana Leite¹, Cecília Alves¹, Daniela Sousa¹, Estrela Neto² & Meriem Lamghari³,¹,²
¹INEB, Porto, Portugal; ²FEUP, Porto, Portugal; ³University of Porto, Porto, Portugal.

Recently, Y1 receptor (Y1R) has arisen as a potential regulator in the local control of bone turnover. BIBP3226 is a potent Y1R selective antagonist that was successfully used in in vitro studies showing a positive impact in the benefit of
bone turnover, thus providing good perspectives for its use as a pharmacological tool for bone regeneration.

However, BIBP3226 behaviour in a complex milieu such as the bone compartment is unknown. As drugs can yield different behaviours depending on the cellular and molecular environment, the design of a successful BIBP3226 delivery system requires the understanding of the antagonist performance within the bone compartment.

In this work we characterize Y1R-BIBP3226 binding and cellular pathways within the mice bone cell microenvironment using a fluoroscent labelled BIBP3226 compound (BIBP3226\(^*\)) and assess its potential as a drug for bone regeneration. Confocal microscopy and Image Flow Cytometry assays, showed that BIBP3226\(^*\) promotes Y1R internalization in osteoblast lineage and osteogenitor cells. Y1R-BIBP3226\(^*\) binding and internalization was found in 10–15% of mice bone marrow cells and occurred upon 20 min of incubation with the antagonist. 22% of the hematopoietic lineage cells bind BIBP3226\(^*\) in contrast with the 4% binding found in stromal cells, suggesting a higher contribution of hematopoietic cells in NPY-Y1R regulation of bone turnover. Bone marrow cell populations targeted by BIBP3226\(^*\) were further identified by immunophenotyp- ing and the intracellular Y1R trafficking pathways upon antagonist binding were investigated.

Results highlight the potential of BIBP3226 in the establishment of an Y1R knock out environment within the bone compartment that can be further explored in the future development of a local drug delivery strategy to promote bone regeneration.

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PP82
Measurement properties of radial and tibial speed of sound for screening bone health and fragility in 10–12 years old boys and girls
Lurdes Rebocho\(^1\), Graça Cardadeiro\(^2\), Vera Zymbal\(^1\), Ezequiel M Gonçalves\(^2\), Luís B Sardinha\(^1\) & Fátima Baptista\(^1\)
\(^1\)Exercise and Health Laboratory, Faculty of Human Movement, Inter-disciplinary Centre for the Study of Human Performance, Technical University of Lisbon, Lisbon, Portugal; \(^2\)Growth and Body Composition Laboratory, Faculty of Medical Sciences, Center for Investigation in Pediatrics, University of Campinas, Campinas, Brazil.

The objective of this study was to analyze measurement properties of BeamMed Omnisense quantitative ultrasound (QUS) of the radial and tibial speed of sound (SoS) for assessing bone health and screening bone fragility in youth. Bone fragility was defined as low whole body large head bone mineral density (WBLH BMD) measured by DXA (first tertile, 95% CI: -1.1, -1.0) and as past history of fractures evaluated by questionnaire. The study was conducted with 319 non obese participants (159 boys and 160 girls) aged 10–12 years old. The degree of agreement between equipment ratings was analyzed by concordance coefficient correlations, linear regressions, and Kappa statistic. For this purpose, both QUS and DXA bone variables were standardized. Accuracy of radial and tibial QUS and WBLH DXA to identify participants with past fractures were analyzed by logistic regression. The results revealed concordance coefficient correlations between WBLH BMD and radial and tibial SoS of 0.129 and 0.038, respectively. The radial SoS explained 1.8% of the variability of the WBLH BMD (P = 0.017) while tibial SoS did not explain any WBLH BMD variability. The regression lines between DXA and QUS variables were different from the identity lines. Cross-classification analysis between QUS and DXA showed that of 113 participants in the first tertile of WBLH BMD only 41 participants (36.3%) were categorized in the first tertile of radial SoS and 38 participants (33.6%) in the first tertile of tibial SoS. Logistic regression adjusted for gender and maturity showed that radial SoS was the only significant variable in predicting OR for identifying participants with past fractures: each SD increase in radial SoS (92 m/s) was associated with a 29.1% decrease in fracture OR (P = 0.020). In conclusion, the BeamMed Omnisense QUS seems to provide significant fracture prediction when measured at the distal radius in youth 10–12 years old revealing to be a valuable tool for screening bone fragility despite the absence of agreement with the DXA WBLH BMD.

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PP83
Trabecular micro-architecture of the proximal femur during post-natal growth
Marija Djuric\(^1\), Petar Milovanovic\(^1\), Danijela Djonc\(^1\), Michael Hahn\(^2\), Bjørn H. Basse\(^2\) & Michael Albrecht\(^1\)
\(^1\)Laboratory for Anthropology, School of Medicine, Institute of Anatomy, University of Belgrade, Belgrade, Serbia; \(^2\)Department of Osteology and Biomechanics, University Medical Center Hamburg-Eppendorf, Hamburg, Germany.

In sharp contrast to the great interest in morphology of the proximal femur in aged individuals, there is a paucity of studies in children. To date, a comprehensive quantitative analysis of trabecular micro-architecture in various biomechanically relevant subregions of the immature proximal femur has been lacking. The aim of this study was to characterize postnatal development of trabecular bone in various regions of the human proximal femur. The study sample was comprised of 18 femora between the ages of 1 month and 12 years from the Laboratory for Anthropology osteological collection. Trabecular architecture was evaluated using micro- computed tomography (Scanco Medical pCT 40) in the following regions of interest: medial, intermediate and lateral femoral neck regions, intertrochanteric region, and femoral head. The data show that the most dramatic changes occur by the end of the first year of life. Namely, during the first year, there is a decrease in bone volume fraction due to a significant reduction in trabecular number, despite slight thickening of trabeculae. The trabeculae mainly had a rod-like shape with decreasing connectivity and increasing separation. In the youngest samples, trabeculae are parallel and mainly longitudinally oriented (high degree of anisotropy), which changes to a less anisotropic arrangement towards the end of the first year. After the first year, due to increased mechanical loading and muscle activity, the trabecular micro-architectural parameters change in a linear manner. Bone volume fraction increases along with the number and thickness of trabeculae. Trabecular separation and connectivity density remained fairly stable, but the rod-like trabeculae gradually changed to a mechanically advantageous plate-like or even honeycomb shape. The degree of anisotropy continuously increased and changed the straight and parallel trabecular arrangement to a distinct trabecular pattern composed of groups of trajectories as seen in adults. Furthermore, all structural changes showed a region-dependent pattern related to differences in stresses/strains experienced in different regions of the proximal femur.

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PP84
Variations in osteotoxic effects of cadmium on femoral bone structure after different routes of exposure
Monika Martiniakova\(^1\), Hana Chovancova\(^1\), Radoslav Omelka\(^1\), Ivana Bobonova\(^1\) & Robert Toman\(^2\)
\(^1\)Constantine the Philosopher University, Nitra, Slovakia; \(^2\)Slovak University of Agriculture, Nitra, Slovakia.

Cadmium (Cd) is regarded as a risk factor for various bone diseases in humans and experimental animals. To compare effects of different routes of Cd administration on femoral bone structure, ten 4-month-old male Wistar rats (group A) were injected intraperitoneally with a single dose of 2 mg CdCl\(_2\)/kg body weight and killed 36 h after Cd had been injected. Ten 1-month-old male rats (group B) were dosed with a daily Cd intake of 30 mg CdCl\(_2\)/l in drinking water for 90 days and then were killed. Both groups were compared to the control group (C) of 10 males without Cd intoxication. Macroscopic structure of femur and detailed histological analyses of compact bone tissue were performed in each group. Our results revealed the significant effect of peroral exposure to Cd on femoral weight and qualitative histological characteristics of the bone in rats from the group B. In these rats, bone microstructure was different in the middle part of substancia compacta where primary vascular radial tissue occurred. Also, some resorption lacunae indicating osteoporotic changes near endosteal surface were observed. The different route of Cd administration induced an opposite effect on the size of the primary ostioens’ vascular canals and Haversian canals in groups A and B. Values of all variables (area, perimeter, maximum and minimum diameter) of these structures were significantly increased (P < 0.05) in rats from the group A. In contrast, they were significantly decreased (P < 0.05) in the group B rats. The size of secondary ostioens was not affected by the route of Cd exposure, it was significantly lower (P < 0.05) in both groups (A and B). Results of this study suggest route- and time-dependent effects of Cd on femoral bone structure in adult male rats. This study was supported by the grants KEGA 025UKF-4/2012; 035UKF-4/2013.

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PP85
The retention and bioactivity of rhBMP-2 released from a bisphosphonate-linked hyaluronan-based hydrogel
Gry Hulsart-Billström1, Pik-Kwan Yuen1, Richard Marsell1, Jöns Hilborn3, Sune Larsson1 & Dmitri Ossipov2
1Department of Orthopedics, Uppsala University, Uppsala, Sweden; 2Department of Materials in Medicine, Uppsala University, Uppsala, Sweden; 3Division of Polymer Chemistry, Department of Materials Chemistry, Uppsala University, Uppsala, Sweden.

Introduction
There are several disadvantages with the present carriers used in rhBMP-2 products including risk for immunological response, inefficient release and poor handling properties. Our aim was to examine the release of rhBMP-2 from a bisphosphonate-linked hyaluronan hydrogel with the hypothesis that it would cause a slower release.

Methods
Triplicates of hydrogels with rhBMP-2 and with (HA-BP) or without (HA) bisphosphonate were prepared, after which cell culture medium was added and refreshed at 1, 3, 6, 12, 24, 48, and 72 h and day 6, 9, 12, and 14. The extracts were analysed using ELISA, after which the gels were degraded and analyzed by ALP- assay on stromal-cells.

Results
6% of rhBMP-2 had been released from the HA-BP hydrogel at the end of the study, compared with 100% from the HA hydrogel. The retained rhBMP-2 induced ALP expression of stromal-cells.

Discussion and conclusions
Bisphosphonate caused a remarkable slower release of rhBMP-2 when linked to the hydrogel. The biological functions of the retained rhBMP-2 were still intact.

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PP86
The effect of incubation time of preformed injectable hydrogels on bone formation when used as carrier of rhBMP-2
Sonya Piskounova2, Gry Hulsart-Billström1, Lars Gedda3, Kristoffer Bergman1, Jöns Hilborn3, Sune Larsson1 & Tim Bowden1
1Department of Materials in Medicine, Uppsala University, Uppsala, Sweden; 2A˚ngström Laboratory, Department of Chemistry, Uppsala University, Uppsala, Sweden; 3Department of Oncology, Radiology and Radiation Science, Uppsala University, Uppsala, Sweden.

Introduction
Hydrogels has demonstrated efficacy as carriers for growth factors. Our aim was to investigate the effect of curing-time of modified hyaluronan on bone formation.

Methods
Hydrogels with rhBMP-2 were cross-linked for 14 and 3 days, 5 h or 1 min before injection. Preformed gels were injected s.c. in 5 rats, the rats were killed after 5 weeks. Explanted samples were radiographed and scanned by pQCT.

Results
Bone formation occurred in all samples. Radiographs revealed higher attenuation for the 5 h cross-linked hydrogel. The same result was seen in the pQCT were both 5 h and 1 min preformed hydrogels had significantly higher bone density compared to 3 days (P = 0.0064, ANOVA Tukey’s multiple comparison test). 5 h yielded higher bone mineral content compared to 1 min cross-linked gel (P = 0.0116 ANOVA Tukey’s multiple comparison test).

Conclusion
A minimum of 5 h curing-time gives the most efficient bone formation concerning density, mineral content and volume.

DOI: 10.1530/boneabs.1.PP86

PP87
Premixed calcium phosphate cement as a carrier for bone morphogenetic protein 2
Nick Walters1, Gry Hulsart-Billström1, Håkan Engqvist1 & Sune Larsson1
1Department of Orthopedics, Uppsala University, Uppsala, Sweden; 2Department of Materials in Medicine, Uppsala University, Uppsala, Sweden.

Introduction
A BMP-2-containing premixed calcium phosphate cement (PCPC) could potentially provide the surgeon with an easy-to-handle alloplastic material with osteoinductive properties, accelerating bone ingrowth by stimulating osteogenic differentiation.

Methods
The release profile of BMP-2 at 0.05 and 0.50 mg/ml from the PCPC was characterized over a period of 1 week using ELISA.

Results
The PCPC released BMP-2 in a biphasic manner, with an initial burst release upon the setting of the material followed by a gradual release. At the first hour was ~1.25% released from the 0.50 mg/ml cement and 0.35% from the 0.05 mg/ml cement. After 1 week was 1.5% of BMP-2 released from the 0.50 mg/ml cement and 0.75% from the 0.05 mg/ml.

Conclusion
This initial burst release could be beneficial by attracting MSCs to the material by chemotaxis. The subsequent gradual release should provide the tissue surrounding the cement with BMP-2, potentially resulting in osteoinduction by stimulating differentiation and activate material resorption by osteoclasts, improving integration of the material by bone-ingrowth.

DOI: 10.1530/boneabs.1.PP87

PP88
Low calcium intake aggravates the deleterious effects of an isocaloric low protein diet on bone material level properties during growth
Carole Fournier, René Rizzoli & Patrick Ammann
Service of Bone Diseases, Geneva, Switzerland.

Low protein or low calcium intake are known to impair bone growth, but their combined effects on determinants of bone strength are not well understood. We investigated the influence of various protein and calcium containing diets on determinants of bone strength in growing rats.

One-month-old female rats were fed isocaloric diets containing 10, 7.5 or 5% casein, with 1.1% (normal; NCa) or 0.2% calcium (low; LCa) during 8 weeks. Tibia midshaft geometry (outer-diameter) was measured by a caliper, BMC by DXA, and cortical tissue hardness by nanindentation.

In the presence of NCa, BMC and outer-diameter were lower in the 5% protein diet than in the 10 or 7.5% groups. In the presence of LCa, lower values were observed already in the 7.5% protein group, but without any difference between NCa and LCa in the 5% protein group. In contrast, in the latter condition, cortical tissue hardness was lower in the LCa, compared to the NCa, suggesting some additive effects on this variable.

These results obtained in growing rats indicate that lowering calcium intakes during an isocaloric low protein diet has some additive deleterious effects on material level properties. Altogether these results point out the important role of adequate protein and calcium intakes to optimize bone development during growth.

Table 1

<table>
<thead>
<tr>
<th>Protein Level</th>
<th>7.5% Casein</th>
<th>5% Casein</th>
</tr>
</thead>
<tbody>
<tr>
<td>10% Casein</td>
<td>Normal Ca</td>
<td>Low Ca</td>
</tr>
<tr>
<td>BMC (mg)</td>
<td>0.13 ± 0.007</td>
<td>0.12 ± 0.002</td>
</tr>
<tr>
<td>Outer-diameter (cm)</td>
<td>2.54 ± 0.04</td>
<td>2.54 ± 0.04</td>
</tr>
<tr>
<td>Tissue Hardness (kPa)</td>
<td>640.5 ± 124.0</td>
<td>638.7 ± 123.1</td>
</tr>
</tbody>
</table>

*P < 0.05 vs 10% casein; †P < 0.05 vs 7.5% casein; ‡P < 0.05 vs normal Ca.
PP89

Spirulina alga prevents impairment of peak bone mass acquisition induced by an isocaloric low protein diet
Carole Fournier, Rene Rizzoli & Patrick Ammann
Service of Bone Diseases, Geneva, Switzerland.

New food strategies should be developed to fight against child malnutrition and growth retardation in developing countries. Spirulina alga, one of the richest sources of vegetable protein, contains all essential amino acids. It easily grows in tropical regions. We hypothesized that impaired peak bone mass acquisition (PBMA) caused by dietary protein deficiency could be prevented by Spirulina supplementation in growing rats.

One-month old female rats were fed an isocaloric diets containing 10% casein (Con10), 5% casein (Con5) or 5% casein + 5% Spirulina (Con5 + Spi5) during 8 weeks. Cortical and trabecular bone microstructure were analyzed by microCT and areal BMD by DXA. Bone strength was evaluated by tibia midshaft three-point binding test and proximal tibia compression test. Serum IGF1 was measured.

As compared with the Con10 group, isocaloric low-protein diet decreased proximal tibia areal BMD (-10%, P < 0.0001), bone trabecular volume (BV/TV: −41%, P < 0.01) and trabecular thickness (Tb.Th: −10%, P < 0.05), resulting in a lower ultimate strength (US: −18%, P < 0.01). All these parameters were significantly higher in the Con5 + Spi5 group which showed similar values as the Con10 group. In tibia cortical middiaphysis, there was a trend towards lower values (areal BMD: −4%, P < 0.056, cortical bone volume (CBV): −7%, P = 0.063, and US: −7%, NS) while Con5 + Spi5 group showed significant higher cortical bone parameters than Con5 group (areal BMD: −6%, P < 0.05; CBV: +12%, P < 0.01; US: +10% NS). Serum IGF1 was also lower in the Con5 group compared to Con5 + Spi5 and Con10 groups (380.5 ± 10.1; 437.0 ± 12.4 and 437.9 ± 18.5 mg/ml, respectively; P < 0.05).

We demonstrate that Spirulina supplementation effectively prevents cortical and trabecular bone alterations, as well as bone strength decrease induced by isocaloric dietary protein deficiency during growth, in association with the maintenance of optimal IGF1 levels. Spirulina is an effective nutrient to prevent impaired PBMA in protein deficient growing rats.

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PP90

The choice of fetal bovine serum influences the degree of spontaneous mineralization on silk fibroin scaffolds in 3D cell cultures
Samantha Paulsen1,2, Jolanda Vetcho1, Ralph Müller1 & Sandra Hofmann1
1ETH Zürich, Zürich, Switzerland; 2University of Wisconsin–Madison, Madison, Wisconsin, USA.

Silk fibroin (SF) sponges are a promising scaffold material for tissue engineering due to their biocompatibility, mechanical properties, and ability to support calcium-phosphate formation in vitro. However, previous studies have shown that SF can mineralize spontaneously in the presence of culture media, which has a detrimental effect on experimental integrity when analyzing how cells deposit bone-like tissue in tissue engineering studies. In this study we analyzed the influence of four types of commercially available fetal bovine sera (FBS) supplied as either control or osteogenic media on mineralization of SF scaffolds seeded with human mesenchymal stem cells (hMSCs) or left acellular, respectively. Calcium assays (n = 3) were performed at weeks three, five, and seven to assess the amount of mineralization per scaffold. By week seven there was no significant difference in calcium content between the cellularized osteogenic groups, which ranged from 581 ± 118 to 648 ± 63 µg per scaffold, nor between the cellularized control groups, which all remained below 15 µg. Though we expected calcium levels in the acellular scaffolds to remain low, by week seven the two most mineralized groups had calcium contents above 200 µg, nearly half the content of their cellularized counterparts. Furthermore, calcium contents were significantly different (P < 0.01) between FBS varieties in both acellular osteogenic and control groups. While two osteogenic groups had average calcium contents higher than 200 µg per scaffold, the remaining two had < 40 µg. A similar trend occurred in the control groups, with the same two groups having more than 300 µg per scaffold, while the others had < 10 µg. These results demonstrate that understanding the role of cell media and the effects of FBS variation on scaffold mineralization is essential for optimizing osteogenic culture conditions, and maintaining experiment integrity by accounting for spontaneous mineralization in acellular scaffolds.

DOI: 10.1530/boneab.1.PP90

PP91

Pediatric differences in bone mineral density according to ethnic background in children: the Generation R Study
Carolina Medina-Gomez, Denise H M Heppe, Albert Hofman, Vincent Jaddoe, André Uiterlinden & Fernando Rivadeneira
Erasmus MC, Rotterdam, The Netherlands.

Aim
Differences in fracture risk between ethnic groups have been documented. The basis for these differences is yet incomplete and the age at which these differences appear is uncertain. Assessment of bone health in pediatric populations could bring insights on factors compromising bone accrual. We describe here differences in total body bone mineral density (TB-BMD) in a unique setting of children of the same age, measured with the same device (iDXA) different ethnic background and in a well-defined geographic region.

Methods
The Generation R Study is a prospective multiethnic birth cohort in Rotterdam, The Netherlands including in this study 6,134 children visiting the research center at 6 years. Up to 45% of the children were of non-Dutch background and belonging to 15 ethnic groups (Dutch Central Office of Statistics) and regrouped into European, Asian and African descent. Differences in TB-BMD were assessed by multivariate regression with multiple comparisons of least-squares (LS) means using the Dutch/ European population as reference, adjusting for age, gender, (followed by) fat mass, lean mass and height.

Results
TB-BMD was highest in groups of African descent and lower in groups of Asian descent as compared with Europeans when adjusting for gender and age. After adjustment for body height and lean mass, BMD levels in Asians were equal to Dutch and Europeans, while differences in children of African descents remained significantly higher even after correction for diverse lifestyle variables.

Conclusion
Ethnic differences in bone mass are already present in childhood. Lower BMD in Asian children (as compared to Dutch and Europeans) results from smaller skeletal frame size and adaptation to loading (i.e. lean mass); while the higher BMD in African children is independent of body size or loading. These findings provide further understanding into the differences in fracture risk observed at a given BMD value across ethnicities.

DOI: 10.1530/boneab.1.PP91

PP92

Identifying scoliosis in population-based cohorts: development and validation of a novel method based on total body dual energy X-ray absorptiometry scans
Hilary Taylor2, Ian Harding2, John Hutchinson2, Ian Nelson2, Ashley Blom1,2, Jon Tobias1 & Emma Clark1
1University of Bristol, Bristol, UK; 2North Bristol NHS Trust, Bristol, UK.

Background
Scoliosis is a lateral curvature of the spine ≥ 10°, as measured on standing spinal radiographs. There are no published studies that have investigated determinants of scoliosis using a prospective cohort design, making the establishment of cause and effect difficult. Several large population-based cohorts exist throughout the world with a wide range of data already collected, and while spinal imaging with radiographs is not generally collected in these cohorts, DXA has been routinely performed at repeated time points for the study of determinants of bone density. We therefore wished to develop and validate a novel method of identifying scoliosis on total body DXA scans.

Methods
Scoliosis was identified on total body DXA scans by triaging to distinguish true curves from positioning errors, followed by a modified-Ferguson method to measure angles. Precision was assessed on 174 children from the Avon Longitudinal Study of Parents and Children (ALSPAC), who underwent repeat DXA scans at age 15, 2–6 weeks apart. In addition, precision of angle estimation was evaluated on 20 scans measured five times. To evaluate accuracy, angle size was compared to spinal radiographs in 13 individuals with known scoliosis. Scoliosis prevalence rates and curve patterns were then identified from DXA scans previously obtained in 5122 ALSPAC participants at aged 15.

Results
There was substantial agreement in identifying those with scoliosis on repeat DXA scans taken 2–6 weeks apart (Kappa of 0.74, 95% CI 0.59 to 0.89). 95% of repeat angle measures were within 5°. Prevalence of scoliosis ≥ 10° in ALSPAC was 3.5% at aged 15, and was higher in girls. Mean ± s.d. curve size was 15 ± 7° at aged 15.

DOI: 10.1530/boneab.1.PP92
Conclusions
We have developed and validated a novel method for identifying scoliosis from DXA scans. Comparison with prevalence data using more established techniques suggests our method provides valid estimates of scoliosis prevalence in population-based cohorts.
DOI: 10.1530/boneabs.1.PP92

**PP93**
Smaller bones at aged 10 predicts scoliosis at aged 15: results from a population-based birth cohort
Hilary Taylor1, Ashley Blom2,4, Ian Harding2, John Hutchinson2, Ian Nelson3, Jon Tobias1 & Emma Clark1
1University of Bristol, Bristol, UK; 2North Bristol NHS Trust, Bristol, UK.

Background
Scoliosis is lateral curvature of the spine, and adolescent idiopathic scoliosis (AIS) accounts for the majority of cases of scoliosis. One potential determinant of scoliosis that is of great interest is bone size and density. However, there are no published studies that have investigated determinants of scoliosis using a prospective cohort design making the establishment of cause and effect difficult.

Methods
This study was based on the Avon Longitudinal Study of Parents and Children (ALSPAC), a population-based cohort. Data on total body (minus head) bone area were collected by DXA in 7333 children aged 10 years. Children with scoliosis already present at aged 10 were excluded. Data was collected on the presence or absence of scoliosis at aged 15. Other potential confounding variables were also measured. At aged 15, peripheral quantitative CT (pQCT) was used to measure bone circumference and cortical thickness. Associations between DXA bone variables and risk of scoliosis developing over the following 5 years were examined by logistic regression. Cross-sectional analyses were also carried out between pQCT bone variables and presence of scoliosis at 15.

Results
Of 4022 children, 175 (4.4%) had developed scoliosis by aged 15. After adjustment for confounders, the OR for scoliosis at aged 15 per SD increase in bone size relative to body size at aged 10 was 0.61 (95% CI 0.42 to 0.89, \(P=0.009\)). Girls with scoliosis at 15 had smaller periosteal circumference (67.81 vs 68.86 mm, \(P<0.05\)) and reduced cortical thickness (5.02 vs 5.16 mm, \(P<0.01\)) compared to those without scoliosis.

Conclusions
Our results show that children with smaller bones at aged 10 are more likely to develop scoliosis. This is the first prospective study in this area and adds weight to our method provides valid estimates of scoliosis prevalence in population-based cohorts.
DOI: 10.1530/boneabs.1.PP93

**PP94**
The healing of fracture of mandible against the chronic nitrate intoxication
David Avetisov, Vitaly Kostenko, Karine Nenporada, Ekaterina Lokes & Stanislav Stavicky
Ukrainian Medical Stomatological Academy, Poltava, Ukraine.

Damages of bones of facial skeleton lay down 8% from all damages, fractures of mandible are 85–90% from it. There are a lot of factors, that make worse the process of reparative regeneration of bone. Using of nitric fertilizers lead to heighten earning of nitric oxide into organism. It makes negative influence on reparative regeneration of bones.

There are dates of research of 40 rats line Vistar in this article. The goal of this study was to examine the effect of chronic nitric intoxication on the healing of fractures of mandible in different terms after trauma. The fracture of mandible was molded at rats after 60 days of nitric intoxication and without it. Histological investigations of callus were carried out on 14-th, 21-th, 28-th and 35-th days after molded of fracture.

The chronic nitric intoxication slows down the course of regenerator processes of experimental fracture of mandible. It is characterized by slower dynamic of differentiation of cellular elements and micro vessels even at later terms of reparative osteogenesis and delay of forming of trabecular bone.
DOI: 10.1530/boneabs.1.PP94

**PP95**
Osteometric parameters of mature rats mandible molars at implantation in the tibia of biogenic hydroxyapatite
Luzin Vladislav, Morozov Vitaly & Morozova Helen
Luhansk State Medical University, Luhans, Ukraine.

Traumatic injuries of the bones of various etiologies are accompanied not only a violation of their integrity, but also the development of a system osteopenic syndrome, which causes disorder neurohumoral regulation of the organism and has a negative effect on the structural and functional state of the skeletal tissues of other parts of the skeleton. The information concerning the characteristics of changes in the parameters of growth of molars of the mandible in the literature there are practically absent and contradictory. In the experiment, 84 adult male rats on days 7, 15, 30, 60, 90 and 180 day attempts to justify the possibility to offset the negative manifestations of the «fracture syndrome» on the growth parameters of molars of the mandible by implantation in the proximal tibial shaft biogenic hydroxyapatite. With the caliper was determined the length and height of the molar row of the mandible. The results were compared with the same parameters with the application of a defect in the same area of the tibia. After statistical analysis of data in the program »STATISTICA 5.5« found, that the length of the molar row was increased by 30 and 90 day observation at 3.23, 2.36%, and its height – only 90 days at 5.03% (\(P<0.05\)). Thus, we can assume that the implantation of biogenic hydroxyapatite accompanied smoothing inhibitory effect of application of the defect in the tibia on the growth processes of the molar row of the mandible in the later period of observation, presumably due to resorption of the implanted material and the release of calcium, phosphorus, boron and silicon, accelerating adaptation in tissues molars.
DOI: 10.1530/boneabs.1.PP95

**PP96**
Macroelements composition of the dentin in rat’s mandibular incisors with implanted biogenic hydroxyapatite into the tibia
Luzin Vladyslav, Morozov Vitaly, Astraikhantsev Dmitry, Golubkoff Pavel & Sokol Marina
Luhansk State Medical University, Luhans, Ukraine.

Objectives
The aim of the study was to establish features of macroelemental composition of the dentin in mature male rat’s mandibular incisors with biogenic hydroxyapatite, implanted in the proximal part of tibial shaft.

Material and methods
One hundred and twenty-six mature male rats were divided into three groups: 1st group – intact animals, 2nd group – animals with the pit defect applied on the proximal part of tibial shaft (systematic osteoporosis model), 3rd group – implanted into the defect area the blocks of biogenic hydroxyapatite. Periods of experiment were 7, 15, 30, 60, 90 and 180 days. Rats were killed under ether mask anesthesia. To measure the macroelement’s content in dentin 10 mg dental ashes were dissolved in 2 ml of 0.1 N HCl, chemically pure, adjusted to 25 ml distilled water. The resulting solution was determined in calcium, sodium, potassium by atomic absorption photometer of the ‘Saturn-2’ in the mode of emission of air-propane flame and phosphorus colorimetrically by Briggs.

Results
Content of calcium and phosphorus in the dentin incisor in 2nd group was similar with 3rd along the whole experimental period. Sodium content increases per day, starting from the 1st month up to the 60th day (at 7.33%, 6.88%), potassium – at 10.89%, 11.66%, but on the 90th and 180th experimental days there was a tendency to reduce the content of these macroelements in the dentin incisor. Fluorine content increased only on the 90th days at 6.68% (\(P<0.05\)) compared with the intact animals.

Conclusions
Thus, in animals with defect in the tibia we establish imbalance of macroelemental composition in dentin of incisor. Presence of the biogenic hydroxyapatite, implanted into the tibia, improved the chemical content of the dentin on the late stages (from 90 to 180 day of observation).
DOI: 10.1530/boneabs.1.PP96

*Bone Abstracts (2013) Vol 1*
PP97
Organogamic parameters of rat’s bones under the effect of toluene vapor
A Skorobogatov, V Luzin, V Morozov & Ye Shutov
Luhansk State Medical University, Luhansk, Ukraine.

Objectives
The aim of this study was establish features changes of organogamic parameters of bones in mature male rats after a 60-day inhalation seed toluene.

Materials and methods
For the experiment were selected 60 mature male rats were divided into two groups: 1st – intact rats, 2nd group – the rats that every day for 2 months to device for inhalation agents received a one-time inhalation of toluene exposure 4 h in 10 maximum permissible concentration. Periods of experiment were 1, 7, 15, 30, and 60 days. Rats were euthanized under ether mask anesthesia after the end of the 2-month impact of toluene. Tibia, hip bone and the third lumbar vertebra were taken and measured by caliper.

The results
The maximum length of the tibia and hip bone were lower than in 1st group from 7 to 60 day at 4.15, 4.47, 2.67, 3.35, 3.41 and 3.84, 3.78, 4.88, 4.16, 2.91% respectively. Height body of third lumbar vertebra was also less than the control, respectively, at 5.12, 6.27, 6.69, 3.28, and 4.80%. The width of the proximal and distal tibial epiphysis decreased in the same period at 3.69, 4.14, 5.77, 4.94, 2.84 and 11.94, 7.78, 10.25, 10.00, and 6.86% and the width and the anterior-posterior size of the middle part of diaphysis – at 6.82, 6.72, 7.01, 5.82, 6.05 and to 12.31, 10.14, 9.80, 8.65, 7.11%. The maximum width of the hip bone was lower than in intact animals from 7 to 60 day at 6.06, 6.19, 6.89, 4.87, and 5.53%, and the width body of third lumbar vertebra – at 7.27, 7.12, 5.90, 5.43, and 5.69%.

Conclusions
Inhaled seed toluene followed by a slowdown in both longitudinal and appositional bone growth. Identified changes manifested by 1, 7, and 15 days after the end of inhalation seed toluene with a tendency to leveling from 30 to 60 days.

DOI: 10.1530/boneabs.1.PP97

PP98
Identification and characterization of a mesenchymal progenitor cell population involved in fracture healing
Brya Matthews1, Danka Grcević2, Liping Wang2, Yusuke Hagiwara1, Douglas Adams1 & Ivo Kalajzic1
1University of Connecticut Health Center, Farmington, Connecticut, USA; 2University of Zagreb, School of Medicine, Zagreb, Croatia.

Fracture healing is a multistep process that involves many cell lineages and is still not fully understood. We aimed to identify and characterize population of mesenchymal progenitor cells during its commitment within a fracture callus. To identify and trace cells in peristeme and bone marrow we used ZsMA promoter-driven inducible Cre expression (ZsMA-CreERT2) combined with a Cre-activated tdTomato reporter (Ai9) to generate ZsMACEreERT2 mice. Tibias, fixed with an intramedullary stainless steel pin, were fractured in 3–4 month old mice treated with tamoxifen the day before and the day of injury. Histological analysis of ZsMACre/Ai9 mice indicated an expansion of osteoblast + cells with fibroblastic shape in the peristeme proximal and distal to the injury 2 days after fracture. Six days after fracture numerous osteoblast +, chondrocytes were observed. By day 12 a population of osteoblasts in the fracture were osteoblast +, periosteum/soft callus and BM were collected 2 days after tamoxifen treatment (unfractured), and 2 and 6 days after fracture and digested using collagenase/trypsin for cell flow cytometry sorting. RNA was extracted from sorted ZsMACEcre labeled populations (tibial +), amplified, and hybridized to Illumina arrays (n = 3).

We observed that Notch signaling components were decreased in tomato + cells following fracture, including Notch 1, 3, 4, Hes1, and Hey1. In order to assess the effect of Notch signaling in progenitor cells, BMSC and peristem cultures from ZsMACEcre/Ai9 mice with and without the Rosa-NICD transgene were treated with hydroxamofxifen then sorted to obtain tomato + cells. In cultures without Notch overactivity cells are capable of differentiation into osteoblast, adipocyte and chondrocyte, however in the presence of Notch activation, chondrogenesis, osteogenesis and adipsogenesis are decreased. This is the first study to characterize a population of mesenchymal progenitor cells that actively participate in fracture callus formation. Downregulation of Notch signaling may be important for commitment of the cells to mature lineages.

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Calcitropic and phosphotrophic hormones and mineral metabolism
Clarisa Marotte1,2, Gabriel Bryk1,2, Magali Zeni Coronel2, Paulina Abrego3, Diego Martín Lucero4, Laura Schreier4, Maria Luz Portela4 & Susana Noemí Zeni1,2
1Catedra de Bioquímica General y Bucal. Fac. de Odontología, UBA, Buenos Aires, Argentina; 2Laboratorio de Enfermedades Metabólicas Óseas, Hospital de Clínicas, Instituto de Immunología, Genética y Metabolismo (INIGEM) CONICET-UBA; Buenos Aires, Argentina; 3Laboratorio de Lípidos y Lipoproteínas. Depto. Bioquímica Clínica, Fac de Farmacia y Bioquímica, UBA, Buenos Aires, Argentina; 4Catedra de Nutrición. Facultad de Farmacia y Bioquímica, UBA, Buenos Aires, Argentina.

It has suggested that BGP is inversely related to body fat. BGP appears to regulate energetic metabolism through the insulin receptor signaling pathway in the osteoblast, which affects bone resorption and BGP activity. Fat tissue is an endocrine organ that secretes different hormones and growth factors; several of them affect bone remodeling and insulin secretion. The present report comparatively studied total BGP levels, glucose homeostasis and body fat mass in spontaneous obese (OB) strain and Wistar (W) rats. Both groups were fed since their mother’s pregnancy until the end of the experience (50 days of age) AIN 93 diet that supplied 0.5 mg%Ca, 0.4 mg%P, 200 IU vitamin D%. Serum BGP, insulin and C-terminal type 1 collagen telopeptide (CTX) were evaluated by ELISA; glucose and triglycerides (TGL) by colorimetric enzymology and 25OH/A by a competitive protein-binding method (DiaSorin).

HOMA-IR was calculated. At the end of experience body composition was evaluated, according AOAC methods. Results of OB vs WN, respectively (mean ± s.e.m): fat (g/100 body weight): 13.1 ± 2.2 vs 10.4 ± 0.8 (P < 0.01); liver weight (g): 12.3 ± 0.9 vs 8.1 ± 1.5 (P < 0.01); TGL (mg/dl): 225 ± 37 vs 59 ± 16 (P < 0.001); glucose (mg/dl): 152 ± 69 vs 99 ± 41 (P < 0.001); insulin (mg/dl): 4.75 ± 2.44 vs 14 ± 0.02 (P < 0.001); CTX (ng/ml): 83 ± 8 vs 94 ± 2.6 (P < 0.01); BGP (ng/ml): 375 ± 18 vs 840 ± 106 (P < 0.001); 25OHID (ng/ml): 19.0 ± 5.4 vs 15.4 ± 2.8 (P < 0.05).

The OB group showed a higher fat content, liver weight, glucose, TGL, insulin and 25OHID levels but lower BGP and CTX levels.

Conclusion
The results confirmed that there was an inverse relationship between levels of BGP and body fat content. The concomitant reduction in bone resorption of OB rats, may suggest a decrease in the biological active BGP that could be the responsible of the observed increment in fasting glucose levels and insulin resistance. The study was partially supported by UBACyT 20021000100320 and CONICET.

DOI: 10.1530/boneabs.1.PP99

PP100
A mixture of GOS/FOS ® added to a low calcium (ca) diet improved ca, phosphorus (p) and magnesium (mg) absorption: experimental model in normal growing rats
Gabriel Bryk1,2, Macarena Gonzales Chaves1,2, Clarisa Marotte1,2, Daniela Medina3, Magali Zeni Coronel2, Maria Luz Portela4 & Susana Noemí Zeni1,2

A mixture of Galacto-oligosaccharides (GOS) and Fructooligosaccharides (FOS) are added to commercial infant formula to promote an intestinal microbiota similar to that prevalent in breast-fed infants to improve Ca bioavailability and general health, but their mechanisms are under debate. Our objective was to evaluate the beneficial effects of the mixture of GOS/FOS added to infant formula, on the absorption of Ca, P and Mg of a low Ca diet. Changes in intestinal pH, absorption percentage (Abs) and in bone mineral density (BMD) were determined in an experimental model of normal growing rats.

Male Wistar rats (n = 10/group) were fed one of the three experimental diets from Metabo for 40 days of age. All diets were prepared according to AIN’93-G rodent diet.
diet except for Ca content. A5 and A3 contained 0.5 and 0.3% of Ca, respectively; GF.3 contained 0.3% Ca and 5.3% of GOS/FOS. Dietary consumption and body weight (BW) were evaluated three times per week and weekly, respectively. Ca, Mg and P absorption percentage was determined at the beginning of the study and during the last 3 days of the study. At the end of the experience (t = 1), caecal pH, total skeleton (tsk) and tibia BMDs were determined by DXA (Lunar corp.)

Results

at t = 1 (mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Caecal Ph</th>
<th>Tibia BMD</th>
<th>tsk BMD (mg/cm²)</th>
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<tr>
<td></td>
<td>%Ca Abs</td>
<td>% Mg Abs</td>
<td>% P Abs</td>
</tr>
<tr>
<td>A5</td>
<td>7.1 ± 0.2</td>
<td>169 ± 4</td>
<td>224 ± 5.7, 5.7</td>
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<td>76.2 ± 1.0**</td>
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<td></td>
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<td>60.1 ± 2.0</td>
<td>77.9 ± 3.7</td>
</tr>
<tr>
<td>A3</td>
<td>7.1 ± 0.2</td>
<td>163 ± 6</td>
<td>221 ± 8.5, 5.0</td>
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<td>82.4 ± 2.2</td>
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<tr>
<td></td>
<td></td>
<td>59.2 ± 1.5</td>
<td>77.1 ± 3.0</td>
</tr>
<tr>
<td>GF.3</td>
<td>6.4 ± 0.2</td>
<td>172 ± 6</td>
<td>236 ± 7.1, 7.11**</td>
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<td></td>
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<td>92.0 ± 2.0</td>
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<td></td>
<td>93.1 ± 2.11**</td>
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<td>92.2 ± 1.6**</td>
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</tbody>
</table>

Table 1

No differences in pH, absorptions and BMD were observed at the beginning of the study. BW increases were not significantly different among groups. At t = 1, GF.3 presented the lowest caecal pH and showed a significant increment of all studied parameters compared to A.3 (** P < 0.05). G.3 also had higher tskBMD, and Ca, Mg, P Abs. than A.5 diet (* P < 0.05).

Conclusion

These results evidence the increment in Ca, Mg and P absorption percentage and suggest the benefit effect of GOS/FOS in bone health during normal growth.


DOI: 10.1530/boneabs.1.PP100

PP102

A genetic polymorphism of osteocalcin is associated with BMI but not with parameters of glucose and lipid metabolism in women with polycystic ovary syndrome

Verena Schweitz, Olivia Trummer, Albrecht Giuliani, Thomas R Pieber, Barbara Obermayer-Pietsch & Elisabeth Lerchbaum

Medical University of Graz, Graz, Austria.

Introduction

Osteocalcin (OC) is a marker of bone formation but also seems to play a hormonal role in the regulation of glucose and energy metabolism. Recently, an association of BMI with a haplotype composed of three single nucleotide polymorphisms (SNPs) in the gene for OC, located on chromosome 1q22, was observed in ethnically homogeneous European pedigrees.

Aim

The aim of the study was to test the association of these three polymorphisms in the gene of OC with BMI in a cohort of women with polycystic ovary syndrome (PCOS). Moreover, as these women show an adverse metabolic profile, we aimed to evaluate a possible association with parameters of glucose and lipid metabolism: A1CInsulin, A1Cglucose, Matsuda, QUICKI (indices for insulin sensitivity), HOMA-IR (index for insulin resistance), levels of triglycerides, total cholesterol, HDL, and LDL.

Methods

Genotypes of SNPs in the OC gene were successfully determined in 680 PCOS women by S1-exonuclease assay. Metabolic and anthropometric characterization was used as well as oral glucose tolerance tests were performed according to standard measurements and biochemical analysis. Results

As for one G > C polymorphism, CC genotype carriers had a significantly higher BMI (25.2 kg/cm² (IQR 22.1–31.1)) compared to CG genotype carriers (23.5 kg/cm² (IQR 20.7–28.9), P = 0.007), but not compared to women carrying the GG genotype (23.7 kg/cm² (IQR 20.7–28.0), P = 0.083). None of the investigated genetic variants was associated with any of the parameters of glucose and lipid metabolism analyzed.

Discussion

We confirm the association of one G > C polymorphism with BMI in a cohort of PCOS women. However, all three OC SNPs did not show any association with parameters of glucose and lipid metabolism in this cohort of PCOS women with an adverse metabolic profile.

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PP103

Abstract withdrawn.

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PP104

Neonatal neuroendocrine alterations impair tooth eruption, enamel mineralization, and leptin and corticosterone secretion in adulthood

Wagner Garcez de Mello1,2, Samuel Rodrigues Lourenço de Morais1, Alberto Carlos Botazzo Delbem1, Rita Cásia Menegati Dornelles2, José Antunes-Rodrigues3 & João Cesar Bedran de Castro1

1Multicentric Graduate Studies Program in Physiological Sciences, Brazilian Physiological Society/Univ. Estadual Paulista, Arac¸atuba, São Paulo, Brazil; 2Department of Basic Sciences, Arac¸atuba Dental School, Univ. Estadual Paulista, UNESP, Arac¸atuba, São Paulo, Brazil; 3Department of Social and Child Dentistry, Arac¸atuba Dental School, Univ. Estadual Paulista, UNESP, Arac¸atuba, São Paulo, Brazil; 4Department of

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Abstract withdrawn*

DOI: 10.1530/boneabs.1.PP103

PP104

Neonatal neuroendocrine alterations impair tooth eruption, enamel mineralization, and leptin and corticosterone secretion in adulthood

Wagner Garcez de Mello1,2, Samuel Rodrigues Lourenço de Morais1, Alberto Carlos Botazzo Delbem1, Rita Cásia Menegati Dornelles2, José Antunes-Rodrigues3 & João Cesar Bedran de Castro1

1Multicentric Graduate Studies Program in Physiological Sciences, Brazilian Physiological Society/Univ. Estadual Paulista, Arac¸atuba, São Paulo, Brazil; 2Department of Basic Sciences, Arac¸atuba Dental School, Univ. Estadual Paulista, UNESP, Arac¸atuba, São Paulo, Brazil; 3Department of Social and Child Dentistry, Arac¸atuba Dental School, Univ. Estadual Paulista, UNESP, Arac¸atuba, São Paulo, Brazil; 4Department of
Physiology, Ribeirão Preto Medical School, University of São Paulo, USP, Ribeirão Preto, São Paulo, Brazil; 2Centro Universitário Toledo, UNI-TOLEDO, Araçatuba, São Paulo, Brazil.

There is a growing body of evidence indicating the important role of the neonatal steroid milieu in programming sexually dimorphic pattern in various physiological systems. We tested the hypothesis that abnormal exposure to steroid hormones within a critical developmental period elicits permanent changes on tooth eruption, enamel mineralization, and leptin and corticosterone concentrations in adulthood. Newborn Wistar rats were divided into four groups, two male groups and two female groups. Male pups were cryoanesthetized and submitted to castration or sham-operation procedures within 24 h after birth. Female pups received a s.c. dose of testosterone propionate (100 μg) or vehicle. Lower incisor eruption was determined between the 4th and the 10th postnatal days, and the eruption rate was measured between the 40th and the 60th days of age. After extraction, performed 60 days, the upper incisors rights were used to obtain data related enamel mineralization, assessed by microhardness testing. The plasma leptin and corticosterone secretions were analyzed by RIA. The results of this study demonstrate that the androgenized female had delay on teeth eruption when compared with control females. The sham-castrated males had higher growth rate of normofunctional tooth eruption than the other groups. The enamel microhardness in both prismatic and aprismatic areas were higher in control females than all the other groups studied. Regarding the profile of hormone secretion, plasma concentrations of leptin in castrated males in the neonatal period were lower when compared to other groups, and plasma concentrations of leptin in castrated females than all the other groups studied. Regarding the profile of hormone secretion, plasma concentrations of leptin in castrated males in the neonatal period were lower when compared to other groups, and plasma concentrations of corticosterone were not statistically different between groups evaluated. In the study, show that neonatal steroids manipulations cause well-defined oral disturbances in adulthood in rats, and suggests the importance of the neonatal sex steroid milieu for normal sexual dimorphic pattern on tooth eruption, enamel mineralization, as well as on hormonal secretion. DOI: 10.1530/boneabs.1.PP104

**PP105**

α2C AR KO mice present opposite bone phenotype in femur and vertebrae

Marilia Bianca Teixeira1, Gisele Martins1, Cristiane Costa1, Patricia Brum1,2 & Cecília Gouveia1

1Biomedical Sciences Institute – IBC III – University of São Paulo, São Paulo, SP, Brazil; 2Physical Education and Sports School – University of São Paulo, São Paulo, SP, Brazil.

α2C Evidences demonstrate that sympathetic nervous system (SNS) activation causes osteopenia via β2-adrenoceptor (β2-AR) signaling. In a recent study, we showed that female mice with chronic sympathetic hyperactivity due to double knockout of adrenoceptors that negatively regulate norepinephrine release, α2A-AR and α2C-AR (α2A/α2C-ARKO), present an unexpected phenotype of high bone mass with decreased bone resorption and increased bone formation. Furthermore, we found that these animals are resistant to the thyrotroopin-releasing hormone (TRH) given in the 1st day of life. The aim of this study was to analyze the effect of Raloxifene (RLX) and RT in bone metabolism of rats on their aging period. Wistar rats (14–18 months), sham or ovariectomized (OVX) were treated with RLX (1 mg/kg per day) or saline by gavage and subjected to RT or not. The animals were subjected to RT-oriented practice by staircase with 80% tilt, with the overload apparatus corresponding to 20% of strength test and weekly increase 10 until 80% using steel balls in tubes attached at the animals tail. Earlier the 3rd and 4th month the maximum strength test was revised to test the load. After 120 days the start of RT and/or RLX, lumbar removed to bone microtomography and immunohistochemistry for Runx2, OSX, OCN, OPG, RANKL and TRAP. For statistical analysis we used a completely randomized design with treatments in a factorial 4 × 2 crossover and post-ANOVA’s test (P < 0.05). The morphometric analysis of bone microtomography shows osteopenia, which was more pronounced in animals in groups of OVX rats. The combination treatment resulted in increased bone formation in both groups. However, the group of sham rats that received both therapies showed trabecular bone organized and more consistent than the other experimental groups. Immunostaining for Runx2, OSX and OCN was higher in groups treated with RLX. In the group Sham/RLX/XE, the immunostaining was higher for OPG and RANKL and lower for TRAP. The results of this study reveal that combination of RT, administration of RLX and especially the combination of these triggered increase bone mass in experimental animals and in different degrees reversed the framework of osteopenia. DOI: 10.1530/boneabs.1.PP106

**PP106**

Bone microtomography and immunohistochemistry of acrylic rats post antiresorptive therapy and resistance training

Camila Stringhetta-Garcia1, Samuel Morais2, Mário Louzada1,3, Edison Evolino4 & Rita Dornelles1,3

1The Multicentric Graduate Studies Program in Physiological Sciences – SBFis, Araçatuba, São Paulo, Brazil; 2Department of Support to Production and Health Animal, Dental School of Araçatuba, Univ Estadual Paulista – UNESP, Araçatuba, São Paulo, Brazil; 3Department of Basic Sciences, Dental School of Araçatuba, Univ Estadual Paulista – UNESP, Araçatuba, São Paulo, Brazil.

Osteoporosis is a growing public health problem. As allies have for preventing physical activity, especially resistance training (RT) and hormone replacement. The aim of this study was to analyze the effect of Raloxifene (RLX) and RT in bone metabolism of rats on their aging period. Wistar rats (14–18 months), sham or ovariectomized (OVX) were treated with RLX (1 mg/kg per day) or saline by gavage and subjected to RT or not. The animals were subjected to RT-oriented practice by staircase with 80% tilt, with the overload apparatus corresponding to 20% of strength test and weekly increase 10 until 80% using steel balls in tubes attached at the animals tail. Earlier the 3rd and 4th month the maximum strength test was revised to test the load. After 120 days the start of RT and/or RLX, lumbar removed to bone microtomography and immunohistochemistry for Runx2, OSX, OCN, OPG, RANKL and TRAP. For statistical analysis we used a completely randomized design with treatments in a factorial 4 × 2 crossover and post-ANOVA’s test (P < 0.05). The morphometric analysis of bone microtomography shows osteopenia, which was more pronounced in animals in groups of OVX rats. The combination treatment resulted in increased bone formation in both groups. However, the group of sham rats that received both therapies showed trabecular bone organized and more consistent than the other experimental groups. Immunostaining for Runx2, OSX and OCN was higher in groups treated with RLX. In the group Sham/RLX/XE, the immunostaining was higher for OPG and RANKL and lower for TRAP. The results of this study reveal that combination of RT, administration of RLX and especially the combination of these triggered increase bone mass in experimental animals and in different degrees reversed the framework of osteopenia. DOI: 10.1530/boneabs.1.PP107

**PP107**

Abstract withdrawn.

**PP108**

A sensitive assay for measuring circulating BMP6

Martina Pauk1, Lovorka Grgurevic1, Jelena Brljačić1, Vera Kufner1, Tatjana Bordukić-Nikšić1, Morana Jankolija1, Hermann Oppermann2 & Slobodan Vukicević1

1Laboratory for Mineralized Tissues, School of Medicine, Center for Translational and Clinical Research, University of Zagreb, Zagreb, Croatia; 2Genera Research, Rakov Potok, Croatia.

Although bone morphogenetic protein 6 (BMP6) is known for its ability to induce growth of bone and cartilage, recent studies identified BMP6 as a key endogenous regulator of hepcidin expression and iron metabolism. Here, we examined BMP6 presence in the serum and investigated whether circulating levels of BMP6 may reflect body iron status. First, we showed by liquid chromatography–mass spectrometry (LC–MS) and western blotting that BMP6 is present in the circulation of healthy humans. We next analyzed biological fluids of mouse and human using commercial ELISAs, but failed to detect BMP6. To enhance the assay sensitivity and simplify the BMP6 measurement procedure, we developed a BMP6 Proximity Extension Assay (PEA) which enabled us to analyze up to 10 pg/ml of BMP6 in

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serum samples before and following iron exposure. We determined that BMP6 circulates at 55.46±9.8 pg/ml, and this is the first demonstration of a physiological range of circulating BMP6 in mice. Interestingly, in untreated mice BMP6 concentrations displayed a diurnal variation, with concentrations being lowest in the early morning and increasing throughout the day before declining during the evening hours. Given the already known hepcidin diurnal rhythm, our results indicate that hepcidin variations are in fact reflecting BMP6 pattern. Furthermore, we found that iron loading was followed by a BMP6 increase in mouse serum at 12 h to 109.96±25.4 and at 24 h to 128.7±21 pg/ml, indicating that circulating BMP6 is likely to play a role in iron metabolism. As circulating BMP6 positively correlates with iron, the measurement of BMP6 in biological fluids presents a promising tool in the diagnosis of conditions in which iron metabolism is affected. Therefore, the development and validation of BMP6 assays would increase our understanding of the physiology of iron homeostasis and iron related bone loss.

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PP109
Biochemical bone markers of bone turnover in diabetics: a meta-analysis
Jakob Starup-Linde1,2, Stine Aistrup Eriksen1 & Peter Vestergaard1,3
1Faculty of Health, Aalborg University, Aalborg, Denmark; 2Department of Endocrinology and Internal Medicine (MEA), Aarhus University Hospital, Aarhus, Denmark; 3Department of Endocrinology and Internal Medicine, Aalborg Hospital, Aalborg, Denmark.

Introduction
Diabetes mellitus may affect bone via bone structure, bone density, and biochemical markers of bone turnover. The aim of this meta-analysis was to examine biochemical markers of bone turnover in diabetics compared to non-diabetics.

Methods
A systematic literature search was conducted using Pubmed, Embase, Cinahl, and Svedmed + with the search terms: ‘Diabetes mellitus’, ‘Diabetes mellitus type 1’, ‘Insulin dependent diabetes mellitus’, ‘Diabetes mellitus type 2’, ‘Non insulin dependent diabetes mellitus’, ‘Bone’, ‘Bone and Bones’, ‘Bone diseases’, ‘Bone turnover’, ‘Hemoglobin A Glycosylated and ‘HbA1c’. After removing duplicates from this search 1188 records were screened by title and abstract and 75 records were assessed by full text for inclusion. After screening, 22 records fulfilled the criteria for the meta-analysis. Revman was used in the data analysis.

Results
From the pooled data in the meta-analysis the bone markers, osteocalcin (P<0.01), and CTX (P<0.01) were significantly lower among diabetics than non-diabetics, however, bone specific alkaline phosphatase (P=0.53), deoxypyridinoline (P=0.99), NTX (P=0.06) and CICP (P=0.54) did not differ. Alkaline phosphatase (P<0.01) was increased in diabetics. A relation to calcitropic hormones; 25-hydroxy vitamin D was lower in diabetics (P=0.02), while PTH (P=0.56) and serum calcium (P=0.54) remained unchanged. All markers displayed very high heterogeneity by I² statistics. No publication bias was present (analyses by funnel plot).

Conclusion
A dissociative pattern of biochemical bone markers of formation and bone resorption was present in diabetics. We speculate that this could point to a measurement error caused by glycation of bone markers, which may alter the configuration of some of the markers thus disrupting the measurement of these.

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PP110
Short-term resveratrol supplementation stimulates serum levels of bone-specific alkaline phosphatase in obese non-diabetic men
Morten Möller Poulsen1, Marie Juul Onstrup1, Torben Harsløf1, Niels Jessen1,2, Bente Lomholt Langjohal1, Bjørn Richelsen1, Jens Otto Lunde Jørgensen3 & Steen Bonløkke Pedersen3
1Department of Endocrinology and Internal Medicine MEO, Aarhus University Hospital, Aarhus, Denmark; 2Department of Pharmacology, Aarhus University Hospital, Aarhus, Denmark; 3Department of Endocrinology and Internal Medicine, Aarhus University Hospital, Aarhus, Denmark.

Despite the substantial preclinical evidence for a positive effect of the polyphenolic compound resveratrol, human data are very scarce, and currently no clinical data addressing the potential impact on bone metabolism have been published. In the present study we addressed this issue by testing a panel of bone specific biomarkers in order to identify potential bone metabolic effects of resveratrol in human subjects. In a randomized, placebo-controlled, double-blinded and parallel-group design, 24 obese (BMI (kg/m²): 34.2±0.7) non-diabetic men were randomly assigned to 500 mg resveratrol or placebo treatment three times daily for four weeks. Biomarkers of bone metabolism, inflammatory parameters, circulating hormones and DXA scans were measured before and after the intervention period. Plasma levels of bone-specific alkaline phosphatase increased significantly in the resveratrol group as compared to placebo (delta changes (U/l); placebo: –1.7±0.7 vs resveratrol: 4.9±0.6; P<0.001). This was paralleled by a tendency of total alkaline phosphatase to rise within the resveratrol group (P=0.061), whereas no changes were detected in other biomarkers of bone metabolism, including PINP, osteocalcin, CTx, or PTH. We suggest that resveratrol represent a primary anabolic modality in preserving bone integrity by possible interference with the mineralization process. The clinical implications remain to be evaluated.

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PP111

The effect of gastric bypass treatment for obesity on hormones related to bone re-modeling and intestinal growth
Bolette Hartmann1, Henrik Vestergaard3, Peter Bonthuis2, Marie Hansen1, Nicolai Albrechtsen1, Peter Eskildsen2, Andrea Floyd2, Annette Lykke Svenningsen2 & Jens Juul Holst1
1University of Copenhagen, Copenhagen, Denmark; 2The Hospital of Roskilde-Koege, Koege, Denmark.

Aim
The aim of the study was to investigate the relationship between gut hormones and bone re-modeling and intestinal growth by measuring simultaneous changes in hormones and bone markers in blood samples collected from patients before and after gastric bypass (GBP).

Methods
Eighteen patients (12 females and 6 males) were included in the study, approved by the Municipal Ethical Committee of Copenhagen. All subjects were studied before surgery (~2 weeks), and 4 weeks and 6 months after. On each study day participants were weighed and given a liquid meal test consisting of 200 ml Fresubin Energy Drink (300 kcal) over 30 min and blood samples (15 ml) were collected before and frequently after the meal. At the first and final visits participants were DEXA scanned for body composition and bone mineral density (BMD) at hip and at spine. Plasma concentrations of glucose-dependent insulinotropic polypeptide (GIP), glucagon-like peptide-2 (GLP-2), and parathyroid hormone (PTH) were measured together with markers of bone re-modeling (CTX-1 and P1NP).

Results
 Pronounced weight losses were seen following GBP. BMI decreased from 41.0 ± 4.7 to 31.3 ± 4.61 kg/m² over 6 months. BMD was reduced at both hip (~5.3 ± 3.4%) and at spine (~2.5 ± 2.0%) 6 months after GBP. Meal stimulated GLP-2 secretion increased significantly (~10 fold) after surgery whereas a minor decrease was seen in GIP secretion. A comparable meal induced reduction in PTH concentration was seen at all three visits with decreasing fasting levels after GBP. Significant meal induced decreases in CTX were observed at all visits, whereas P1NP was not influenced by the meal. However, levels of both CTX and P1NP were significantly increased after GBP.

Conclusion
GBP induced weight loss together with BMD loss. Increased bone resorption was seen after GBP despite increased GLP-2 and decreased PTH.

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PP112

Plasma triiodothyronine levels are positively associated with BMD and bone strength: a cross-sectional study
Emil Moser, Tanja Sikjaer, Leif Moskilde & Lars Rejnmark
Department of Endocrinology and Internal Medicine, Aarhus University Hospital, DK-8000 Aarhus C, Denmark.

In previous studies, discrepant effects of TSH and thyroid hormones on bone have been reported. While low TSH and high thyroxine (T4) levels increase bone turnover, a recent study suggested a positive association between triiodothyronine (T3) levels and mineral deposition in bone. No data are available on bone architecture and strength in relation to thyroid status.

Aim
Using a cross-sectional design, we determined whether bone density, structure, and strength are affected in patients with post-surgical hypothyroidism on T4 substitution therapy.

Patients and methods
We compared 45 patients with well-substituted hypothyroidism with 45 age- and sex-matched controls. Areal BMD was assessed by DXA. Volumetric BMD and bone geometry was measured by HR-pQCT scans (distal radius and tibia). Bone strength was estimated by finite element analysis.

Results
Mean age was 55 years. 80% were women. Patients had been on treatment with T4 for 11.5 (range 3–41) years. Patients and controls had similar levels of TSH. Patients had significant higher T4, but lower T3 levels. Areal BMD did not differ between groups at the lumbar spine, hip, forearm or the whole body. Neither did cortical or trabecular volumetric BMD or indices of bone geometry differ between groups. Finite element analysis showed no significant difference in failure loads. For the entire population, there was no significant correlation between BMD and TSH or T4, but T3 was significantly positively correlated to BMD at the hip (P = 0.324, P < 0.01), spine (P = 0.225, P < 0.05), and the distal forearm (P = 0.278, P < 0.05). Furthermore, T3 correlated positively to failure load at radius (P = 0.386, P < 0.01) and tibia (P = 0.302, P < 0.01).

Conclusion
If patients with hypothyroidism are well-substituted with T4, the disease does not affect bone to any major degree. Our findings of an increased BMD and improved bone strength with increased T3 levels call for further studies on potential beneficial effects of T3 on bone metabolism.

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PP113

Lower bone turnover in obesity: is there a link to energy metabolism?
Heli Viljakainen1, Kaisa K Ivaska2, Marita Lipsanen-Nyman3, Tero Saukkonen3, Sture Andersson1, Kalevi Latinen3 & Outi Mäkitie1,4
1Children’s Hospital, Helsinki University Central Hospital, University of Helsinki, Helsinki, Finland; 2Department of Cell Biology and Anatomy, Institute of Biomedicine, University of Turku, Turku, Finland; 3Department of Obstetrics and Gynecology, Helsinki University Central Hospital, Helsinki, Finland; 4Folkhalsan Research Center, Helsinki, Finland.

Background
Observations in rodents suggest that osteocalcin (OC) participates in glucose metabolism. Data from human studies are inconclusive and it remains unclear whether OC is simply a marker of bone turnover (BTM) or if it also mediates interrelations between the skeleton and glucose homeostasis. This study determined the responses of BTMs, including OC, to oral glucose tolerance test (OGTT) in obese and normal-weight subjects.

Materials and methods
Patients with early-onset severe obesity were recruited from Children’s Hospital, University of Helsinki, and controls from civil register. Subjects underwent a standard 2-h OGTT. Glucose, insulin and six BTMs (BALP, P1NP, TRACP, CTX, and total, and carboxylated OC) were determined at baseline and at 30, 60, 90, and 120 min.

Results
Study included 34 sex- and age-matched case-control pairs (mean age 19 ± 2.3 years). Heights were similar but other anthropometrics were greater in the obese subjects; mean BMIs being 40.4 and 21.9 kg/m². HOMA index was 2.7 times greater in the obese and none were diabetic. All BTMs, except BALP, were significantly lower in the obese compared with the controls: the differences at baseline were 43, 16, 37, 29 and 27% for P1NP, TRACP, CTX, total OC and carboxylated OC (P < 0.05 for all). All BTMs decreased during OGTT. When adjusted for baseline values, the OGTT-responses in total and carboxylated OC (measured as AUC) were different between the two groups (P = 0.031 and P = 0.043 respectively) and the decrease during OGTT was less pronounced in the obese subjects. Responses in other BTMs were similar between the groups (P > 0.05).

Conclusions
Bone turnover is substantially lower in obese subjects compared with controls. Total and carboxylated OC showed less pronounced decrease during OGTT in obese subjects compared with controls, while other BTMs responded similarly in the two groups. This supports a role for OC in glucose homeostasis.

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PP114

Pro-angiogenic and pro-survival functions of glucose in human mesenchymal stem cells upon transplantation
Michaël Deschepper, Joseph Faquet, Mathieu Manassero, Morad Bensidhoum, Karim Ouidna, Delphine Logeat-Avramoglou & Herve Petite

A major limitation in the development of cellular therapies using human mesenchymal stem cells (hMSCs) is cell survival post-transplantation. In this study, we challenged the current paradigm of hMSC survival, which assigned a pivotal role to oxygen, by testing the hypothesis that exogenous glucose may be key to hMSC survival. We demonstrated that hMSCs could endure sustained near-anoxia conditions only in the presence of glucose. In this in vitro cell model, the protein expressions of Hif-1α and angiogenic factors were up-regulated by the presence of glucose.

Ectopically implanted tissues constructs supplemented with glucose exhibited four- to fivefold higher viability and were more vascularized compared to those without glucose at day 14. These findings provided the first direct in vitro and in vivo demonstration of the pro-angiogenic and pro-survival functions of glucose.
PP115
On the importance of selenium for bone physiology
Nicole Pietzschmann, Eddy Rijntjes, Antonia Hoeg & Lutz Schomburg
Charité–Universitätsmedizin Berlin, Berlin, Germany.

The essential micronutrient Selenium (Se) plays an important role for bone formation and homeostasis. This notion is mainly derived from animal experimental studies showing impaired bone development and reduced measures of bone quality in animals on diets with low Se supply. Selenoprotein P (SePP) functions as the central Se storage and transport protein. SePP-knockout mice have a growth deficit. SePP is taken up by a receptor-mediated mechanism. We hypothesize that impaired SePP expression affects regular bone development, bone Se status and bone turnover. We have compared gene expression in SePP wild-type, heterozygous and knockout mice. Of the two alleged SePP receptors, only ApoER2 but not megalin was expressed in bone. Notably, ApoER2 expression was inversely associated with SePP levels, indicating that this SePP receptor might be involved in feedback regulation of Se metabolism in bone. When analyzing Se concentrations in bone with total reflection X-ray fluorescence (TXRF), a gender difference became apparent. Male mice appeared more sensitive to differences in SePP-genotype than females. This result supports the notion that SePP represents a Se transporter to bone affecting Se status in a sex-specific way. Our data indicate that bone is a preferably supplied target tissue of SePP ensuring its high Se concentrations, similar to e.g. brain or endocrine glands, and highlight the importance of selenoproteins for bone physiology.

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PP116
Effect of high doses of vitamin D on arterial properties, adiponectin, leptin, and glucose homeostasis in type 2 diabetic patients
Marina Sharogradsky
Wolfson Medical Center, Holon, Israel.

Background and aims
Vitamin D supplementation has the potential to alleviate the cardiovascular damage in diabetic patients. The present study was designed to evaluate long-term impact of high doses of vitamin D on arterial properties, glucose homeostasis, adiponectin and leptin in patients with type 2 diabetes mellitus.

Methods and results
In randomized, placebo-controlled study 47 diabetic patients were assigned into two groups: Group 1 received oral daily supplementation with vitamin D at a dose of 1000 U/day. Group 2 received matching placebo capsules. Blood sampling for metabolic parameters, including fasting glucose, lipid profile, HbA1c, insulin, hs-CRP, 25 OH VitD, adiponectin, and leptin was performed at baseline and at the end of the study. Insulin resistance was assessed by homeostasis model assessment (HOMA-IR). Central aortic augmentation index (AI) was evaluated using SphygmoCor (version 7.1, AtCor Medical, Sydney, Australia). The two groups were similar at baseline in terms of hemodynamic parameters. After 12 months, AI decreased significantly during the treatment period in patients received vitamin D (P<0.0001) and did not change in placebo group. Glucose homeostasis parameters, leptin as well as leptin adiponectin ratio did not change in both groups. 25 OH Vit D level significantly increased (P=0.022) and circulating adiponectin marginally increased (P=0.065) during 12-month treatment period in active treatment and did not change in placebo group.

Conclusions
High doses of vitamin D supplementation in diabetic patients was associated with significant decrease in AI during one year treatment. This beneficial vascular effect wasn’t associated with improvement in glucose homeostasis parameters.

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PP117
Relationship between bone mineral density, body composition, skin sclerosis, and serum 25(OH) vitamin D levels in systemic sclerosis
Addolorata Corrado, Anna Neve, Arcangela Marucci, Ripalta Colia, Angiola Mele & Francesco Paolo Cantatore
Rheumatology Clinic, Department of Medical and Surgical Sciences, University of Foggia, Foggia, Italy.

Introduction
Hypovitaminosis D is observed in several rheumatic autoimmune diseases, including systemic sclerosis (SSc); nevertheless, data concerning the possible determinants of reduced serum vitamin D levels in this disease are not fully investigated. The aim of this study is to evaluate the relationship between BMD, body mass composition, skin sclerosis, and serum vitamin D levels in two subsets of SSc patients.

Patients and methods
55 Post-menopausal SSc patients, classified according to Leroy as limited cutaneous (IISc) or diffuse cutaneous (dSSc) SSc, were studied. Clinical parameters were evaluated, including the extent of skin involvement (Rodnan skin score). Bone mass density (BMD) at spine and hip, and body mass composition were determined by dual-energy X-ray absorptiometry. Serum calcium, phosphorus, alkaline phosphatase, osteocalcin, urine pyridinium cross-links, intact parathyroid hormone (PTH), and 25-hydroxvitamin D (25 OHD) were also measured. The study protocol was approved by Local Ethical Committee.

Results
In dSSc, BMI, and BMD (spine, femoral neck, and total hip) were significantly lower compared to IISc (P<0.05). Total body mass was significantly lower in dSSc (P<0.05), with no differences in both fat and lean mass in the two study groups; conversely, body mineral content (BMC) was significantly reduced in dSSc patients (P<0.05). In both groups, hypovitaminosis D was observed (mean 25OHD 16.8±9.7, but 25OHD serum levels were significantly lower in dSSc (P<0.01) and inversely correlated with the extent of skin thickness (r = −0.46, P<0.05). No differences between IISc and dSSc in serum calcium, phosphorus, alkaline phosphatase, osteocalcin, urine pyridinium cross-links and PTH were found.

Conclusions
These results support the hypothesis that the extent of skin involvement in SSc patients could be an important factor in determining low circulating levels of 25OHD, which in turn could play a significant role in the reduction of BMD and BMC.

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PP118
Role of estrogen replacement therapy in the control of immune system in postmenopausal osteoporosis
Patrizia D Amelio, Francesca Sassi, Ilaria Buondonno, Giorgia Fornelli, Elena Bonardo & Giovanni Carlo Isia
Department of Internal Medicine, Torino, Italy.

Introduction
We have recently shown that T cells play a key role in postmenopausal bone loss, here we investigate the influence of estrogen replacement therapy in the control of the immune system and osteoclastogenesis.

Description of methods
We enrolled in the study 30 women with postmenopausal osteoporosis randomized to estrogen replacement therapy (HRT) or raloxifene (RLX) associated with calcium and vitamin D or calcium and vitamin D alone. Osteoclast precursors (OCP) in peripheral blood, regulatory T cells (Tregs) and cytokines production were evaluated at basal level and after 3, 6 and 12 months of therapy.

Results
OCP were significantly reduced by the sole RLX. Tregs were reduced by HRT, and not by other therapies. HRT ameliorates T cells immune response. TNFα, IL7, and IFNg and RANKL:OPG ratio were significantly reduced by HRT.

Conclusion
Here we demonstrate that estrogens have an immunomodulatory effect on T cells, reduce Tregs and ameliorates T cells response to immune stimulation.

HRT reduce the production of pro-inflammatory cytokines as TNFα, IL7, and IFNg. These cytokines are also responsible for increased osteoclastogenesis. HRT reduces the RANKL:OPG which is the main driver of osteoclast formation and activity, whereas it has no direct effect on OCP number.
PP119

Vitamin D in fragile elderly women with severe osteoporosis and uncontrolled hypertension

Ferdinando D'Anzico1,2, Francesco Caronzo1, Giovanni Gaglio1, Antonino Granata1, Giuseppina Lombardo1, Tiziana Pipicella1, Pippo Sapatol1, Enzo Russo1 & Alessandro Grippa1

1Department of Geriatrics Hospital of Patti, Patti, Messina, Italy; 2School of Medicine University of Messina, Messina, Italy.

Introduction

Aim of this study was to evaluate connections between Blood Pressure values and hypovitaminosis D states in elderly women with severe osteoporosis.

Design and methods

The subjects were all >80: 63 women (mean age 84±3) affected by severe osteoporosis. In 26 women we discovered a new spinal fracture after treatment. Thirty-seven women had multiple spinal fractures (>3). Among subjects with severe osteoporosis we selected 37 patients affected by hypertension treated with RAS non-interfering drugs. The design of the study included the evaluation of: i) clinical measurement of blood pressure; ii) renin; iii) 25 hydroxycolecalciferol; and iv) parathormone (PHT). Blood Pressure (BP) control was considered with RAS non-interfering drugs. The mean age of patients was 54.76 years and the mean BMI was 30.07 kg/m². The clinic situation of secondary hyperparathyroidism and hypovitaminosis D also shows an increase in renin values. A correction in treatment for hyperparathyroidism and hypovitaminosis D can influence the RAS systemic activity and can also influence the secondary hyperparathyroidism treatment.

Results

In the women examined we detected: mean systolic blood pressure (SBP) 134 mmHg, mean diastolic blood pressure 92 mmHg. In 31 women with hypovitaminosis we detected secondary hyperparathyroidism. Thirty-four women had uncontrolled hypertension with mean values of SBP 141 and of DBP 93 mmHg. Subjects with hypovitaminosis D and hyperparathyroidism showed an increase of renin values compared to women with normal levels of parathormone. Consequently we detected that there was a connection in elderly patients with severe osteoporosis between variable factors like age, blood pressure, vitamin D, and PTH (P <0.001).

Conclusions

Through this study we can state that in subjects affected by severe osteoporosis there is a direct pathogenetic link between parathormone and regenerative mechanism of blood pressure. The clinic situation of secondary hyperparathyroidism and hypovitaminosis D also shows an increase in renin values. A correction in treatment for hypovitaminosis D can influence the RAS systemic activity and can also influence the secondary hyperparathyroidism treatment.

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PP121

Calcium dietary intake in southern lebanese women

Yasser Yaghi1,3, Fatiha El Hoor2, Nancy Mann1 & Kinda Yaghi1

1Bone and Joint Decade, Saida, Lebanon; 2Lebanese Welfare Association for the Handicapped, Sarafand, Lebanon; 3Hammoud Hospital-Beirut Arab University, Saida, Lebanon.

Aim

The aim of this study was to assess the calcium daily food intake in Lebanese women living in South Lebanon, an under privileged area.

Subjects and methods

Assessment of calcium food intake was performed with a validated questionnaire in 290 female patients attending clinics in two major referral health centers in South Lebanon.

Results

The mean age of patients was 55.76 years and the mean BMI was 30.07 kg/m² (s.d. 6.56 kg/m²). The mean dietary intake of calcium in this study group was 632.78 mg/day (s.d. 286.02 mg/day), the 25th, 50th, and 75th percentiles were 397.69, 596.64, and 827.22 mg/day. The mean daily calcium intake was under the recommended daily amount of 900–1200 mg/day.

Conclusion

The study confirms the prevalence of overweight and low calcium food intake in women living in Southern regions of Lebanon, and this implies that the average need for exogenous calcium supplementation should be above 500 mg daily whereas health professionals should play a role to influence nutritional norms aimed at better health awareness.

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PP122

Effects of long-term oral administration with methimazol on femur properties in male Wistar rats

Marcin Tatara1, Marcin Golnyński2, Radosław Radzki1, Marek Bienko1 & Witold Krupski3

1Department of Animal Physiology, University of Life Sciences in Lublin, Lublin, Poland; 2Department and Clinic of Animal Internal Medicine, University of Life Sciences in Lublin, Lublin, Poland; 3II Department of Radiology, Medical University of Lublin, Lublin, Poland.

Methimazol is an antithyroid drug for treatment of hyperthyroidism. Methimazol inhibits the enzyme thyroid peroxidase participating in thyroid hormone synthesis and reduces triiodothyronine (T3) and thyroxine (T4) blood concentrations. The study was performed to determine effects of 90-day long oral administration with methimazol on femur properties isolated from male Wistar rats. The first group (n = 6) consisted of control rats. The experimental rats (Met group, n = 6) received methimazol in 0.05% water solution ad libitum. At 6 months of life, serum concentration of osteocalcin (OC) was determined and right femurs were isolated to measure bone length and weight. Total bone volume (BV/TV) and mean volumetric bone mineral density (MvBMD) of femur was determined with a use of quantitative computed tomography (QCT). Using peripheral QCT (XCT Research SA Plus apparatus, Stratec Medizintechnik GmbH, Pforzheim, Germany) and dual-energy X-ray absorptiometry (DEXA; Norland Excell Plus Densitometer, Fort Atkinson, WI, USA), bone mineral content (BMC), and bone mineral density (BMD) of the trabecular and cortical bone were evaluated. Furthermore, trabecular (TBA) and cortical (CBA) bone areas, periosteal (PC)
and endosteal (EC) circumferences, axial (AMI) and polar (PMI) moments of inertia, and moment of resistance (SSI) were determined. Final body weight of experimental rats was significantly lowered by 30% when compared to the control. (P<0.05). Serum concentration of OC was significantly decreased in experimental rats (3.06±0.46 ng/ml) when compared to the controls (3.89±0.46 ng/ml; P=0.03). Significantly decreased values of bone weight, length, Bvol, MvBMD, BMC, BMD, TBA, CBA, PC, AIM, PIM, and SSI of femur were found in the experimental rats when compared to the controls (P<0.05). In conclusion, this study showed negative effects of long-term oral administration with methimazol on bone formation process and morphological and densitometric properties of femur. Long-term antithyroid treatment may lead to growth inhibition and development of osteopenia or osteoporosis.

PP123
Vitamin D supplementation during lactation: effect on maternal and offsprings’s vitamin D status and bone mass—double-blind randomized control trial
Jastyna Czech-Kowalska1, Maciej Jaworski1, Julita Latka-Grot1, Dorota Bulsiewicz2, Pawel Pludowski1, Bogdan Chazan2, Beata Pągus2, Grazyna Wygledowska1, Anna Zochowska1, Maria K Kornacka1, Edyta Kryśkiewicz1, Elżbieta Karczmarewicz2 & Anna Dobrzańska1
1The Children’s Memorial Health Institute, Warsaw, Poland; 2Holly Family Hospital, Warsaw, Poland; 3Miedzyleski Hospital, Warsaw, Poland; 4Public Hospital, Otwock, Poland; 5Warsaw Medical University, Warsaw, Poland.

Introduction
Optimal vitamin D intake for lactating women remains controversial. We hypothesized that 1200 IU/day (vs 400 IU/day) of vitamin D during breastfeeding will enhance maternal vitamin D status and bone mass.

Methods
Healthy mothers after term, singleton delivery were randomized to receive vitamin D3: 1200 IU/day (800 IU/day from multivitamins) during lactation. Serum 25-hydroxyvitamin D (S-25-OHD), PTH, and densitometry were performed in mothers and infants after delivery (V0) and 3 months later (V1). Calcinemia and calcitriol (urinary calcium:creatinine ratio) were assessed at V1.

Results
174 mother–infant pairs were recruited. Intention to treat analysis was performed for 137 pairs completed the study (1200 IU/day group (n = 70), 400 IU/day group (n = 67)). Baseline maternal and neonatal (cord blood) S-25-OHD, PTH and anthropometric measurements were similar among groups. Maternal S-25-OHD increased from 13.65 to 25.7 ng/ml (P<0.0001) and from 16.1 to 24.5 ng/ml (P<0.0001). Maternal S-25-OHD (V1) was significantly higher in 1200 than 400 IU/day group (25.7 vs 24.5 ng/ml; P=0.049) but comparable among infants (33.9 vs 32.9 ng/ml; P=0.165). While percentage of maternal S-25-OHD >30 ng/ml was similar in both groups (22.85 vs 22.99%) at V1, Maternal PTH decrease from 28.6 to 22.1 pg/ml (P<0.0001) and from 30.4 to 23.3 pg/ml (P<0.0001) in the 1200 and 400 IU/day groups respectively. There were no differences between groups in maternal and neonatal PTH, bone mass, serum, and urinary calcium at V1.

Conclusions
Vitamin D intake at a dose 1200 IU/day is not effective in achieving maternal S-25-OHD >30 ng/ml and reducing bone mass loss during lactation. Breastfed infants receiving 400 IU/day of vitamin D have adequate S-25-OHD irrespective of maternal supplementation up to 1200 IU/day.

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PP124
Polyphenols from berries of Aronia melanocarpa improve the antioxidative capacity of the bone tissue in cadmium-exposed rats
Joanna Rogalska, Małgorzata M Brzóśka, Alicja Roszczenko, Małgorzata Galazyn-Sidorczak & Marija Jurczuk
Department of Toxicology, Medical University of Białystok, Białystok, Poland.

Cadmium is characterized by oxidative properties and this heavy metal-induced oxidative stress has been recognized to be involved in its injurious impact on the skeleton. Oxidative/reductive processes are an integral component of bone remodeling; however, destroying of the bone tissue oxidative/antioxidative status with excessive production and accumulation of reactive oxygen species has detrimental impact on bone metabolism. Thus, the aim of this study was to investigate whether polyphenolic compounds, known to possess strong antioxidative properties, can improve the antioxidative capacity of the bone tissue under chronic exposure to cadmium. For this purpose, chosen indices of the enzymatic antioxidative barrier (glutathione peroxidase and catalase), total antioxidative status (TAS), and total oxidative status (TOS) as well as the level of oxidative stress (OSS=O2/TAS) of the bone tissue at the distal femoral end (trabecular bone region) of the female Wistar rats administered as the only drinking fluid 0.1% water extract of polyphenols from the berries of Aronia melanocarpa or cadmium in diet (5 mg/kg) for 3, 10, 17, and 24 months were estimated. The exposure to cadmium decreased the activities of glutathione peroxidase and catalase, and TAS of the bone tissue and increased the bone TOS resulting in the development of oxidative stress. The administration of polyphenolic compounds under the exposure to cadmium protected from this heavy metal-induced weakening of the bone antioxidative capacity and from oxidative stress in the bone tissue. Based on the results, it can be concluded that consumption of polyphenol-rich products such as Aronia melanocarpa berries under long-term exposure to cadmium may have beneficial impact on the skeleton via improving the bone tissue antioxidative/antioxidative status. This study was financially supported by the grant (no. N N405 051140) from the National Science Centre (Poland).

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PP125
The serum serotonin and 25-OH vitamin D levels: a study in 97 menopausal women
Mara Carsote1,2, Mihaela Popescu3, Ramona Sanojla1, Rene Baloescu2, Madalina Muler1, Adriana Gruia1 & Catalina Poiata1,2
1Davila UMF, Bucharest, Romania; 2Parhon Institute, Bucharest, Romania; 3Medlife Center, Bucharest, Romania.

Introduction
The vitamins as B6, C, or D are involved in serotonin metabolism but mostly in central neurotransmitter pathways. There are very few clear data related to 25-OH vitamin D status and serum serotonin (SS) levels.

Aim
We analyze the SS and 25-OH D.

Materials and methods
We included women in menopause. The serum serotonin (SS; normal 100–400 ng/ml) and 25-OH vitamin D (normal 30–100 ng/ml) were performed in fasting status. The statistical analyse was performed with Student’s t-test.

Results
97 Women (w) were included with mean age of 57.03 years. Based on 25-OH D levels four groups were formed: 1–3 with vitamin D deficiency and group 4 (control) with normal D levels. Group 1 had 31 w with 25-OH D between 1–9.9 ng/ml (mean 6.52 ng/ml), and mean SS of 180.97 ng/ml, group 2 with 40 w having 25-OH D between 10–10.9 ng/ml (mean 14.42 ng/ml), and a mean SS of 153.78 ng/ml, group 3 with mean 25-OH D of 23.47 ng/ml (between 20–29.9) and mean SS of 171.53 ng/ml, including 19 w; group 4 with 7 w, a mean 25-OH D of 37.28 ng/ml (between 30–39.9) and mean SS of 109 ng/ml. Statistically significant differences were obtained between each group of D deficiency (groups 1, 2, or 3) and control group (P=0.02, P=0.01, and P=0.03 respectively); and between all the women with D deficiency (mean SS 166.89 ng/ml) and control group (mean SS of 171.53 ng/ml, including 19 w; group 1 with vitamin D deficiency and group 4 with normal D levels).

Discussion
The SS might not be very adequate to analyze its complex metabolism since different levels are found in brain and periphery.

Conclusions
Based on our observations higher levels of serotonin is found in different levels of vitamin D deficiency.

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PP126
Vitamin D: light side and best time of sunshine in Riyadh, Saudi Arabia
Fahad Alsahari1, Mussa Almaliek2, Najj Aljohani1, Abdullah Alzahrani1, Youssef Alsalih1 & Michel Holick3
1King Abdulaziz Medical City, National Guard, Riyadh, Saudi Arabia; 2King Fahad Medical City, Riyadh, Saudi Arabia; 3King Abdulaziz Medical, Jeddah, Saudi Arabia; 4Boston University Medical Center, Boston, Massachusetts, USA.

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Low levels of 25-hydroxyvitamin D have been documented among inhabitants of the wider Middle East and North African countries. Sunlight has long been recognized as a major provider of vitamin D. In this study we aimed to determine the optimum time for sun exposure in the Central region of Riyadh, Saudi Arabia. Ampoules containing 7-dehydrocholesterol in ethanol were exposed to sunlight every hour starting from sunrise until sunset in July and December. Our results demonstrated that the time of the day has a major influence in vitamin D production. In this study, summer conversion of previtamin D, was observed to be elevated between 0900 and 1500 h with peak hours between 1000 and 1200 h. During wintertime however, the conversion begins later at around 0930 h until 1400 h, with peak hour around 1100 h. In conclusion, the optimum time to get sun exposure for vitamin D production in Riyadh, during summer time is from 0830 h and before 1030 h, as well as after 14 h until 16 h while during wintertime it’s from 1000 h to 1400 h. These timings are important on a public health perspective, as it’s free, safe and enjoyable. Furthermore it’s a highly efficacious way for management and prevention of vitamin D deficiency.

**PP127**

**Hypercalcemia following high vitamin D loading dose**

Paul Lips, Aegida Nenadova, Nakasja van Schoor & Mark Vervloet

VU University Medical Center, Amsterdam, The Netherlands.

Vitamin D deficiency is common in older persons and non-western immigrants. Vitamin D is often started in loading doses of 50 000 IU/ml solution. Though, generally considered safe, this highly concentrated solution carries some risks as is illustrated by the following cases. A woman of >80 years old was admitted with hypercalcaemia, calcium 3.27 mmol/l. She complained of nausea, thirst and polyuria for 3 months. History included cholecystitis, atrial fibrillation, myocardial infarction, type 2 diabetes, polyneuropathies, and moderate renal insufficiency (precontrast creatinine 213 μmol/l). Medication included tolbutamide, pantoprazol, and metoprolol. Physical exam was unremarkable except for a known abdominal ultrasound and mammography did not reveal a cause for the hypercalcaemia. Protein electrophoresis did not show an M-protein. Calcium 3.27 mmol/l, albumin 34 g/l, creatinine 254 μmol/l, alkaline phosphatase 95 U/l.

The patient was rehydrated with saline and received pamidronate i.v. Skeletal radiographs showed osteoarthritis of both hips, but no osteolytic lesions. The patient was discharged on calcium carbonate with good dietary advice.

**PP128**

**Mass spectrometric immunoassay for intact parathyroid hormone: correlation with immunoassay and application to clinical samples**

L. Couchman1, D. Taylor1, B. Kyriatsis2, M. Lopez3, A. Prakash4, D. Sarracino1,2, A. Garces1,2, M. Vogelsang1,2, S. Peterman1,2, G. Vadali1,2, S. Robinson1,3 & C. Moniz2

1Kings College Hospital, Clinical Biochemistry, London, UK; 2ThermoFisher Scientific, BRIMS, Boston, Massachusetts, USA; 3ThermoFisher Scientific, Hemel Hempstead, UK.

**Introduction**

Parathyroid hormone (PTH) measurement is of use in i) differential diagnosis of hypercalcaemia and ii) patients with renal impairment at risk of bone disease. PTH immunoassays are complicated by cross-reactivity with truncated (inactive) variant forms, which accumulate in patients with renal impairment. PTH assay variability is a critical governance issue in renal medicine, suggesting an MS-based reference method is required.

**Aim**

To develop a mass spectrometric immunoassay (MSIA) for intact PTH quantification, and to compare results with a PTH immunoassay.

**Methods**

 Plasma PTH was immunocaptured onto MSIA pipette tips pre-bound with anti-PTH antibody, using an automated liquid handler (Versette). Captured antibodies were eluted from the tips, digested and specific tryptic peptides analysed by LC–MS/MS, using labelled peptides and recombinant PTH standards for assay calibration. Samples (n=357) analysed by immunoassay (Advia Centaur) then re-analysed by MSIA for comparison.

**Results**

The MSIA assay demonstrated excellent linearity (R² = 0.90–0.99), sensitivity (LOQ, 16 pg/ml), specificity and precision (CV < 10%). Significant findings were i) poor correlation (R² = 0.67) between the immunoassay and the surrogate N-terminal tryptic peptide (aa 1–13) for intact PTH, ii) better correlation (R² = 0.86) of ‘mean PTH’ by MSIA (i.e. the mean concentration of all tryptic peptides, including variant forms), iii) identification of novel variant forms in samples from patients with renal disease, and iv) the commonly-cited variant form PTH 7–84 was not detected.

**Conclusions**

This approach allows rapid, automated immunoenrichment achieving high sensitivity and selectivity. MSIA allows the simultaneous monitoring of intact and variant PTH forms. Correlation of the PTH MSIA assay using only the N-terminal tryptic peptide (aa 1–13) with immunoassay demonstrated that the immunoassay overestimates the amount of active PTH. This difference was greater in patients with renal impairment, in whom PTH concentrations direct clinical decisions.
Calcium-phosphorus metabolism and calcium-regulating hormones in osteoporosis associated with liver cirrhosis

Iryna Golovach1, Zinovij Mytnyk1,2 & Diana Vershyhina1,2
1Clinical Hospital Feofania, Kyiv, Ukraine; 2National Medical University, Ivano-Frankivsk, Ukraine.

Chronic liver diseases (CLD) leads to an imbalance of bone remodeling, bone mass decrease with the development of hepatic osteodystrophy, which is most often seen in osteoporosis. In the development of structural and functional deficiency of bone plays an important role of calcium–phosphorus homeostasis and coherence calcium-regulating hormones (CRH), which include parathyroid hormone (PTH) and active metabolites of vitamin D.

The aim of this study was to determine violations of mineral metabolism, the concentration of calcium-regulating hormones, as well as to establish the influence of disturbances in these systems of osteoporosis associated with liver cirrhosis LC.

We observed 172 patients with LC, the average age – 49.3 ± 7.7 years. The men were 108 (62.8%), women – 64 (37.2%). Bone mineral density (BMD) was determined by DXA ‘Challenger’ (DNS, France). We also determined the serum concentration of calcium and phosphorus, urinary excretion, the level of PTH, and 25-hydroxyvitamin D (25(OH)D).

We found significant violations of the calcium-phosphorus metabolism, changes in the CRH, and reduced bone mineral density in patients with LC, but the severity of the imbalance depended on the severity of the disease and correlated with the main laboratory criteria of liver dysfunction. We observed decrease serum concentration of total and ionized calcium, as well as the expressive tendency to hypercalcemia.

The concentration of 25(OH)D was 9.88 ± 4.36 ng/ml (in control 26.76 ± 9.27 ng/ml; P < 0.001). The level of deficit depended on the degree of liver dysfunction. In the case of compensated of LC the level of 25(OH)D was 12.93 ± 5.42 ng/ml and decompensated – 4.53 ± 2.22 ng/ml (a decrease of 2.8 times). It emphasizes that with abnormal liver function significantly affected the formation of this 25(OH)D, i.e. mechanism of the first hydroxylation of vitamin D, which emphasizes that with abnormal liver function significantly affected the formation of this 25(OH)D, i.e. mechanism of the first hydroxylation of vitamin D.

In patients with cirrhosis of the liver, the daily dose of 25(OH)D was 3.1 pg/ml (in control 11.7 pg/ml; P < 0.001). PTH correlated with bone mineral density of the radius and lumbar spine.

Thus, in patients with cirrhosis of the liver, the daily dose of 25(OH)D was 3.1 pg/ml (in control 11.7 pg/ml; P < 0.001). PTH correlated with bone mineral density of the radius and lumbar spine.

In conclusion, it is determined that osteoporosis in patients with liver cirrhosis associated with liver cirrhosis LC.

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Cancer and bone: basic, translational and clinical

The central role of the histone demethylase JHDM1D in the regulation of tumor associated genes in bone tumor-related cells

Roman Thaler1, Silvia Spitzer1, Florian Haider1, Heidrun Karlic2, Klaus Klaushofer1 & Franz Varga1
1Ludwig Boltzmann Institute of Osteology, AUVA Trauma Center Meilding, Hanusch Hospital of WKGK, Vienna, Austria; 2Ludwig Boltzmann Institute for Leukemia Research and Hematology, Hanusch Hospital, and Ludwig Boltzmann Cluster Oncology, Vienna, Austria.

Tumor development occurs often by over-activation of members of the RAS-oncogene family (small GTPases (sGTPs)). By blocking the mevalonate pathway, aminobisphosphonates (BPs), and statins prevent activation of GTPs by inhibiting their post-translational prenylation. As we have shown, this induces apoptosis in U2OS osteosarcoma cells by re-activation of FAS expression via epigenetic DNA demethylation (1).

The histone demethylase JHDM1D exerts a tumor-suppressive function by down-regulating angiogenesis (2). Furthermore, weak JHDM1D expression correlates with Rho-GTPase dependent increased cell motility and invasiveness of MDA-MB-231 and MCF-7 breast cancer cells (3)

Bone metastatic human PC-3 prostate and MDA-MB-231 breast cancer and U2OS and MG63 osteosarcoma cells were treated up to 72 h with increasing concentrations of ibandronate (Ibn) or with the statin simvastatin (Sim). JHDM1D expression was suppressed by siRNA techniques, cDNA over-expressing cell lines where created. Gene expression was analyzed by Affymetrix gene array, RT-qPCR, and immunoblotting. Caspases activities and cell proliferation were measured.

While Sim strongly repressed proliferation in all cell-lines tested, Ibn showed a similar effect only in osteosarcoma cells having a considerable effect on PC-3 and MDA-MB-231 cells.

For all cell lines, both compounds increased significantly the expression of JHDM1D. Knock down of JHDM1D largely abrogated Simv or Ibn dependent inhibition of cell proliferation in PC3 and MDA-MB-231 cells or in osteosarcoma cells respectively.

Tumor related genes like FAS, CEACAM1, DRAM1, ESM1, or PTX3 where strongly up-regulated by both drugs in the cell-lines tested. Re-expression of all these genes by Sim or Ibn was JHDM1D dependent. Cell proliferation rates where halved in Esm1 or PTX3 over-expressing osteosarcoma cells. For PTX3 this may be due to increased FAS expression.

Our data demonstrate a central role for JHDM1D in suppression of RAS-oncogene family mediated bone tumor development.

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**PP133**
Identification of tumorigenic sarcoma cancer stem cells based on high aldehyde dehydrogenase 1 activity
Birgit Lobberger1, Beate Rinner2, Nicole Stuendl1, Sonja Maria Walzer3, Reinhard Windhager1 & Andreas Leitlmeir1
1Department of Orthopaedic Surgery, Medical University of Graz, Graz, Austria; 2Center for Medical Research, Core Facility Flow Cytometry, Medical University of Graz, Graz, Austria; 3Department of Orthopaedic Surgery, Medical University of Vienna, Vienna, Austria.

Tumors contain a small population of cancer stem cells (CSC) proposed to be responsible for tumor maintenance and relapse. Aldehyde dehydrogenase 1 (ALDH1) activity has been used as a functional stem cell marker to isolate CSCs in different cancer types. This study used the Aldefluor assay and fluorescence-activated cell sorting (FACS) analysis to isolate ALDH1high cells from five human sarcoma cell lines and one primary chordoma cell line. ALDH1high cells range from 0.3% (MUG-Chor1) to 4.1% (SW-1353) of gated cells. Immunohistochemical staining of the clone formation efficiency, and xCELLigence microelectrostatic sensor technology revealed that ALDH1high cells from all sarcoma cell lines have an increased proliferation rate compared to ALDH1low cells. By investigating important regulators of stem cell biology, real-time RT-PCR data showed an increased expression of c-Myc, ß-catenin, and SOX-2 in the ALDH1high population and a significant higher level of ABCG2. Statistical analysis of data demonstrated that ALDH1low cells of SW-982 and SW-1353 showed higher resistance to commonly used chemotherapeutic agents like doxorubicin, epirubicin, and cisplatin than ALDH1low cells. Using a NOD/SCID mouse xenograft model, ALDH1high cells showed a greater tumor formation capacity compared to ALDH1low cells. The ALDH1high tumors were significantly larger than the ALDH1low tumors after 4–6 weeks. This study demonstrates that in different sarcoma cell lines, high ALDH1 activity can be used to identify a subpopulation of cells characterized by a significantly higher proliferation rate, increased colony forming, increased expression of ABC transporter genes and stemness markers compared to control cells. In addition, enhanced drug resistance and a greater tumor forming capacity were demonstrated.

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**PP134**
Isolation of ALDH1high cells by flow cytometry and investigation of the expression pattern of Wnt pathway genes in primary chordoma cell lines
Birgit Lobberger1, Nicole Stuendl1, Katharina Meditz2, Berndadette Liegl1, Andreas Leitlmeir1 & Beate Rinner2
1Department of Orthopaedic Surgery, Medical University of Graz, Graz, Austria; 2Center for Medical Research, Core Facility Flow Cytometry, Medical University of Graz, Graz, Austria; 3Institute of Pathology, Medical University of Graz, Graz, Austria.

Chordomas are rare, low to intermediate grade malignant bone tumors of the axial skeleton. Current treatment options are limited to surgical procedures as chordomas are largely resistant to conventional radiation and chemotherapy. Cell lines are valuable tools to explore molecular mechanisms involved in tumorigenesis and they have a fundamental impact on the development of new anticancer agents. We established a novel chordoma cell-line, MUG-Chor1, from a recurrent morphologically ‘classic’ sacrococcygeal chordoma of a 58-year-old Caucasian female. In this current study, we first used the Aldefluor assay (Stemcell Technologies) and fluorescence-activated cell sorting (FACS) analysis to assess the presence and size of the cell population with ALDH1A1 enzymatic activity in three primary chordoma cell lines. ALDH1high chordoma cells range from 0.35 ± 0.34% (UCH1) to 1.39 ± 0.56% (MUG-Chor1; n = 10) of gated cells. ALDH1high and ALDH1low cells differed significantly in logarithmic growth velocity measured in a label-free real-time cell electronic sensing assay (RT-CES). By investigating of important regulators of stem cell biology, the pluripotent stem cell progenitor proliferator and real-time RT-PCR data showed an increased expression of Sox2, Sox17, E-cadherine, oct4, and gossecoid (GCS) in the ALDH1high population. In the analysis of genes, which play an important role in the Wnt pathway, a significant difference in the expression of six genes between ALDH1high and ALDH1low cells could be demonstrated. We have successfully used the Aldefluor assay for the isolation of ALDH1high and ALDH1low chordoma cells and showed significant differences in cell proliferation properties and the expression pattern of stem cell- and Wnt pathway genes.

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**PP135**
Anti-RANKL nanobody ALX-0141 shows sustained biomarker inhibition in a Phase I study in healthy postmenopausal Women
Pieter Schoen, Sandy Jacobs, Katrin Verschueren, Ingrid Ottevaere, Sigrid Sobry & Josefin-Beate Holz

Introduction
The interaction between RANK/RANKL is critical for the regulation of osteoclastogenesis and bone resorption. Inhibition of this interaction helps restore the balance between bone resorption and formation. ALX-0141, a novel biological agent (Nanobody) that specifically targets RANKL, was studied in a Phase I trial to assess the safety, tolerability, immunogenicity and PK after single injection.

Methods
Forty-two healthy postmenopausal women (53–77 years, mean 66 years) were included in this study, which was approved by the local Ethical Committee. Participants received a single s.c. injection of ALX-0141 (n = 31) at six dose levels, ranging from 0.003 to 1 mg/kg, or placebo (n = 11). PK, PD, and safety parameters were monitored for 3 months at the lowest dose level and for more than a year in the higher dose levels.

Results
The safety analysis indicated that ALX-0141 was well tolerated. No serious adverse events related to ALX-0141 or dose-limiting toxicity occurred. The frequency of treatment emergent adverse events (TEAE) was similar in placebo-treated subjects (16 events in 7 subjects (64%)) and in subjects treated with ALX-0141 (193 events in 23 subjects (74%)). The most frequent TEAE were musculoskeletal and connective tissue disorders (n = 27, reported by 14 subjects) and all TEAE were transient, of mild intensity, and did not result in any study withdrawals. ALX-0141 showed a favourable PK profile, triggering a prolonged PD response. Serum levels of the lead biomarker for bone resorption CTX-1 decreased rapidly and stayed suppressed for up to 390 days after a single administration of 1 mg/kg.

Conclusions
The results from this Phase I trial indicate that ALX-0141 is a potent RANKL inhibitor that is well tolerated over a wide range of doses. This data supports the further development in bone-resorptive diseases with reduced BMD and increased fracture risk, such as in cancer-related bone diseases, osteoporosis and other disorders.

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**PP136**
Modulation of macrophage activation status by bisphosphonates and breast cancer cells
Sofia Sousa1, Jukka Mönkkönen1 & Jorma Määtta1,2
1University of Eastern Finland, Kuopio, Finland; 2University of Turku, Turku, Finland.

Tumour stromal macrophages differentiate into tumour associated macrophages (TAMs), with characteristics resembling the immunosuppressive M2 polarization instead of the pro-inflammatory M1. TAMs have a central role in promoting tumour vascularization, cancer cell dissemination and suppression of anti-cancer immune response. Cancer cell dissemination leads to metastasis formation, e.g. in breast cancer often happens in bone marrow. We have studied the in vitro modulation of that polarization by bisphosphonates (BPs) and breast cancer cell conditioned medium (CM). The effect of CM from the murine breast cancer cell line 4T1, on the J774 murine macrophage cell line response to LPS, was studied. 4T1 CM, but not control 3T3 CM decreased NO2 production and increased IL6 and MMP-9 mRNA expression by J774 cells upon LPS stimulus. BPs uptake by macrophages is improved by liposome encapsulation. The analysis of the effects of free and liposome encapsulated BPs on J774 cells, revealed the expected increase in potency of the liposome encapsulated drugs to induce apoptosis. At sublethal doses, clodronate (CLO) led to the intracellular accumulation of AppCl2p and zoledronate (ZOL) to isopentenyl pyrophosphate (IPP), triphosphoric acid l,4,5,6-yl ester 3-c(3-methylbut-3-enyl)ester (AppI) and unpreynlated proteins. To establish if these drugs also affect the cell polarization status, free and liposome drugs were tested prior to LPS stimulus.
According to our preliminary analysis, in the presence of 4T1 CM liposome encapsulated ZOL enhanced the expression of M1-type mediators, but did not downregulate the expression of M2-type mediators. In conclusion, in our model breast cancer cells were able to alter macrophage polarization and liposome encapsulated ZOL was able to modulate that effect. The relevance of this in tumour propagation needs further studies.

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PP137

Clusterin inhibition using OGX-011 synergistically enhances zolendronic acid activity in osteosarcoma
Francois Lamoureux1,2, Marc Baud’huin1,2, Benjamin Ory1,2, Martin Gleave1, Dominique Heymann1,4 & Francoise Redini1,2
1LUNAM Université; INSERM, UMR 957, Nantes, France; 2Laboratoire de Physiopathologie de la Résorption Osseuse et Thérapie des Tumeurs Osseuses, Université de Nantes, Nantes atlantique universités, Primitives, Nantes, France; 3The Vancouver Prostate Centre, University of British Columbia, Vancouver, British Columbia’s, Canada.

Despite recent improvements in therapeutic management of osteosarcoma, ongoing challenges in improving the response to chemotherapy warrants new strategies still needed to improve overall patient survival. Among new therapeutic approaches, zolendronic acid represents a promising adjuvant molecule to chemotherapy to limit the osteolytic component of bone tumors. However, zolendronic acid triggers the elevation of heat shock proteins (Hsp), including Hsp27 and clusterin (CLU), which could enhance tumor cell survival and treatment resistance. We hypothesized that targeting clusterin (CLU) using siRNA or the antisense drug, OGX-011, will suppress treatment-induced CLU induction and enhance zolendronic acid-induced cell death in osteosarcoma (OS) cells.

The combined effects of OGX-011 and zolendronic acid were investigated in vitro on cell growth, viability, apoptosis and cell cycle repartition of zolendronic acid-sensitive or zolendronic acid-resistant human cell lines (SaOS2, U2OS, MG63 and HOS). In OS cell lines, zolendronic acid increased levels of HSPs, especially CLU, in a dose- and time-dependent manner by mechanism including increased HSF-1 transcriptional activity. The OS resistant cells to zolendronic acid exhibited higher CLU expression level than the sensitive cells. Moreover, CLU overexpression protects OS sensitive cells to zolendronic acid-induced cell death by modulating the farnesyl diphosphate synthase expression. OGX-011 suppressed treatment-induced increases in CLU and synergistically enhanced the activity of zolendronic acid on cell growth and apoptosis. These biologic events were accompanied by decreased expression of HSPs, Akt, and HSF-1 transcriptional activity. In vivo, OGX-011, administered three times a week (i.p., 20 mg/kg), potentiated the effect of zolendronic acid (s.c.; 100 mg/kg), significantly inhibiting tumor growth by 50% and prolonging survival in HOS xenograft model compared to zoledronic acid alone.

In OS cell lines, zolendronic acid decreases the proliferation of hMCS in a dose-dependent manner and significantly inhibiting tumor growth by 50% and prolonging survival in HOS xenograft model compared to zolendronic acid alone.

These results indicate that zolendronic acid-mediated induction of CLU can be attenuated by OGX-011, with synergistic effects on delaying progression of osteosarcoma.

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PP138

New chondrosarcoma cell lines and mouse models to study the link between chondrogenesis and chemoresistance
David Monderer1,2, Alexandrine Luseau2, Ame´lie Bellec3, Marie-Francoise Heymann1,8, Ronan Le Bot2,4, Franc¸ois Gouin 1,8 & Francoise Redini1,2
1Laboratoire de Physiopathologie de la Résorption Osseuse et Thérapie des Tumeurs Osseuses Primitives, Université de Nantes, INSERM, UMR 957, Equipe Labellisée LIGUE 2012, Nantes, France; 2Atlantic Bone Screen (ABS), St Herblain, France; 3Unit of Cell and Gene Therapy, Nantes University Hospital, Nantes, France; 4Laboratoire de Physiopathologie de la Résorption Osseuse et Thérapie des Tumeurs Osseuses, Université de Nantes, Nantes atlantique universités, Primitives, Nantes, France; 5Department of Medical Genetics, Nantes University Hospital, Nantes, France; 6Hematology Laboratory, Nantes University Hospital, Nantes, France; 7Osteoarticular Diseases Unit, Nantes University Hospital, Nantes, France.

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Chondrosarcomas are cartilage-forming, poorly vascularized tumors. With an estimated annual incidence of 1 in 200,000, they represent the second malignant primary bone tumor of adults after osteosarcoma. These tumors are resistant to chemotherapy and radiotherapy, surgical excision remaining the only therapeutic option. However, very few cell lines and animal models are available, and the mechanisms behind their chemoresistance remain largely unknown. Our goal was to establish new cell lines and animal cancer models from human chondrosarcoma biopsies. These models were then used to study chondrosarcoma chemoresistance.

During the last 5 years, 10 chondrosarcoma biopsies were collected at the Nantes hospital and used for cell culture and transplantation in Nude mice. Only one transplanted biopsy and one injected cell line developed in immunodeficient mice, producing conventional central high grade chondrosarcoma. In culture, three new cell lines were obtained from high grade chondrosarcoma biopsies. Their genetic characterization revealed (hyper)triploid karyotypes, mutations in IDH1, IDH2, TP53, deletion in P16Ink4a / P14arf and/or MMM2 amplification. These cell lines expressed mesenchymal membrane markers (CD44, 73, 90 and 105) and were able to produce a cartilaginous matrix only in 3D chondrogenic cultures. Using a high throughquantitative RT-PCR approach, we observed that cell lines in monolayer culture (2D) lost expression of genes implicated in cartilage development (COL2A1, COMP, AGC, SOX5/6, etc.) but regained expression in 3D cultures. Chondrosarcoma cells in monolayer culture were not resistant to mafosfamide, cisplatin or doxorubicin but in 3D culture, they were resistant to low doses of cisplatin and doxorubicin. In fact, low doses of doxorubicin could not accumulate in chondrosarcoma cells when cultured in 3D, indicating that impaired diffusion of the drugs through the cartilaginous matrix would lead to chemoresistance. Therefore, 3D pellets constitute a relevant model to study chondrosarcoma chemoresistance and could be a valuable alternative to animal experimentations.

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Osteosarcoma is the most common primary malignant bone tumor, characterized by osteoid production and/or osteolytic lesions of bone. Despite recent improvements in chemotherapy and surgery, the problem of non-response to chemotherapy remains and constitutes a poor prognosis parameter. Consequently, new therapeutic strategies aim to improve the overall rate of survival. The present work investigated the therapeutic interest of a dual phosphatidylinositol 3-kinase (PI3K)/mammalian target of rapamycin (mTOR) inhibitor, NVP-BEZ235 (Novartis Pharma). This inhibitor targets both PI3K and mTOR kinase activity, in normal cells as in cells in which PI3K is mutated or PTEN is lost, two events frequently observed in oncologic process. *In vitro* effects of NVP-BEZ235 on proliferation, apoptosis and cell cycle have been assessed in five osteosarcoma cell lines (human: MG-63, HOS; rat: OSRGA; mouse: MOS-J, POS-1). Moreover *in vivo* experiments have been performed to establish the *in vivo* effects of NVP-BEZ235 on osteosarcoma development. More precisely, the tumor volume and the bone microarchitecture have been analyzed in the murine MOS-J osteoblastic osteosarcoma model. The results showed that *in vitro* NVP-BEZ235 exerts a dose-dependent anti-proliferative effect and induces a cell cycle arrest in G0/G1 phase in all cell lines studied. However, the drug does not induce apoptosis of osteosarcoma cells. In the MOS-J osteosarcoma model, oral administration of NVP-BEZ235 (45 mg/kg per day) significantly inhibits the tumor development. Furthermore, NVP-BEZ235 reduces the tumor ectopic bone formation as shown by radiography and micro-CT. Overall, the present work demonstrates that the dual PI3K/mTOR inhibitor NVP-BEZ235 represents a promising drug in the therapeutic arsenal against osteosarcoma.

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### PP141

**Therapeutic interest of Imatinib Mesylate in osteosarcoma**

Bérengère Gobin1,2, Gatien Moriceau1,2, Benjamin Ory1,2, Régis Brion1,2, Françoise Rédini1,2 & Dominique Heymann1,2

1INSERM UMR 957, Nantes, France; 2Laboratoire de Physiopathologie de la Résorption Osseuse et Thérapie des Tumeurs Osseuses Primitives, Université de Nantes, Nantes Atlantique Universités, Nantes, France.

Osteosarcoma is the most common primary malignant bone tumor, characterized by osteoid production and/or osteolytic lesions of bone. A lack of response to anti-tumor drugs is observed, leading to development of metastases and to the patient’s death. Because TGF-β promotes metastases from many solid epithelial tumors, we investigated the effect of inhibitory Smad7 overexpression on osteosarcoma behavior. *In vitro*, using three human osteosarcoma cell lines (HOS, SAOS2 and U2OS), we generated osteosarcoma cell clones constitutively expressing Smad7. By transfection of cells with (CAGA),lux or -800PAI1-lux, by measuring the level of Smad3 phosphorylation, and by measuring the PAI-1, CTGF and COL1A1 expression by qPCR, we demonstrated that the overexpression of Smad7 inhibits the transcriptional response mediated by Smad3/4 in osteosarcoma cell lines. In addition, expression of Smad7 efficiently reduced the capacity of osteosarcoma cells to invade Matrigel in Boyden migration chambers. Gelatin zymography identified reduced MMP-2 secretion by Smad7-expressing osteosarcoma cells as compared with their control counterparts. *In vivo*, using a xenograft model of osteosarcoma induced by parabola injection of HOS or SAOS2 cells overexpressing Smad7 in mice, we showed that inhibition of the TGF-β signaling pathway significantly slows primary tumor growth and increases mice survival. The microarchitectural parameters which were assessed by radiography and by microscanner analysis showed an increased trabecular bone volume when Smad7 was over-expressed. In addition, Smad7 overexpression in osteosarcoma cells inhibits the development of lung metastasis. These results suggest that the inhibition of TGF-β signaling pathway could be a new therapeutic strategy against the tumor progression of osteosarcoma.

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### PP143

**Bone remodelling in patients with an IgM monoclonal gammapathy (Waldenström disease – MGUS)**

Daniel Chappard1, Béatrice Bouvard1,2, Mathieu Royer2, Emmanuel Hoppé2, Erick Legrand1,2, Norbert Irihaj1,2 & Maurice Audran1,2

1Germ–Lhea, Iris - Ibs; CHU d’Angers, Angers, France; 2Germ – Rheumatology Unit, Angers, France; 3Hematology Unit, Angers, France.

An IgM monoclonal gammapathy (MGUS) is often the first sign of a lymphomonoctytic B-lymphoma (Waldenström macroglobulinemia-WD). Osteolytic lesions can occur in B cell malignancies (WD, hairy cell leukemia, LLC...) but are less frequent than in myeloma. In addition, bone remodeling in WD is poorly understood. However, an osteoporosis is often observed in MGUS patients. We studied a series of bone biopsies performed in patients with an IgM gammapathy by histomorphometry, microCT and immunohistochemistry. All patients had a double tetracycline labeling; identification and counting of mononuclear TRAcP appeared due to microresorption. Bone fragility observed in these patients may reflect the altered osteoclasts was done after TRAcP staining. 45 patients (9 women and 36 men) had a double tetracycline labeling; identification and counting of mononuclear TRAcP appeared due to microresorption. The presence of a significant contingent of small BFR/BV, BFR/BS). The eroded surfaces were increased in almost all patients and were a reduction in bone formation with decreased osteoid parameters, cortical thickness were not significantly decreased. The most typical findings were a reduction in bone formation with decreased osteoid parameters, mineralization surfaces (MS/BS) and bone formation rates (Aj.Ar, BFR/TV, CD45, CD138 and IgM which characterized the lymphoma cells. Trabecular volume and cortical thickness were not significantly decreased. The most typical findings were a reduction in bone formation with decreased osteoid parameters, mineralization surfaces (MS/BS) and bone formation rates (Aj.Ar, BFR/TV, BFR/BV, BFR/BS). The eroded surfaces were increased in almost all patients and appeared due to microresorption. The presence of a significant contingent of small mononuclear TRAcP+ osteoclasts was seen in >80% of patients. MicroCT aspects of trabecular erosion were visible only when the eroded surfaces were considerably increased. During WD, there are marked alterations in bone remodeling characterized by an intense osteoblastic depression associated with microresorption. Bone fragility observed in these patients may reflect the altered bone quality with inability to restore vertebral microdamages due in part to the microresorption associated with a dramatic reduction of the osteoblast activity.

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PP144
Selective BET bromodomains epigenetic signaling inhibition as a therapeutic strategy in primary bone tumors
Francois Lamoureux1,2, Marc Baudhuin1,2, Lidia Rodriguez1,2, Camille Jacques3,4, Martine Berre1,2, James E Bradner3,4, Francoise Redini1,2, Dominique Heymann1,2, & Benjamin Ory1,2
1INSERM, UMR-S 957, 1 Rue Gaston Veil, Nantes, France; 2Physiopathologie de la Resorption Osseuse et Therapie des Tumeurs Osseuses Primitives, Université de Nantes, Nantes Atlantique Universités, EA3822, Nantes, France; 3Department of Medical Oncology, Harvard Medical School, Dana-Farber Cancer Institute, 44 Binney Street, Boston, Massachusetts, USA; 4Department of Medicine, Harvard Medical School, 25 Shattuck Street, Boston, Massachusetts, USA.

Osteosarcoma is the most frequent primary bone tumor that develops mainly in young adults. The survival rate at 5 years is below 30% for patients with poor response to treatment or with metastasis. The histones modifications are of critical importance in maintaining the transcription program of both normal and tumor cells. 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 present the therapeutic opportunity to pharmacologically target the BET bromodomain family in primary bone tumors.

The consequence of this pharmacologic inhibition of bromodomains is a wide gene expression alteration, but it is believed to selectively target malignant cells by disrupting transcription at intense activity, most notably MYC and Runx2 in our model. Considering MYC and Runx2 are of particular importance for the oncogenic potential of primary bone tumors, a therapeutic strategy targeting those networks might be extremely relevant and potent.

In osteosarcoma tumor cell lines, BET inhibitor reduced cell growth in a dose-dependent manner and induced apoptosis with an increase of sub-G1 fraction and PARP cleavage. These biological events were accompanied by decreased proliferation process by disrupting both osteoclast and osteoblast differentiation. In vivo, BET inhibitor (i.p.; 50 mg/kg) significantly inhibits tumor growth by 70% and prolongs survival in both POS-1 syngeneic and HOS xenograft models compared to control. Additionally, these results were accompanied by a decrease of associated bone lesions. These findings demonstrate that dual pharmacologic inhibition of MYC and Runx-2 is achievable through targeting BET bromodomains to treat osteosarcoma.

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PP145
Synergistic anti-tumour effects on human breast cancer cells by mevalonate pathway inhibitors atorvastatin and zoladronic acid
Andy Göbel1, Stefanie Thiele1, Martina Rauner4, Lorenz C Hofbauer1,2 & Tilman D Rachner1
1Division of Endocrinology, Diabetes and Bone Diseases, Department of Medicine III, University Hospital Carl Gustav Carus, Dresden, Dresden, Germany; 2Center of Regenerative Therapies Dresden, Technical University Dresden, Dresden, Dresden, Germany.

Introduction
Bone metastases represent a frequent complication of breast cancer and are characterized by increased tumour-driven activation of osteoclasts and subsequent bone loss. Amino- and acyl-CoA synthetases inhibit osteoclast function and are established therapies of skeletal metastases. Similar to statins, they block the mevalonate pathway and are thought to have direct anti-tumour effects. Here, we report on the anti-tumour potential of a sequential inhibition of the mevalonate pathway by combining atorvastatin and zoladronic acid in human breast cancer cell lines.

Materials and methods
Successful inhibition of the mevalonate pathway using zoladronic acid and atorvastatin was assessed by detection of unprenylated Ras and Rap1A in the hormone-receptor negative MDA-MB-231 breast cancer cell line. Caspase 3/7 activation assay, annexin V/PI staining and detection of cleaved caspase 3 and poly ADP ribose polymerase (PARP) were used to quantify apoptosis.

Results
Atorvastatin and zoladronic acid in concentrations ranging from 1 to 100 μM led to dose-dependent increase of apoptosis (up to sixfold). The observed effects could be reversed by geranylgeranylpyrophosphate, but not by farnesylpyrophosphate. Concordantly, treatment with geranylgeranyl transferase I inhibitor GGTI-298 but not farnesyl transferase I inhibitor FTI-277 evoked apoptosis highlighting that geranylation of proteins is the main affected process. Interestingly, the combination of 10 μM zoladronic acid and 1 μM atorvastatin induced a threefold increase of apoptosis (P < 0.01) and accumulation of cleaved caspase 3 and PARP after 48 h, whereas only mild effects were observed with individual treatment (1.2-fold each). Annexin V/PI staining revealed 4.58% of apoptotic cells upon combination treatment in comparison to single treatment with atorvastatin and zoladronic acid (1.76 and 2.59%).

Conclusion
Our results indicate the mevalonate pathway as a potential therapeutic target that is amenable to a combination of commonly available and approved drugs. Such strategy could be useful to treat breast cancer-induced bone metastases.

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PP146
Metabolomics identifies plasma biomarkers of multiple myeloma development and progression
Elisabetta Mariani1,2, Francesca Fontana1,2, Silvia Mari3, Jose Manuel Garcia Mantegazza1,2, Magda Marcati3, Nicola Napoli1,3, Francesco Camnasio6, Gianfranco Fraschini3, Enrico Caneva3, Roberto Siti6, Giovanni Musco6, Fabio Ciceri1 & Simone Cenci1,2
1San Raffaele Scientific Institute, Milan, Italy; 2Università Vita-Salute San Raffaele, Milan, Italy; 3Dulbecco Telethon Institute, San Raffaele Scientific Institute, Milan, Italy; 4Hematology and Bone Marrow Transplantation Unit, San Raffaele Scientific Institute, Milan, Italy; 5Università Campus Bio-Medico di Roma, Rome, Italy; 6Department of Orthopaedics and Traumatology, San Raffaele Scientific Institute, Milan, Italy; Centro Interdipartimentale Grandi Apparecchiature, Università di Milano, Milan, Italy.

Multiple myeloma is an incurable neoplastic disorder of plasma cells, which invade the bone marrow, secrete monoclonal immunoglobulins, and induce bone lesions, hypercalcemia, anemia and renal failure. The development of myeloma relies on vicious interactions with the bone microenvironment, a deeper knowledge of which is needed to identify prognostic markers and potential therapeutic targets. To achieve an unbiased, comprehensive assessment of the extracellular milieu of myeloma, we performed metabolic profiling of patient-derived peripheral and bone marrow plasma by ultra high performance liquid/gas chromatography and mass spectrometry (UHPLC/GC–MS). Moreover, in order to address the local heterogeneity of myeloma bone disease, we also set up to investigate myeloma lesions by HR-MAS NMR on primary tissue specimens.

In multivariate analyses, UHPLC/GC–MS metabolic profiling of both peripheral and bone marrow plasma successfully discriminated active disease from control conditions (health, MGUS or remission), and correlated with bone marrow plasma cell counts. Independent disease vs control comparisons consistently identified a panel of metabolic alterations hallmarking active disease, including increased levels of the complement C3i peptide, HWE/ASSL, of specific aminoacid metabolites, including sarcosine and hydroxy-kynurenine, and decreased lysophosphocholines. At hoc in vitro tests on cell lines and patient-derived myeloma cells revealed a previously unsuspected trophic function of lysophosphocholines on malignant plasma cells. HR-MS NMR metabolic fingerprinting of primary specimens efficiently matched histological findings, clustering according to tissue identity, with high concentration of lipids in tumor-rich areas, holding prognostic potential.

By providing the first metabolic fingerprinting of the bone marrow environment, our metabolomic study offers relevant information on the complex interactions established by multiple myeloma with the bone marrow environment. In particular, it identifies unanticipated disease markers for development of more accurate early diagnostic strategies, and discloses previously unpredicted pathogenic pathways as possible therapeutic targets.

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PP147
Involvement of the co-receptor RAMP2 in the progression of breast cancer-induced osteolytic lesions
Alfredo Cappariello1, Nadia Rucci2, Mattia Capulli2, Maurizio Muraca2 & Anna Testi2
1Children Hospital Bambino Gesù, Rome, Italy; 2University of L’Aquila, L’Aquila, Italy.

Bone is the primary site of metastasis for breast cancer, which leads mainly to osteolytic lesions. Cancer cells can expand into the bone for their ability to ‘dialogue’ with resident cells, interfering with the physiological processes of bone turnover. In this study, a large-scale analysis comparing gene expression of biopsies of bone and visceral metastases from human breast cancer patients showed that the receptor (G protein-coupled) activity modifying protein-2 (RAMP2) gene, encoding for a co-receptor calcitonin-receptor-like receptor, was overexpressed 2.7-fold in bone metastases. Gene expression also showed a significant increase of components of the RAMP2-pathway, both receptors (calcitonin-receptor-like receptor, +2.08-fold) and ligands (amylase +1.22-fold). To elucidate the potential role of RAMP2 in osteolytic lesions, we stably transduced the human osteotropic breast cancer cell line MDA-MB-231 with RAMP2 (MDA-RAMP2) and found an increased ability of in vitro migration and proliferation, compared to empty vector transfected (MDA-empty) cells. Moreover, osteoclast precursors treated with conditioned medium (CM) from MDA-RAMP2 cells showed a significant increase of osteoclast differentiation (+2.1-fold, P<0.01) and function (pit index: +6.1-fold, P=0.0001) compared to MDA-empty-CM treated preosteoclasts. Semi-quantitative RT-PCR revealed an increase in Rank/Jtrp ratio in primary osteoblasts treated with MDA-RAMP2-CM, indicating further pro-osteoclastogenic action of tumour cells mediated by osteoblasts. We also observed that MDA-RAMP2 cells formed oncospheres larger (+2.61-fold, P=0.04) but less numerous (+2.87-fold, P=0.02) than MDA-empty cells, indicating a reduced stemness in favour of proliferation and differentiation. Finally, in vivo experiments of intratibial injection of MDA-RAMP2 cells in Balb/c nu/nu mice showed an increased osteolytic area (+1.6-fold, P=0.048) compared to MDA-empty cell injected tibias. In conclusion, our data suggest that RAMP2 plays a role in tumour aggressiveness and promotes the growth of cancer cells in bone through their ability to communicate with the resident cells, thus contributing to the osteotropism of breast cancer cells.

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PP149
Cytotoxicity of picocyanobacteria strains of the genera Cyanobium on osteosarcoma cells
Rosário Martins1,2, Margarida Costa1, Mónica Garcia1, Piedade Barros1, João Costa-Rodrigues1, Vitor Vasconcelos1,2 & Maria Fernandes1
1Laboratory for Bone Metabolism and Regeneration, Faculdade de Medicina Dentária, Universidade do Porto, Porto, Portugal; 2CISA – Centro de Investigação em Saúde e Ambiente, Escola Superior de Tecnologia da Saúde do Porto, Instituto Politécnico do Porto, Portugal;

Marine cyanobacteria have been recognized as an important source of bioactive compounds. The cytotoxicity on cancer cell lines has been extensively explored and several cyanobacteria metabolites are already described as potential anticancer compounds or are considered useful templates for the design of new anticancer drugs. The majority of compounds have been isolated from filamentous or colonial cyanobacteria that grow in high densities along shores. In contrast, picoplanktonic forms have rarely been explored since, for these strains, there is a need for culture for biomass production. From our LEGE cyanobacteria culture collection we selected a panel of seven strains of the picocyanobacteria genera Cyanobium in order to explore it’s potential as anticancer agents. Strains were cultured under laboratory conditions. Freeze-dried biomass was extracted using methanol and dichloromethane to a crude extract and then fractionated using hexane, ethyl acetate and methanol. The cytotoxicity of crude extracts and fractions was evaluated in the osteosarcoma cell line MG63 by the reduction of the bromide 3-(4,5-dimethyl-2-tiazol-2-yl)-2,5-difenil-tetrazolio (MTT) and confirmed by the lactate dehydrogenase (LDH) assay. From the results, four of the seven Cyanobium strains were found to induce a significant decrease in cell viability. The highest percentage of inhibition of tumor cells growth was observed with the ethyl acetate, which is therefore, promising in terms of isolation of bioactive compounds.

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PP148
Direct administration of zoledronic acid improves bone structure in local osteoporotic lesion of ovariectomized rats
Yohei Matsuo, Masafumi Kashti, Tsuyoshi Sugiuara, Tokimitsu Morimoto, Hirotsugu Honda, Takashi Kaito, Motoki Iwasaki & Hideki Yoshikawa
Department of Orthopedic Surgery, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka, Japan.

Objective
To examine the efficacy and safety of direct administration of zoledronic acid (ZOL) on local osteoporotic lesion of ovariectomized rats.

Methods
Six weeks later after ovariectomy, 16 6-month-old female s.d. rats were divided into the two groups with no differences of body weight and BMD of the proximal tibia. In the group L, 50 μl ZOL at a dose of 67 μg/kg were locally injected into the bone marrow between the two drilled holes and 50 μl saline was systemically administrated by s.c. injection. In the group S, 50 μl saline was locally injected, and 50 μl ZOL at a dose of 67 μg/kg was systemically administrated. Local osteoporotic lesions induced by ovariectomy (Area 1: cancellous bone area of right proximal tibia between the two holes, Area 2: left side mirror area) were analysed using in vivo micro-CT at 2, 4, 6, and 8 weeks later after administration.

Results
In the group L, BMD of the locally injected Area 1 continuously increased until week 8 (+41%), but BMD increased and stayed constant in the group S (+17%). In the group L, BMD of the Area 2 continuously decreased until week 5 (<12%), but BMD maintained at the pre-treated level in the group S. In the group L, BMD and microstructural parameters of the Area 1 were significantly higher than the group S at week 4, 6, 8, and these parameters of the Area 2 were significantly lower than the group S at week 6, and 8.

Conclusions
ZOL is the most potent bisphosphonate that strongly inhibits osteoclast function with high binding affinities for bone. Taking advantage of these characteristics, we showed that direct administration of high-dose ZOL to local osteoporotic lesion have more beneficial effects on local bone structure than the systemic administration, and have no influence on other bone tissue.

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PP150
Inhibition of osteoclastogenesis by proton pump inhibitors on co-cultures of human osteoclasts and breast cancer cells
Sara Reis1,2, Maria Fernandes1 & João Costa-Rodrigues1
1Laboratory for Bone Metabolism and Regeneration, Faculdade de Medicina Dentária, Universidade do Porto, Porto, Portugal; 2Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal.

Proton pump inhibitors (PPIs) are a class of drugs particularly used in gastric disorders. They promote a decrease on gastric acid secretion by inhibiting the H+/K+ ATPases. Osteoclasts are cells specialized in bone resorption by H+ translocation to the bone surface. Thus, PPIs might be regarded as potential tools to modulate osteoclast resorption activity, particularly in conditions that are associated with a hyperactivation of osteoclasts, like it happens, in bone osteolytic metastasis. Breast cancer is one of the most frequent tumours that originate bone metastasis. In this context, this work intended to characterize the effects of three PPIs on human osteoclastogenesis in co-cultures of human osteoclasts and breast cancer cells.

Osteoclastic precursors were isolated from human peripheral blood and were co-cultured with two different breast cancer cell lines (T47D and SK-BR-3). Cell cultures were treated with a concentration range (10-7 to 10-3 M) of omeprazole, esomeprazole and lansoprazole. Cell cultures were characterized throughout a 21-day period for total protein content, tartrate-resistant acid phosphatase (TRAP) activity, TRAP+ multimecelates and the presence of cells with actin rings and expressing vitronectin and calcitonin receptors. The presence of breast cancer cells, particularly T47D cells, greatly induced
osteoclastogenesis. The tested PPIs caused a dose-dependent inhibition of osteoclast development. The osteoclastogenic inhibition was verified at levels higher than 10^{-6} M for the three PPIs. Although the highest concentrations seemed to be toxic for osteoclastic cells, the inhibition observed at lower levels appeared to result from specific effects on the osteoclasts, rather than to a significant decrease on the cellular viability. Taken together, PPIs had the ability to decrease human osteoclastogenesis, when osteoclastic precursors were co-cultured with breast cancer cells. Understanding the subject mechanisms can open new perspectives in the utilization of such compounds in pathological conditions characterized by a hyperactivation of osteoclastic cells. 

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PP151
Troxol inhibits breast cancer bone metastasis and bone destruction through suppression of PGE2 production
Jong-Ho Lee, Hyunil Ha, Won Jong Jin, Sun-Don Kim, Jin Suk Jung, Hong-Hee Kim & Zang Hee Lee
Department of Cell and Developmental Biology, School of Dentistry, Seoul National University, Seoul, Republic of Korea.

The skeleton is a prefered site of metastasis in patients with advanced breast cancer, and bone loss is one of the major complications of breast cancer metastasis. Therefore, prevention of bone metastasis is clinically important. Our previous observation of an anti-osteoclastic activity of Troxol, a vitamin E analogue, led us to investigate whether Troxol could inhibit bone metastasis and bone destruction induced by breast cancer. I.P. administration of Troxol markedly inhibited osteolytic lesions and preserved bone volume in intracardially injected breast tumor-bearing mice. Histological analysis revealed decreased tumor burden as well as reduced osteoclast number by Troxol treatment. In vitro, Troxol inhibited breast tumor-induced prostaglandin E_2 (PGE_2) synthesis and mRNA expression of RANKL in primary osteoblasts. This reduction of RANKL expression was attributed to a decrease in PGE_2 production, because exogenous addition of PGE_2 to the osteoblasts restored the RANKL expression inhibited by Troxol. Also, we found that Troxol decreased the invasiveness of breast cancer cells through down-regulation of the PGE_2 level. The inhibitory effect of Troxol on PGE_2 synthesis as well as osteoclast formation was confirmed in triple cell co-cultures of breast cancer cells, osteoblasts, and bone marrow cells. In line with the in vitro results, in bone marrow fluid, breast tumor-induced PGE_2 production was decreased by Troxol treatment, which resulted in a reduction of osteolysis and preservation of bone volume in an intra-tibal injection experiment. We have identified that Troxol has anti-metastatic and anti-osteolytic activities on breast cancer metastasis to bone through the suppression of PGE_2 production. Therefore, Troxol may be a potent therapeutic agent for patients with bone metastasis of advanced breast cancer.

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PP152
Carcinoid tumors and DXA assessment: a study in 222 menopausal women
Catalina Poiana1,2, Mara Carsoțe1,2, Rodica Petris1,2, Raluca Trifinescu1,2, Gabriela Voicu1 & Diana Paun1
1Davila UMPh, Bucharest, Romania; 2Parhon Institute, Bucharest, Romania.

Introduction
The bone mineral density loss may be related to cancer. A specific correlation in the neuroendocrine tumors (NET) is not fully described yet.

Aim
The analyze DXA in patients with or without NET.

Material and method
We performed central DXA (spine and hip) with a GE Lunar device in post-menopausal women. None of them were previously treated with anti-osteoporotic drugs. The study groups included women with carcinoid tumors (pathological confirmation of the diagnosis) and clinical symptoms of the carcinoid syndrome. This is a transversal study.

Results
Twenty-two patients with confirmed carcinoid tumors were included. The control group consisted in 200 carcinoid free women. The two groups were age-matched: mean age was 57 vs 56.77 years. The two groups were BMI - matched: mean BMI was 23.61 vs 24.01 kg/m^2. The mean BMD was: 1.006 vs 1 g/cm^2. No statistically significant difference was registered between lumbar BMD (P=0.9). In carcinoid group the WHO categories (normal DXA /osteopenia /osteoporosis) were: 34% / 46% / 20%, while in control group: 25% / 52.5% / 22.5%.

Discussion
The interpretation of this data is limited to the small number of women with carcinoid tumors.

Conclusions
Based on our observations, the DXA-BMD was not statistically significant different in women with carcinoid tumors to non-carcinoid tumors patients. Probably the skeletal assessment is these women is a multi-factorial equation, including not only age, BMI, BMD but different other parameters as 25-OH vitamin D levels, etc.

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PP153
The DXA results in 41 patients with neuroendocrine tumors: a transversal study
Mara Carsoțe1,2, Andreia Geleriu2, Roxana Duscaean2, Roxana Miron3, Cristina Enescu4, Valentin Radu1, Gabriela Voicu1 & Catalina Poiana1,2
1Davila UMPh, Bucharest, Romania; 2Parhon Institute, Bucharest, Romania; 3Constanta Hospital, Constanta, Romania.

Different results might be registered in DXA assessment in patients with neuroendocrine tumors (NET) since various factors induce bone disturbances as bone metastases, vitamin D hypovitaminosis, etc.

Aim
The analyze DXA in NET.

Material and method
The patients (p) were evaluated between 2008 and 2013. The diagnosis of NET was histological confirmed. We also included medullar thyroid cancer (MTC) with distance metastases and carcinoid syndrome. The WHO/ENETS classification was used for NET grading. The central DXA (GE Lunar device) was used. This is a pilot transversal study. The informed consent of the patients was obtained.

Results
41 NET p were included: 24 women and 17 men. The mean age was: 56.5 years. The most frequent type of NET were: 44% G1, 27% G2 and 29% G3. The most frequent primary NET sites were: unknown 19%, lung 14% and MTC 13%. The mean time between NET confirmation and DXA was 14.54 months. The mean age was 57 vs 56.77 years. The two groups were BMI – matched: mean BMI was: 23.61 vs 24.01 kg/m^2. The mean BMD was: 1.006 vs 1 g/cm^2. No statistically significant difference was registered between lumbar BMD (P=0.9). In carcinoid group the WHO categories (normal DXA /osteopenia /osteoporosis) were: 34% / 46% / 20%, while in control group: 25% / 52.5% / 22.5%.

Discussion
Based on our observations, the DXA-BMD was not statistically significant different in women with carcinoid tumors to non-carcinoid tumors patients. Probably the skeletal assessment is these women is a multi-factorial equation, including not only age, BMI, BMD but different other parameters as 25-OH vitamin D levels, etc.

Conclusion
The BMD decreased in the groups of NET from G1 (the best prognosis tumors) to G3 and then G3 NET (the most aggressive tumors). 1\% of women or men have low BMD.

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PP154
miR-192 impairs invasion and tumor-induced osteolysis by repressing CCL2 in bone metastatic colonization
Karmele Valencia1, Diego Luis-Ravelo1, Nicolas Bovy2, Susana Martinez-Carrazia1, Cristina Ormaza1, Carolina Zuñiga1, Iker Antón1, Ingrid Struman1, Sébastien Tably1, Victor Segura1, Javier De Las Rivas1 & Eva Bandrés1
1Center for Applied Medical Research, Pamplona, Spain; 2University of Liègè, Liègè, Belgium; 3University of Salamanca, Salamanca, Spain.

Emerging evidence suggests that miRNAs (miR) can modulate a complex gene network in a cell-intrinsic and non-cell autonomous manner. We previously identified by transcriptomic analysis miR-192 to be heavily downregulated in different highly metastatic subpopulations (HMS) isolated from bone metastases in a lung cancer mouse model, but its mechanistic contribution to the prometastatic activity remains unknown.

To delineate the pleiotropic functions elicited by miR-192 in metastatic activity we retrovirally overexpress miR-192 in HMS. Forced expression of miR-192 led to stunted decrease invasiveness in Boyden chamber assay and a diminished metatropic osteolytic activity using a fluorogenic assay as compared to mock transduced cells. Next, we inoculated miR-192 overexpressing cells by i.c. injection in nude mice. Animals inoculated with miR-192 cells showed a dramatic decrease in bone tumor burden assessed by bioluminescence imaging (BLI) and a marked reduction in osteolytic lesions assessed by X-rays and μCT scans as compared to mock cells. In contrast miR-192 was ineffective decreasing cell proliferation and subcutaneous tumor growth. To explore its role in bone colonization, we intratibially injected miR-192 overexpressing cells. In agreement with previous findings, miR-192 tumors showed a significant decrease in BLI indicating a marked decrease in tumor burden as compared to mice injected with mock cells. Interestingly, immunohistochemical analysis showed no in vivo effect of miR-192 on growth kinetics and apoptosis. Moreover, the number TRAP+ cells at tumor-bone interface were impaired in mice i.e. injected with miR-192 cells. Transcriptomic analysis identified the pro-osteoclastogenic cytokine CCL-2 as a factor severely repressed in miR-192 derived tumors. This finding was validated by real time PCR. Consistently, incubation with conditioned medium derived from miR-192 tumor cells showed a decreased TRAP+ cells in vitro. Thus, miR-192 appears bone metastatic colonization by a novel mechanism involving tumor cell-dependent functions and non-cell autonomously regulating tumor-induced osteolysis.

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PP155
Influence of sex steroids on sclerostin levels in patients with prostate cancer
Manuel Muñoz-Torres1, Rebeca Reyes-Garcia1,2, Beatriz Garcia-Fontana1, Sonia Morales-Santana1,2, Mariela Varsavsky1, Maria Dolores Aviles-Perez1 & Antonia Garcia-Martin1
1Bone Metabolic Unit, Endocrinology Division, Hospital Universitario San Cecilio, RETICEF, Granada, Spain; 2Endocrinology Unit, HGU Rafael Méndez, Murcia, Spain; 3Proteomic Research Service, Hospital Universitario San Cecilio, Granada, Spain.

There is increasing evidence for the key role of osteocytes in the regulation of bone remodeling. One of the main products of these cells, sclerostin, inhibits bone formation and may also stimulate bone resorption. To our knowledge, there are few data in prostate cancer (PC) patients especially in patients with hypogonadism related to androgen deprivation therapy (ADT). The aim of this study was to compare serum levels of sclerostin in ADT-treated and untreated PC patients with healthy controls, and to evaluate their relationship with sex steroids and bone metabolism. Our study was a cross-sectional one including 81 subjects: 25 patients with PC treated with ADT, 34 PC patients without ADT treatment, and 22 healthy controls. We measured serum sclerostin concentrations, bone turnover markers and BMD in all subjects, and also sex steroids levels in PC patients. The corresponding adjusted OR for vitamin D deficiency was 3.02 (1.90 to 4.86) (P<0.001). A significant association between vitamin D and risk of postmenopausal breast cancer was found: adjusted OR for vitamin D deficiency was 3.02 (1.90 to 4.86) (P<0.001). The corresponding adjusted OR for 25(OH)D concentration was 0.93 (0.91 to 0.95) (P<0.001) per each ng/ml increment in serum 25(OH)D. A borderline interaction between age and 25(OH)D (P=0.05) was detected. When stratified by median age we found an OR of 3.30 (1.71 to 6.46) among the older (aged 60 years or above), compared to an OR of 2.87 (1.50 to 5.68) for those aged <60 years. Conclusion Our results suggest an inverse association between 25(OH)D serum levels and early breast cancer prevalence among postmenopausal women from a Mediterranean region. In our data, vitamin D deficiency is related to an almost three-fold higher risk of breast cancer.

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PP157
Bone metastatic prostate cancer cells regulate their growth via impairing osteoblast differentiation
Marjolein van Driel1, Iris Robbesom1, Ruben Koster1, Bianca Boers-Sijmons2, Hideki Chiba3 & Hans van Leeuwen4
1Erasmus MC, Rotterdam, The Netherlands; 2Fukushima Medical University, Fukushima, Japan.

Metastases to the bone are the incurable final outcomes of cancer, reducing both length and quality of life in an aggressive way. Despite the discoveries of many
PP158
Role of receptor activity modifying proteins in skeletal regulation
Suiruchi Pacharne1, Gareth Richards1, Ning Wang1, Timothy Skerry2 & Kathleen Caron1
1University of Sheffield, Sheffield, UK; 2University of North Carolina, Chapel Hill, North Carolina, USA.

Receptor activity modifying proteins (RAMPs 1, 2 and 3) are a class of important accessory proteins that interact and regulate several G-protein coupled receptor (GPCR) activity by finely modulating ligand interaction and in some cases trafficking receptors to cell surface. Predominant roles of RAMPs include ligand selectivity in receptors for calcitonin receptor (CT) family of peptides that comprise calcitonin, calcitonin gene related peptide, amylin and Adrenomedullin. Functional receptors to these peptides result from heterodimer formed between a RAMP and CT receptor or calcitonin-like receptor.

To test our hypothesis that altering RAMP expression alters skeletal phenotype; we conducted skeletal analysis of RAMP1/2/3 transgenic mice. We observed that RAMP2−/− mice are not viable, but heterozygotes exhibit a haploid insufficiency phenotype with aberrant endochondral and in the skeletus, thinner cortices than WT controls. Whereas, RAMP3−/− (R3KO) mice had a significant increase in cortical thickness and bone volume.

MicroCT analysis of postnatal day 5 (WT: n = 14, R3KO: n = 15), 27-day-old mice (WT: n = 16, R3KO: n = 15), and 8 week old (WT: n = 6, R3KO: n = 6) mice revealed an age dependent skeletal phenotype with evidence of accelerated skeletal formation until 27days of age in the R3 KO mice. Dynamic histomorphometry revealed increased bone apposition rate in the endocortical region of tibia at 8 weeks. Ovariotomy at the age of 12 weeks showed significant increase in trabecular pattern factor and thickness of tibia of R3 KO's. Primary osteoblast cultures from neonatal calvaria revealed significant increase in total β-catenin in R3 KO cultures.

Our data provides evidence to significant role of R3 in skeletal regulation and suggests that data implicating R3 as an early response gene in Wnt stimulation in cancer cells has wider physiological consequences and is an interesting drug target.

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PP159
A novel antagonist of the canonical Wnt-signalling pathway, Sostdc1, is expressed in experimental models of myeloma and suppresses bone formation
Clive Buckle1, Zahra Farahab2, Michelle Lawson3, Colby Eaton2, Karin Vanderkerken2 & Peter Croucher1
1Department of Oncology, Faculty of Medicine, Dentistry and Health, University of Sheffield, Sheffield, UK; 2Department of Human Metabolism, Faculty of Medicine, Dentistry and Health, University of Sheffield, Sheffield, UK; 3Department of Haematology and Immunology, Vrije Universiteit Brussel (VUB), Brussels, Belgium; 4Garvan Institute for Medical Research, Sydney, New South Wales, Australia.

Introduction
Patients with multiple myeloma (MM) commonly present with devastating bone disease mediated by increased bone resorption and suppressed bone formation.

We have previously shown that blocking activity of the Wnt antagonist Dkk-1 promotes osteoblastogenesis and inhibits development of bone lesions in experimental models of MM. In the 5T murine models of MM, tumour cells home to the bone marrow. Injection of 5T2MM cells into C57BL/6 mice results in bone disease whereas injection of 5T3MM cells does not. Microarrays revealed that the Wnt antagonist, Sostdc1, is significantly upregulated in 5T2MM-bearing animals (+ 4.6-fold, FDR < 0.005), compared to 5T3MM-bearing mice. We hypothesise that elevated levels of secreted Sostdc1 in the bone microenvironment reduce osteoblastogenesis and bone formation, and that this contributes to the bone disease associated with MM.

Methods
Six-week-old mice were injected subcutaneously, above the calvaria, with rhSOSTDC1 or vehicle and skulls were examined using μCT and histomorphometry. In a second study, 9-week-old C57BL/6 mice received intravenous rhSOSTDC1 or vehicle and tibiae were examined, using μCT and both static and dynamic histomorphometry.

Results
In the initial study, μCT analysis of calvariae revealed a reduction in bone volume, which was accompanied by a significant reduction in osteoblast (OB) number (P < 0.05) and perimeter (P < 0.05). In the second study, reduced tibial bone volume was accompanied by significantly reduced OB number (P < 0.01) and OB perimeter (P < 0.01) in treated animals. In addition, rhSOSTDC1-treated animals exhibited reduced bone formation and significantly reduced mineralisation rate (P < 0.05). Interestingly, no effect on osteoclast number was observed in either study.

Conclusion
These data suggest that Sostdc1 is a significant inhibitor of OB activity in vivo. Together with separate studies, which demonstrate that rhSOSTDC1 inhibits Wnt- and BMP-induced OB differentiation in vitro, they suggest that blocking myeloma-derived/d-induced SOSTDC1 may be of therapeutic value in patients with myeloma bone disease.

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PP160
Glucose ceramide synthase inhibitors prevent osteoclast activation and limit myeloma-induced osteolytic lesions
Adel Ersic1, Ke Xu2, Anastasios Karadimitris3 & Nicole J Horwood1
1University of Oxford, London, UK; 2Imperial College London, London, UK.

Glycosphingolipids (GLS) are essential structural components of mammalian cell membranes and lipid rafts that exert pleiotropic effects on cell survival, proliferation, and differentiation. Cancer associated GLS have been shown to promote tumor growth, angiogenesis, and metastasis; however their role in osteoclast (OC) activation and the development of osteolytic bone diseases such as multiple myeloma are not known. We investigated the hypothesis that GLS contribute to OC activation and inhibitors of GLS biosynthesis would antagonise GLS-dependent osteoclastogenesis.

Exogenous addition of GM3, the prevailing GSL produced by myeloma plasma cells, synergistically enhanced the ability of the pro-osteoclastogenic factors RANKL and IGF1 to induce the maturation of OC in vitro. However, these effects were inhibited by the glycosphingolipid synthase inhibitor N-butyl-deoxynojirmycin (NB-DNJ). In vivo administration of GM3 increased OC numbers and activity; this effect was reversed by treatment with the GLS synthase inhibitor NB-DNJ. NB-DNJ prevented OC development and activation by disrupting RANKL-induced localisation of TRAF6 and c-Src into lipid rafts thus attenuating MAPK signalling.

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To prove the therapeutic potential of NB-DNJ, the STGMI mouse model of multiple myeloma was used and we were able to demonstrate a significant improvement in bone parameters compared to the PBS treated mice. These data demonstrate a novel role for tumor-derived, as well as de novo-synthesized GSL in OC differentiation and activation and suggest that glycosphingolipid synthesis inhibitors, such as the clinically approved NB-DNJ, may be beneficial in reducing OC activation and bone destruction associated with multiple myeloma.

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**PP161**

**Effect of zoledronic acid on bone mineral density in premenopausal women receiving neoadjuvant or adjuvant therapies for breast cancer: the ProBone II Study**

Peyman Hadji1, Anette Kauka1, Thomas Bauer1, Katrin Birkholz2, Monika Baier1, Mathias Muth Muth1 & May Ziller1

1Philipps-University of Marburg, Universitätsklinikum Giessen und Marburg, Marburg, Germany; 2Novartis Pharma GmbH, BU Oncology, Nuenberg, Germany.

**Introduction**

Bone mineral density (BMD) evaluations have shown that adjuvant chemother-apy or endocrine therapy (ET) for early breast cancer (BC) is associated with accelerated BMD loss and increased fracture risk. In recent studies, zoledronic acid (ZOL) increased BMD in premenopausal and postmenopausal women with BC, and improved disease-free survival in some patient subsets compared with no ZOL. The purpose of the current study was to investigate the effect of adjuvant treatment with ZOL on BMD in premenopausal women with early BC treated with chemotherapy or ET.

**Methods**

In this randomized, double-blind, placebo-controlled study, 71 patients receiving adjuvant chemotherapy and/or ET were randomly assigned to also receive ZOL (4 mg i.v. q 3 months) or placebo for 24 months. The primary endpoint was change in BMD at lumbar spine (LS) at 24 months relative to baseline. Secondary endpoints included change in femoral neck and total femoral BMD, course and change in bone turnover marker levels, assessment of potential correlations between BMD and bone turnover, development of metastases, pathologic fractures, and safety and tolerability.

**Results**

At 24 months, LS BMD substantially increased (+3.13%) with ZOL, and decreased (−6.46%) with placebo relative to baseline (P < 0.001, between-group comparison). Femoral neck and total BMD also increased with ZOL, vs decreases with placebo at 24 months relative to baseline (P < 0.001, between-group comparisons). By month 3, mean bone marker levels decreased (−65% for C-telopeptide of type I collagen and −61% for N-terminal propeptide of type I procollagen, relative to baseline) with ZOL, with significant (P < 0.001) between-group differences in levels of both bone markers at 24 months vs baseline. Overall, ZOL was well tolerated, and only one case of osteonecrosis of the jaw was reported.

**Conclusions**

Early initiation of ZOL is well tolerated and preserves BMD and reduces bone turnover biomarker levels in premenopausal women with early BC undergoing chemotherapy or ET.

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**PP162**

**Effect of zoledronic acid on bone metabolism in patients with bone metastases from prostate or breast cancer: the ZOTECT Study**

Peyman Hadji1, May Ziller1, Tobias Maurer2, Michael Auenterrih1, Mathias Muth1, Amelia Ruebel1, Christoph May2, Katrin Birkholz2, Erhardt Diebel1, Iochen Giesseuer1, Peter Rothe1 & Haugen E Giswendi1

1Philippus-University of Marburg, Universitätsklinikum Giessen und Marburg, Marburg, Germany; 2Urologische Klinik, Klinikum rechts der Isar, Muenchen, Germany.

**Introduction**

The prospective, single-arm, open-label ZOTECT study was designed to assess the effect of zoledronic acid (ZOL) on bone-marker levels and potential correlations with disease outcomes in bisphosphonate-naive patients with bone metastases.

**Methods**

Patients with bone metastases from prostate cancer (PC; n = 301) or breast cancer (BC; n = 99) who have not received bisphosphonates for ≥6 months were enrolled at 98 sites in Germany (from May 2006 to July 2008). Patients received ZOL (4 mg i.v. every 4 weeks for 4 months, with a final follow-up at 12 months). The primary endpoint was change in bone marker levels at 12 months relative to baseline. Secondary assessments included skeletal-related event (SRE) rate, pain, quality of life (QoL), and prostate-specific antigen (PSA) levels. Endpoints were assessed using summary statistics by visit/tumor (type and Kaplan–Meier analyses).

**Results**

ZOL treatment significantly decreased bone-marker levels vs baseline (aminoterminal propeptide of type I collagen (PINP), C-terminal cross-linking telopeptide of type I collagen (CTX); P < 0.0001), and this decrease was maintained through the final 1-year follow-up visit. Baseline PINP and CTX levels correlated with the extent of bone disease (P < 0.0001, each) and on-treatment decreases in marker levels. Skeletal disease burden and bone-marker levels were similar between PC and BC patients, and ZOL did not significantly influence osteoprotegerin/receptor activator of nuclear factor-κB ligand levels. During the 12-month period, only 13 SREs occurred in 11 patients. On-treatment bone-marker level changes did not correlate with SRE rate, pain scores, or QoL. Mean PSA levels were lower at study end (120 days; 92.5 μg/l) than at baseline (168.5 μg/l; Wilcoxon’s signed-rank test, P = 0.27). In general, ZOL was well tolerated and adverse events were consistent with its established safety profile.

**Conclusions**

This study confirms that ZOL therapy significantly reduces bone turnover (measured as PINP and CTX levels) in patients with bone metastases from PC or BC.

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**Cell biology: osteoblasts and bone formation**

**PP163**

**Hepatic lipase is expressed by osteoblasts and modulates bone remodeling in obesity**

Alexander Bartelt1, F Timo Beil1, Brigitte Müller1, Till Kohne1, Markus Heine1, Tayfun Yilmaz2, Joerg Heeren1, Thorsten Schinke1 & Andreas Niemeier1

1University Medical Center Hamburg-Eppendorf, Hamburg, Germany; 2University Medical Center, Freiburg, Germany.

Here we identify the lipolytic enzyme hepatic lipase (HL, encoded by Lepc) as a novel cell-autonomous regulator of osteoblast function. In an unbiased genome-wide expression analysis, we find Lepc – which was formerly thought to be expressed almost exclusively by the liver – to be highly induced upon osteoblast differentiation, as verified by quantitative Tagman analyses of primary osteoblasts in vitro and of bone samples in vivo. Functionally, loss of HL in vitro leads to increased expression and secretion of osteoprotegerin (OPG), while osteoblast differentiation is mildly impaired. When challenging energy metabolism in a diet-induced obesity (DIO) study, lack of HL leads to a significant increase in bone formation markers and a decrease in bone resorption markers. Accordingly, in the DIO setting, we observe in Lepc−/− animals but not in wild-type controls a significant increase in lumbar vertebral trabecular bone mass and an increase in bone formation rate. Taken together, here we demonstrate that HL expressed by osteoblasts has an impact on osteoblast OPG expression and that lack of HL leads to increased bone formation in DIO. These data provide a novel and completely unexpected molecular link in the ever more complex interplay of osteoblasts and systemic energy metabolism.

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**PP164**

**Histologic evaluation of direct pulp capping by the using of calcium hydroxide and octacalcium phosphate in dental pulp of cats**

Fereydoon Sargolzaee-aval, Mohammad Reza Arab & Eshagh Ali Saberi

Zahedan University of Medical Sciences, Zahedan, Shstan and Baluchestsn, Iran.

**Objective**

The aim of this study was to evaluate the pulpal responses to octacalcium phosphate (OCP) and calcium hydroxide (CH) used as direct pulp capping (DPC) materials.

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Study design
The pulp of 72 premolars of nine cats were selected for this experiment. After the cats had been anesthetized, the teeth were exposed and capped directly with OCP, CH, and no capping materials used as control group. The cavities of all three groups were filled with glass ionomer cement (GI). Histological evaluations were performed at 2, 4, and 8 weeks after the pulp capping. The results were analyzed statistically by using the Mann–Whitney U and $\chi^2$ test ($P<0.05$).

Results
Two weeks after the pulp capping, all specimens in three groups showed mild to severe inflammation. The formation of the hard tissues (dentinal bridge) initiated in the exposed areas of the experimental groups that was more noticeable in the calcium hydroxide than in the octacalcium phosphate group. These differences was statistically significant ($P<0.001$). At 4 weeks, hard tissues were observed in both groups which was more evident in the CH group and there was statistically significant differences between two experimental groups same as the 2 weeks.

At 8 weeks, continuous hard tissues were observed more frequently in the OCP group. In vivo, APC increased BV in ectopic bone pellets by 73% ($P<0.01$) and TV by 104% ($P<0.001$) but did not alter BV/TV. APC inclusion led to 20% enhancement of Oc.N ($P<0.05$), suggesting that APC was pro-anabolic and not anti-resorptive. In vitro, APC increased MTT incorporation over 72 h by 15% ($P<0.05$) and similarly increased cell count. APC stimulated pERK activation and calcium deposition. PAR1 and PAR2 were expressed by MG-63 cells, and PAR antagonists abolished all effects of APC.

Conclusion
APC increased rhBMP2 induced ectopic bone formation, consistent with the results of increased osteoblast proliferation and matrix mineralization in cultured cells. PAR antagonists blocked the effects of APC, suggesting PAR1 and PAR2 directly mediate the effects of APC on bone.

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PP167
Adipogenesis occurs at the expense of osteoblast differentiation in primary osteoblasts deficient in protease-activated receptor 2
Fereydoon Sargolzaei Aval, Mohammad Reza Arab & Forough Sargolzaei Aval
Zahedan University of Medical Sciences, Zahedan, Sistan and Baluchestan, Iran.

Background and aim
This study was designed to investigate the process of bone formation caused by implantation of octacalcium phosphate (OCP) at alveolar ridge.

Materials and methods
In this descriptive study we used 20 male Sprague–Dawley rats. Synthetic OCP was implanted into the bony defect measuring 3 mm in diameter and 2 mm in depth was surgically created with a bur in the rat mandible. Bone formation at the alveolar ridge was examined histologically between 1 and 4 weeks after implantation.

Results
Osteogenesis was initiated on the center of the defect between the OCP particles and multinucleated giant cells appeared on the implanted materials in 1 week. More apposition of new bone was observed on the implanted materials in week 2. In addition to bone formation locally around the OCP particles, more apposition of new bone was observed near the defect margin in week 3. At week 4, the defect was almost completely filled with bone, which was in close contact with host bone and implanted OCP was surrounded by newly formed bone. In the control group, bone formation was observed only along and near the defect margin.

Conclusion
The present results demonstrate that OCP could be used to enhance atrophic alveolar ridge for filling a tooth socket after extraction.

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PP168
Distinct potential of osteoblast differentiation of adipose tissue- and bone marrow-derived mesenchymal stem cells
Rodrigo Abuna, Fabiola de Oliveira, Rogerio Kato, Adalberto Rosa & Marcio Beloto
Cell Culture Laboratory, School of Dentistry of Ribeirao Preto, University of Sao Paulo, Ribeirao Preto, Sao Paulo, Brazil.

Adult mesenchymal stem cells (MSCs) are of interest in the fields of cell therapy and tissue engineering thanks to their potential of differentiating into distinct cell lineages, e.g. osteoblast, chondrocyte, myoblast, and adipocyte. As the capacity of differentiation may vary according to the cell source, here, we compared the potential of osteoblast differentiation of MSCs derived from either bone marrow or adipose tissue. MSCs from rat bone marrow and adipose tissue were cultured...
under osteogenic conditions for periods of up to 17 days. Cell proliferation was evaluated by counting the number of cells using an automated cell counter, extracellular matrix mineralization by Alizarin Red Staining, and gene expression of key bone markers by real-time RT-PCR. Data were obtained in triplicate (n = 3) and compared by Mann-Whitney U test (p < 0.05). Cell proliferation was higher in cultures from bone marrow compared with adipose tissue at days 4, 10, and 17 (p < 0.05). At day 17, we noticed more extracellular matrix mineralization in cultures from bone marrow compared with adipose tissue (p < 0.05). Gene expression of Runx2, collagen type 1, alkaline phosphatase, bone sialoprotein, and osteocalcin was higher in cultures from bone marrow compared with adipose tissue at days 4, 10, and 17 (p < 0.05). We have shown the higher potential of proliferation and osteoblast differentiation of bone marrow MSCs compared with adipose tissue MSCs under the same osteogenic culture conditions. These findings indicate that MSCs source is of relevance and that bone marrow MSCs should be chosen for future research on cell therapy and bone tissue engineering.

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PP169
Increase of mineral nodules and alkaline phosphatase levels in osteoblasts cultures by using disordered carbon nanotubes and titanium discs
Daniela Cervelle Zancanela, Ana Maria Sper Simão, Elaine Yoshiko Matsubara, José Maurício Rosolen & Pietro Ciancaglini
Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, FCLRP-USP, Ribeirão Preto, São Paulo, Brazil.

Biological calcification is a regulated process in which different types of tissues, cells and biomolecules participate in the coordination and regulation of the metabolic events involved in accumulating large amounts of calcium phosphate. This process could be speeded up using carbon nanotubes (CNTs) systems. The objective of our study was to compare cell growth and formation of mineralized matrix nodules by osteoblasts grown in plastic and in titanium (Ti) discs surfaces. The four sets of CNTs with diameter distribution size and disorder relatively large were prepared employing (Co, Mn) and (Fe) as catalysts, two sources of carbon precursors (methanol and ethanol) and NaCl substrate. Alkaline phosphatase activity and formation of mineral nodules were evaluated after addition of CNTs in different phases of cell growth. Better results for alkaline phosphatase activity and formation of mineral nodules were obtained when the cells were incubated with CNTs prepared with set (Fe, methanol) or (Co/Mn, ethanol) mainly in the presence of Ti surface. For alkaline phosphatase activity, CNT (Fe, methanol) showed 35% of increase in the intermediate phase of growth and 13% in the stationary phase, and CNT (Co/Mn, ethanol) showed 54% of increase in the intermediate phase of growth and 26.7% in the stationary phase, when compared to the control. Observing the Ca/Pt molar ratios, the values closer to the hydroxyapatite ratio (1.666) were obtained for CNT (Fe, methanol) (1.95) and CNT (Co/Mn, ethanol) (1.64) in the presence of Ti surface, showing a great possibility of hydroxyapatite formation in these nodules. This study provides information for the application of different types of CNTs associated with Ti in processes of biomineralization stimulation, suggesting that depending on the CNT type there is an interaction between CNTs and Ti that favors the formation of mineral nodules on Ti surface.

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PP170
Calcium transport and phosphomonohydrolase activity by proteoliposomes harboring annexin V and alkaline phosphatase
Mayté Boleán1,2, Ana Maria Sper Simão1,2, Tánia Kiffer-Moreira2, Marc Hoyaerta5, José Luis Millán1,2 & Pietro Ciancaglini1,2
1FFCLRP-USP, Ribeirão Preto, São Paulo, Brazil; 2Sanford-Burnham Medical Research Institute, La Jolla, California, USA; 3University of Leuven, Leuven, Belgium.

The biomineralization process is initiated inside matrix vesicles (MV), with phosphate and calcium ions crystalizing as hydroxyapatite. This process is accomplished by the activities of several proteins, such as annexins (e.g. Annex V) that mediates Ca2+ influx into MVs and tissue-nonspecific alkaline phosphatase (TNAP), a phosphomonohydrolase that uses ATP and PPi as substrates. Dipalmitoylphosphatidylcholine/choleline (DPPC) and dipalmitoylphosphatidylethamine (DPPS) are found in MVs membranes and play a crucial role in the biomineralization process, regulating both Ca2+ entry into the MVs and formation of hydroxyapatite crystals. We studied the incorporation of Annex V and TNAP into DPPC and DPPC:DPPS (10% molar ratio) liposomes and their ability to transport Ca2+. Proteoliposomes harboring Annex V were reconstituted using 1:100 protein/lipid (molar ratio). When DPPS was used, we had 80% of increase in protein incorporation. Proteoliposomes containing TNAP and Annex V were reconstituted using a 1:15 000 and 1:100 protein/lipid (molar ratio), respectively. The presence of both (70% Annex V and 30% TNAP) into proteoliposomes was confirmed by western blot. The proteoliposomes (10 μg protein) were incubated with a fixed 10 mM Ca2+ concentration (5.5 μM Ca2+) and increasing Ca2+ concentrations (from 1 to 5 mM), resulting in a linear increased uptake, reaching a maximum with 2 mM Ca2+. Around 0.8 μmol Ca2+ was incorporated, with a similar profile for all proteoliposomes curves. The presence of TNAP in the proteoliposomes containing both proteins did not affect significantly Annex V-mediated Ca2+ transport. However, the presence of Annex V affected significantly the hydrolysis of PPi, ATP, and ADP by TNAP. When both proteins are present, the V/m for PPi hydrolysis decreased around 19 times and Km was not affected significantly. For ATP, V/m decreased around seven times and Km also decreased (nine times). Finally, V/m for ADP decreased two times and Km was not affected. These studies will help us in the development of mineralization-competent MV biomimetics.

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PP171
In vitro effect of prolactin on the osteogenic potential of bone marrow mesenchymal stem cells of rats
Natalía de Melo Ocarino, Silvia Silva Santos, Lorena Rocha, Joneu Freitas, Amanda Maria Serafim Reis & Rogério Serakides
Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.

The effects of prolactin on bone metabolism have been the subjects of several studies. It is believed that prolactin acts directly influencing the synthesis of bone matrix by stimulating the osteoblastic activity, since receptors for this hormone have been identified in osteoblasts and human mesenchymal stem cells (MSCs). However, no study on the effects of prolactin on the osteogenic differentiation of MSCs was found in the literature. The objective of this study was to verify the in vitro effect of prolactin under osteogenic potential of bone marrow mesenchymal stem cells (BMSCs) of young female rats. BMSCs were grown in osteogenic medium and were separated into two groups: i) BMSCs of young rats (control) and ii) BMSCs of young rats treated with prolactin (100 ng/ml). At 7, 14, and 21 days of osteogenic differentiation of BMSCs, 3-4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) conversion, gene expression for collagen I, osteocalcin, osteopontin, BMP-2, osterix and the cells number/field were analyzed. The percentage of mineralized nodules was analyzed at 21 days. The addition of prolactin in the BMSCs culture increased the expression of osteons at 7 days and alkaline phosphatase at 14 days. However the expression of osteopontin in the prolactin group was lower at 21 days when compared to the control group. Expression of BMP-2, osteocalcin, type I collagen was not different between groups. Also no significant difference between groups in the conversion of MTT into formazan crystals, cell number and percentage of mineralized nodules. It was concluded that the prolactin in a dose of 100 ng/ml does not alter the osteogenic potential of BMSCs of young female rats.

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PP172
Differential gene expression of matrix metalloproteinases (MMPs), MMP inhibitors (TIMPs and RECK), and MMP-activator (EMMPRIN/CD147) during osteogenic differentiation from human dental pulp stem cells
Katiucia Paiva1, Luiz Silva1,2 & Mari Sogayar1
1Department of Biochemistry, Chemistry Institute, University of São Paulo, São Paulo, São Paulo, Brazil; 2Department of Oral Pathology, Dental School, University of São Paulo, São Paulo, São Paulo, Brazil.

Constant remodeling of extracellular matrix (ECM) 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. MMPs, MMP-activator (EMMPRIN/CD147) and MMP-inhibitors (TIMPs and RECK) are responsible for bone matrix remodeling and, probably, determine the level of its turnover. Mesenchymal stem cells derived from dental pulp are multipotent and have the capacity to differentiate into several mesenchymal
tissues, such as bone, fat and cartilage, under inductive conditions in vitro. However, it is unknown In this study, we evaluated differential gene expression of MMPs (25 members), TIMPs (four members), RECK, and MMPRIND/CD147 of dental pulp stem cells (DPSCs) exposed to osteogenic induction. 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 mM dexamethasone, and 50 μg/ml ascorbate) for 35 days. We measured bone formation markers (osteoclastin, alkaline phosphatase, and mineral nodules) using western blot, colorimetric assay and Alizarin Red S dye, respectively, and gene expression by qRT-PCR. After osteogenic differentiation, bone formation markers, matrix mineralization and differential gene expression were observed. This is the first evidence that MMPs, TIMPs, RECK, and MMPRIND/CD147 are differentially expressed in osteoblast differentiation from DPSCs in vitro.

Keywords
Dental pulp stem cells, MMP, TIMP, RECK, MMPRIND/CD147, and Osteoblast Differentiation.

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**PP173**

Exogenous polyphosphate is not readily utilized for mineralization in vitro

Marianne Ariganello1, Sidney Omelon2, Rima Wazen1, Fabio Vario1 & Antonio Nanci1

1Université de Montréal, Montréal, Québec, Canada; 2University of Ottawa, Ottawa, Ontario, Canada.

Polyphosphates (polyPs) are inorganic phosphate chains found in many cell types with higher concentrations in bone cells. As a source of inorganic phosphate (Pi) and an effective calcium reservoir due to chelation, PolyPs enable total Ca2+ and PO43− concentrations above those required for apatite saturation. Alkaline phosphatase (ALP) cleaves Pi from polyP, thus polyPs may be involved in apatite mineralization.

**Aim**
To investigate the role of exogenous polyP as a Pi source for mineralization.

**Methods**
We conducted experiments with osteoblastic cells expressing different endogenous ALP levels and also utilized lentiviral vectors (LV) to overexpress the ALP transgene. SAOS-2 cells (high ALP), MC3T3-E1 (typical ALP) and MC3T3-E1 LV-ALP (ALP overexpression) were cultured in the presence of either β-glycerophosphate (βGP) alone or polyP alone.

**Results**
Control (βGP-treated) SAOS-2 cells were von Kossa (VK, Pi staining) and alizarin red (AlzR, Ca-staining) positive. Despite an endogenously high level of ALP expression, SAOS-2 cells treated with polyP did not mineralize, as determined by negative VK and AlzR. PolyP-treated MC3T3s and LV-ALP MC3T3s similarly displayed negative VK staining. However both MC3T3s cell types (control and LV-ALP), when treated with polyP yielded a uniform AlzR stain atypical of the standard punctate AlzR pattern observed with βGP-treated cells. Scanning electron microscopy and energy dispersive X-ray spectroscopy suggest that in these polyP-treated cultures AlzR binds to residual Ca-polyP, not mineral, resulting in non-specific, 'false positive' staining.

**Conclusions**
Our results highlight the caution required when evaluating mineralization with AlzR. They also demonstrate that, under standard cell culture conditions, exogenous polyP does not promote extracellular mineralization, suggesting incomplete metabolism of polyP by ALP and/or their involvement in inhibitory events. Understanding the role(s) of polyP in physiological nucleation and subsequent regular mineral deposition may provide perspective for regulation of normal and pathological mineralization. Supported by CIHR, FRQ QT, NSERC and RBIO-FRQQT.

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**PP174**

Elevated levels of serotonin decrease bone volume by direct effects on bone turnover in rats

Igor Erjavec1, Tatjana Bordukalo-Niksic1, Jelena Brkljacic1, Martina Pauk1, Lovorka Grgurevic1, David D Thompson2, Vishwas M Paralkar2

1Institute of Molecular and Translational and Clinical Research, University of Zagreb, Zagreb, Croatia; 2Laboratory of Molecular Tissues, School of Medicine, Center for Translational and Clinical Research, University of Zagreb, Zagreb, Croatia.

Elevated levels of circulating serotonin have been reported to decrease bone mineral density. Conversely, reduced serotonin (5HT) in mice lacking TPH1, the rate limiting enzyme for 5HT synthesis, was reported to be anabolic to the skeleton with high osteoblastic activity. However, in other studies TPH1 deletion led to either an initial increase in BMD due to inhibition of osteoclastic bone resorption1, or had no bone effect2. To address this issue, we used selective breeding to identify rats with elevated (high-5HT) and low (low-5HT) plasma levels of platelet 5HT and high and low levels of platelet 5HT transporter activity. In high-5HT animals platelet serotonin levels and uptake were about 100% higher than in animals with low 5HT. Serotonin was analyzed with μCT, DEXA, histomorphometry and in vitro methods to evaluate the effects of high and low levels of serotonin on bone tissue.

In high-5HT rats, bone volume was significantly decreased due to increased bone turnover and an enhanced osteoclastogenesis paralleled by increased serum CTX and osteocalcin values. PTH, 1,25(OH)2D3, insulin, FGF23, BMP6, and leptin were similar in the plasma of both groups. Cultured primary osteoblasts and osteoclasts from high-5HT and low-5HT rats produced 5HT and 5HT receptors that can locally regulate bone turnover. These results suggest that systemically elevated 5HT increased bone turnover leading to bone loss. Further research is required to delineate the 5HT role in the skeleton and to determine the role of serotonin on bone metabolism.

**References**

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**PP175**

Identification of a small molecule kinase inhibitor that enhances osteoblast differentiation of human skeletal (mesenchymal) stem cells through regulation of TGFβ signaling

Majken Storm Siersbaek1,2, Abbas Jafari1,2, Walid Zaher1,2, Li Chen1,2 & Moustapha Kassem1,2

1Endocrine Research Laboratory (KMEB), Department of Endocrinology and Metabolism, Odense University Hospital, University of Southern Denmark, Odense, Denmark; 2Faculty of Health Sciences, Danish Stem Cell Center (DanStem), University of Copenhagen, Copenhagen, Denmark.

Identifying novel molecules that enhance human skeletal (mesenchymal) stem cells (hMSC) differentiation into osteoblastic bone forming cells (OB), may lead to development of new bone anabolic drugs. We have identified Kix, a small molecule kinase inhibitor that enhanced hMSC to increase bone formation.

Kix targeted undifferentiated hMSC, effectively inhibited osteoprotegerin (OPG) receptor signaling and thus downregulated RANKL/OPG and p21 and BHLHB2. Furthermore, Kix treatment of hMSC for 15 minutes enhanced canonical TGFβ signaling pathways, e.g. BMP2/4/6, TGFBR1/2/3 and their downstream targets e.g. IGFBP3/5, p21, and BHLHB2. Furthermore, Kix increased in vivo heterotopic bone formation and hMSC survival in an in vivo bone regeneration model of mouse calvarial defect. In order to determine molecular mechanisms, we carried out DNA microarray analysis which revealed that Kix treatment up-regulated gene expression of different components of TGFβ and BMP signaling pathways, e.g. BMP2/4/6, TGFBR1/2/3 and their down-stream target genes e.g. IGFBP3/5, p21, and BHLHB2. Furthermore, Kix treatment of hMSC for 15 minutes enhanced canonical TGFβ signaling, as shown by approximately twofold upregulation of p-smad2 as well as enhancing TGFβ3 effects on p-Smad3. Treatment of hMSC with SB-431542; an inhibitor of TGFβ signaling abolished Kix-mediated increase in alkaline phosphatase (ALP) activity and ex vivo matrix mineralization. Active-site-directed competition kinase binding assay (Discovera Research, University of Zagreb, Croatia) revealed KINOMiX kinase A, C, G, and D, known inhibitors of TGFβ receptor signaling and thus induced in increased TGFβ3 signaling, from a clinical perspective, Kix may represent an attractive molecule for further development for in vivo targeting of hMSC to increase bone formation.

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The effect of fibroblast growth factor 2 on mesenchymal stromal cell differentiation
Tina Kähkönen, Kaisa K Ivaska & Pirikko Härkönen
Cell Biology and Anatomy, Institute of Biomedicine, University of Turku, Turku, Finland.

Mesenchymal stromal cells (MSCs) have a potential to differentiate to osteoblasts and adipocytes. Differentiation can be stimulated or inhibited by different growth factors, including fibroblast growth factors (FGFs). In this study we evaluated the effect of FGF2 on the osteoblastic and adipogenic differentiation of MSCs in vitro. Mouse MSC-derived cells were cultured in differentiation medium that led to differentiation to osteoblasts in 14 days and to adipocytes in 7 days. The cells were treated during the differentiation with FGF2 (25 ng/ml) for 24 h before sample collection (short-term treatment) or continuously through the whole culture period (long-term treatment). Samples were collected for qRT-PCR and western blot analysis.

Both short- and long-term treatment with FGF2 had an inhibitory effect on MSC differentiation. FGF2 decreased osteoblastic differentiation, as evaluated by a decrease in the expression of osteoblast marker genes (collagen I, osteocalcin, alkaline phosphatase, and RUNX2). We also observed changes in the expression of FGF-receptors as the mRNA levels of FGFR2, FGFR3, and FGFR5 were downregulated. Interestingly, the mRNA level of FGFR1 was increased by both treatments. FGF2 treatment also decreased adipogenic differentiation evaluated by the expression of the adipocyte markers (fatty acid binding protein 4 and PPARγ). During adipocyte differentiation FGF2 induced FGF1 expression in undifferentiated cells but decreased it in later stages of differentiation. The mRNA levels of FGFR2, FGFR3, and FGFR5 were downregulated by short- and long-term treatment of FGF2.

We conclude that FGF2 treatment inhibits osteoblastic and adipogenic differentiation of MSCs in vitro. FGF2 inhibition of MSC differentiation was associated with differential alterations of the expression of the FGFs.

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Extracellular glucose alters mesenchymal stromal cell growth and differentiation
Anna-Reeta Virta & Kaisa K Ivaska
Department of Cell Biology and Anatomy, Institute of Biomedicine, University of Turku, Turku, Finland.

Disorders of glucose metabolism are associated with adverse skeletal effects. Hyperglycemia impairs the function of osteoblast-like cells but the mechanisms underlying glucose toxicity are poorly understood. In this study we determined the effect of elevated extracellular glucose levels on the proliferation and osteogenic differentiation of mesenchymal stromal cells (MSC).

Bone marrow cells were isolated from rat long bones, plastic-adherent MSCs were enriched in vitro and differentiated in osteogenic conditions for up to 14 days. Culture medium (containing 5.5 mM glucose) was supplemented with different doses of glucose at various stages of differentiation for 24 h (acute exposure) or continuously through the culture period (chronic elevation). Mannitol was used as iso-osmolar control. Cultures were evaluated for cell viability, glucose utilization, bone formation, and the expression of osteoblast marker genes (Runx2, alkaline phosphatase, and osteocalcin) at the mRNA and protein levels. High extracellular glucose significantly and dose-dependently impaired the proliferation of MSCs (p < 0.001). Chronic exposure to high glucose resulted in reduced number of osteoblasts, as evaluated by alkaline phosphatase activity and osteocalcin secretion (p < 0.001). Extracellular glucose also had an effect on the expression of osteoblast marker genes and glucose utilization during osteogenic differentiation. Treatment of MSCs with an equal concentration of mannitol partially mimicked the effects seen with glucose, but the changes in proliferation and differentiation were observed at higher concentrations than with glucose. This suggests that MSCs are sensitive to osmotic stress during differentiation and it may partially mediate the inhibitory effects of high glucose.

We conclude that MSCs are sensitive to increasing extracellular glucose levels, causing reduced growth and altered differentiation. The findings further suggest that the effects of high glucose may be partially mediated through osmotic response pathways. Modulation of the growth and osteogenic differentiation of MSCs is a potential component of the bone loss associated with hyperglycemia.

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Regulation and function of immunosuppressive molecule human leukocyte antigen G5 in human bone tissue
Frederic Deschaseaux1, Julien Gaillard2, Alain Langonne2, Chantal Chevache, Abderrahim Naji3,4, Annya Bouacida1,3, Philippe Rosset5, Dominique Heymann6, Gonzague de Pinieux6, Nathalie Rouas-Fraissé6 & Luc Sensé6
1Rue de l’Alma, UMR UPS-CNRS 5273, U1031 Inserm, EFS-Pyrénées-Méditerranée 31432, Toulouse, France; 2Laboratoire de Biologie des Cellules et Génomes du Cirque, EA3855, Université François-Rabelais, Tours, France; 3EA 4490, Physiopathologie des Maladies Osseuses Inflammatoires, Lille 2-ULCO, IR114, P RHS Université Lille Nord de France, Boulogne/Mer, France; 4Laboratoire Immuno- Hématoto DSV-DRM, CEA Hôpital St-Louis, Paris, France; 5CHRU de Tours – Université de François-Rabelais de Tours, PRES Centre, Val de Loire Université, Tours, France; 6Inserm UMR957, Physiopathologie de la Résorption Osseuse et Thérapie des Tumeurs Osseuses Primitives, Université de Nantes, Nantes, France.

Bone-marrow mesenchymal stem cells (MSCs) are the origin of bone-forming cells with immunomodulation potential. Among the generated immunosuppressive molecules there is HLA-G. HLA-G proteins play a crucial role in promoting the acceptance of allografts. However, the mechanisms regulating the expression of HLA-G in human MSCs are unknown. We induced differentiation of human MSCs (harvested from iliac crests of healthy volunteers after their informed consent following approved Ethical Local Committee of Tours Hospital) and found that HLA-G5 was greatly upregulated only in osteoblastic cells (+63% for mRNA). Growth plates and bone callus post-fracture in adults showed that only bone lining cells and mesenchymal progenitors were positive for HLA-G5. Use of gene silencing and dominant-negative factors revealed that HLA-G5 depends on SHP1. However, in mature osteoblasts, the expression of HLA-G5 protein was greatly suppressed (above 100% suppression) whereas the pro-osteoclastogenic factor, RANKL, was concomitantly increased. Down-regulation of HLA-G5 expression during the maturation of osteoblasts was due to binding of the repressor GLI3, a signal transducer of the Hedgehog pathway, to the GLI binding element within the HLA-G promoter. Our findings show that bone tissue specifically expresses HLA-G5, with a key role in bone homeostasis.

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Mineralizing properties of DMP1 studied in vitro with cellular and acellular 3D collagen model systems mimicking the bone tissue
Jérémie Silvent1, Nadine Nassif2, Thierry Azaïs3, Christophe Hélay4, Sidney Delgado1, Fabrice Soncin1, Marie Madeleine Giraud-Guille1 & Jean-Yves Sire1
1Université Pierre et Marie Curie, Paris, France; 2Institut de Biologie de Lille, Lille, France.

Bone is a complex structure associating cells to an extracellular organic phase, including collagen and non-collagenous proteins (NCPs), in close association with apatite mineral platelets. Although bone has given rise to extensive studies, the exact part played by NCPs in nucleating or inhibiting the mineral phase remains controversial. The present study aimed to better understand the functions of a major mineralizing protein, dentin matrix phosphoprotein1 (DMP1), an acidic, highly phosphorylated protein secreted during dentin, and bone formation.

In a first step, in order to identify a correlation between the expression of various NCP genes and apatite crystal deposition, we performed a 60 days cell culture experiment using primary human osteoblasts seeded on dense 3D collagen matrices mimicking the osteoid tissue. We show that i) the cells displayed features characteristic of osteoblasts in vivo (mineralization, protein, and gene expression) and ii) DMP1 expression correlated with the first hydroxyapatite crystallization at day 21.

In a second step, in order to target conserved motifs that could be involved in the mineralization altered differentiation, we underwent an evolutionary analysis of mammalian DMP1. Among various evolutionary conserved motifs we identified in the C-terminal region several new motifs rich in acidic residues. This region was predicted to play a role in the mineralization process.

In a third step, we tested the possible function of these highly conserved motifs using a recombinant DMP1 peptide, designed in the C-terminal region, which comprised also two collagen binding sites. The recombinant was added at two concentrations (2.5 and 25 μg/ml) to a dense collagen-matrix system mimicking the compact bone matrix. In the two conditions, our first data strongly suggest that
N-cadherin governs age-related osteoprogenitor cell determination in mice through modulation of Wnt5a and Wnt10b

Eric Hay, Francois-Xavier Dieudonne, Caroline Marty & Pierre J Marie
INSERM U606, and University Paris Diderot, Sorbonne Paris Cité, Paris, France.

Semile osteoporosis and age-related osteopenia are associated with decreased osteoblastogenesis and increased bone marrow adipogenesis. The mechanisms controlling the fate determination of osteoblast to adipocyte differentiation of bone marrow stromal cells (BMSC) during aging are not known. We and others previously showed that the cell-cell adhesion molecule N-cadherin (N-Cadh) expressed in osteoblasts controls bone formation, but little is known about its role in BMSC fate determination. Here, we tested the hypothesis that N-Cadh governs BMSC fate during skeletal aging in mice. We found that N-Cadh overexpression in osteoblasts leads to increased BMSC adipogenic differentiation and increased bone marrow fat associated with decreased BMSC osteoblast differentiation and bone formation in young (1.5 months) transgenic (TG) mice, whereas in aging (18 months) N-Cadh TG mice, BMSC adipogenic differentiation was reduced while osteogenic differentiation was increased, which resulted in increased bone formation and bone mass. This change in BMSC determination was associated with an age-related decrease in endogenous N-Cadh expression associated with increased Wnt5a, Wnt10b expression in bone. Conditioned media from old N-Cadh TG osteoblasts which express high Wnt5a and Wnt10b restored osteoblast differentiation in young N-Cadh TG osteoblasts, and this effect was abrogated by Wnt5a and Wnt10b silencing, demonstrating that the age-related BMSC fate is controlled by N-Cadh-mediated changes in Wnt5a and Wnt10b. Transplantation of BMSC derived from old N-Cadh TG mice into young recipient TG mice resulted in increased bone volume compared to wild type BMSC, demonstrating the intrinsic role of N-Cadh in the control of bone mass. These data support a model by which N-cadherin-mediated modulation of Wnt5a and Wnt10b in the bone marrow governs the age-related switch in osteoblast to adipocyte differentiation of mesenchymal cells, which in turn regulates bone formation and bone mass during aging.

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The impairment of bone formation and mineralization in BSP−/− mouse calvaria cell cultures is partly rescued by increasing cell density

Guénanèle Bouet, Wafa Bouleftour, Marchat David, Linossier Marie-Thérèse, Thomas Mireille, Aubin E Jane, Vico Laurence & Malavial Luc
1INSERM 1059, Laboratoire de Biologie du Tissu Osseux, IFR143-IFRESIS, Jean Monnet University, Saint-Etienne, France; 2Ecole Nationale Supérieure des Mines de Saint-Etienne, Center for Health Engineering, IFR143-IFRESIS, Saint-Etienne, France; 3Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada.

Bone sialoprotein regulates osteoblast activity and bone formation. In knockout (BSP−/−) mouse bone marrow (BM) stromal cell cultures, the pool of osteoprogenitor (OP) cells (CFU-F number) is not different from wild-type (+/+), nor is their early differentiation (same numbers of alkaline phosphatase positive colonies=CFU-ALP, although these are smaller), while the number of osteoblast, mineralized colonies (CFU-OB) is dramatically reduced. Because ossification of newborn BSP−/− mouse calvaria is delayed, we analysed the impact of the mutation on in vitro osteogenesis in cultures of mouse calvaria cells (MCC), isolated from 6 days old mice by collagenase digestion. In contrast to BM, CFU-F, CFU-ALP, and CFU-OB numbers were lower in BSP−/− MCC cultures. Consistent with less OP, BSP−/− cultures displayed lower proliferation and delayed growth. In MCC cultures seeded at 5000 cells/cm² osteoblast marker expression did not differ between genotypes until D6. By D14 (= first CFU-OBs) ALP, Coll1, OSX, Runx2 as well as terminal differentiation markers, OCN, PHEX, DMP1, and MEPE increased strongly in BSP−/− cultures but was low/absent in BSP−/−, with no mineralization. In contrast, osteopontin (OPN) was over-expressed in BSP−/− dishes. At high density (≥25000cells/cm²), marker levels were similar for both genotypes, and BSP−/− cultures mineralized. OPN is a potent inhibitor of mineralization, and was reported to be a substrate for PHEX. Very low PHEX expression in low density BSP−/− cultures suggests that OPN is less degraded and might inhibit mineralization. Increased PHEX expression at higher density would permit OPN degradation and mineralization. Lack of BSP thus reduces MCC culture clonogenicity, differentiation and activity, consistent with lower bone formation in vivo. A BSP−/− bone microenvironment may alter proliferation/cell fate in early OP, explaining the smaller size of CFU-ALP observed in BM cultures.

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Evaluation of bone formation capacities of human adipose-derived stromal cells cultured in platelet growth factor-enriched plasma medium

Fabien Guilloton, Vahideh Rabaji, Meudhbir Brennan, Giuilio Bassi, Mauro Krampera, Pierre Layrolle, Luc Sensèbe & Frédéric Deschaseaux
1StromalLab, UMR CNRS 5273, EFS PM, UPS, INSERM U1031, Toulouse, France; 2INSERM U957-LPRO, Nantes, France; 3Stem Cell Research Laboratory, University of Verona, Verona, Italy.

Human adipose-derived stromal cells (ASCs) exhibit strong plasticity and proliferation potentials. In addition, ASCs are easy to harvest and are found at high frequency in adipose tissue samples. This gives us opportunities for their use in bone regeneration therapy. We thus evaluated the bone formation potential of ASCs in vitro and in vivo. ASCs were isolated from subcutaneous adipose tissue (following Local Ethical Guideline and after patient informed consent) and expanded in vitro in medium containing either 10% fetal calf serum (FBS) or 2% platelet growth factor-enriched plasma (PGP). Cells were then subjected for osteoblastic differentiation by using osteogenic medium containing bone morphogenetic protein 4 (BMP4), β-glycerophosphate, and ascorbic acid. Bone marrow mesenchymal stromal cells (MSCs) were also used for comparison. We chose RUNX2, DLX5, and OSX/SP7 as transcription factors and PTHR1, ALPL, BGLAP as well as calcium deposition capacities as functional read-out to assess in vitro osteoblastic differentiation. Before differentiation basal expressions of RUNX2 and ALPL proteins were strongly increased in PGP-derived ASCs when compared to 10% FBS–ASCs. After induction of differentiation, phenotypic and functional analyses showed that 2% PGP-derived ASCs were more prone to differentiate into osteoblastic cells than 10% FBS–ASCs. Besides these in vitro studies, bTCP discs were loaded by PGP–ASCs and MSCs alone or in combination (90% MSCs/10% ASCs and 10% MSCs/90% ASCs) and then inserted in the back of Nude mice. After 8 weeks, transplants were harvested and analysed for evaluation of bone formation. Only transplant containing 100 or 90% MSCs contained new bone. On the contrary, ASCs formed fibrous tissue. Therefore, ASCs were able to differentiate into osteoblastic cells in vitro but were not spontaneously capable to induce bone formation in vivo. Better pre-conditioning protocols should solve such defect.

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Development of mice models to study implant osseointegration and failure in alveolar bone

Sylvain Mouralet, Claire Bardet, Dan J Hunter, Antoine Popelet, John B Brunski, Catherine Chauvin, Philippe Bouchard & Jill A Helms
1Division of Plastic and Reconstructive Surgery, Department of Surgery, Stanford School of Medicine, Stanford, California, USA; 2Service of Odontology, Department of Periodontology, Rothschild Hospital, AP–HP, Paris 7, Denis Diderot University, U.F.R. of Odontology, Paris, France; 3Dental School, University Paris Descartes PRES Sorbonne Paris Cité, EA 2496, Montrouche, France.

Many of our assumptions concerning oral implant osseointegration are extrapolated from experimental models studying skeletal tissue repair in long bones rather than in oral bones. This discrepancy between clinical practice and experimental research hampers our understanding on how alveolar bone forms or resorbs around implants and how osseointegration of oral implants can be improved. To overcome this disconnect, we have developed a mouse model which mimics oral implant placement in the human jaws. It consists in the placement of a Ø 0.6 mm titanium implant in the edentulous ridge anterior of the first molar. In this study, we performed two protocols of implant placement in adult male mice, mimicking different clinical situations. First, implants were firmly screwed down in a Ø 0.45 mm implant bed to obtain a successful osseointegration. Second,
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Activation of β-catenin signalling enhances the osteogenic gene response to mechanical loading in mesenchymal stem cells

Claudia Nemitz¹, Franz Jakob², Anita Ignatius³ & Astrid Liedert¹

¹Institute of Orthopedic Research and Biomechanics, Ulm, Germany; ²Orthopedic Department, Würzburg, Germany.

Introduction

Wnt/β-catenin signalling and mechanical loading are able to inhibit adipogenesis and to stimulate osteoblastogenesis of mesenchymal stem cells. The involvement of β-catenin signalling in mechanically induced bone formation has already been shown in vivo using a tibia loading model. The aim of this study was to investigate the influence of the activation of β-catenin on the osteogenic and adipogenic response of mesenchymal stem cells to mechanical loading in vitro.

Methods

C3H10T1/2 cells were cultivated in adipogenic medium. SB415286 was added for activating β-catenin signalling. Cells were loaded by daily homogenous cyclic stretching for 5 days. Real-time RT-PCR and western blotting were performed for expression analysis. Three independent experiments in duplicate (n = 6) were performed. Data were analysed for significance (value P ≤ 0.05) using Student’s t-test.

Results

Mechanical loading and the β-catenin signalling activator SB415286 significantly upregulated the relative gene expression of the osteogenic markers Runx2, Ptg2, and Cyp61, as well as the expression of Wnt10b. Mechanical loading and SB415286 downregulated the adipogenic markers Cebpa and Pparg. Mechanical loading in addition to SB415286 treatment enhanced the mechanically induced expression of Runx2, Ptg2, Cyp61, Wnt10b, and reduced expression of Pparg and Cebpa. Real-time RT-PCR results were verified by western blotting.

Discussion

SB415286 and mechanical loading led to an increase of osteogenic marker expression and to a reduction of adipogenic marker expression. SB415286 provoked a sensitizing effect on the mechanically induced osteogenic gene expression as well as on the mechanically reduced adipogenic gene expression. Interestingly, the expression of Wnt10b, which is known as an inhibitor of adipogenesis and a stimulator of osteoblastogenesis, was upregulated by SB415286 and mechanical loading. Sensitizing mechanosensitive pathways, which contribute to the enhancement of osteogenesis and simultaneous impairment of adipogenesis might represent a therapeutic target for osteoanabotic therapy in patients with osteoporosis.

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PP185

DNA-damage, survival, differentiation, and matrix mineralization in vitro of a murine multipotent mesenchymal precursor cell line

Patrick Lau, Yueyuan Hu, Christine Hellweg, Christa Baumstark-Khan & Günter Reitz

German Aerospace Center (DLR), Institut of Aerospace Medicine, Cologne, Germany.

Radiation therapy is one of the most effective and indispensable treatment modalities for cancer patients. Known tissue complications caused by radiation-induced stem cell depletion, may result in structural and functional alterations of the surrounding matrix. Although, studies have demonstrated that ionizing radiation can induce apoptosis and senescence, little is known about the effects of therapeutic irradiation concerning the commitment of mesenchymal stem cells to the osteoblastic lineage. C3H10T1/2 clone eight cells were used reflecting an early stage of differentiation. Notably, radiation doses of 2 Gy reduced proliferation, but had no significant effect on cell viability. Cell cycle analysis revealed that the yield of cells captured in the G2/M phase of the cell cycle was markedly and dose-dependently increased. Instead of apoptosis we detected increased activity of stress-induced premature cellular senescence. Histochemical staining and quantification of the hydroxyapatite content of the extracellular bone matrix revealed positive staining for alizarin red S. Expression of TP53 encoding for tumour suppressor protein p53 and its downstream target cyclin-dependent kinase inhibitor 1A (p21WAF1/CIP1) were significantly increased. Gene expression analysis of the osteoblast specific genes, Runx2 and osteocalcin were assessed. Here, we confirmed that exposure to X-rays was dose dependently effective in decreasing cellular survival. Our results indicate that the direct impairment of proliferation and osteogenic differentiation potential of MSCs by irradiation may contribute partly to post-irradiation osteoporosis.

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Involvement of Runx2 in the differentiation process of osteoblastic precursor cells after radiation exposure

Yueyuan Hu, Patrick Lau, Christine Hellweg, Christa Baumstark-Khan & Günter Reitz

German Aerospace Center (DLR), Institut of Aerospace Medicine, Cologne, Germany.

Astronauts on exploratory space missions will experience a complex environment that includes microgravity and radiation. While the deleterious effects of unloading on bone are well established, fewer studies have focused on the effects of radiation. Space radiation produces distinct biological damages which, up to now, little is known about the correlation between radiation exposure and bone tissue. In our study we used osteoblastic precursor cells to investigate the radiation response of bone cells. Effects of radiation on differentiation were investigated by their ability to deposit extracellular matrices that mineralize under in vitro culture conditions using the histochemical Alizarin Red Staining (ARS). Calcium precipitation was detected in a bright red color already ten days after exposure to X-rays for doses up to 10 Gy. Notably, our results indicate that exposure to higher radiation doses could be correlated to a pronounced staining of the extracellular matrix. In order to gain more detailed insights into the osteoblast specific mineralization process, the transcriptional expression level of Runx2 was analysed. Our studies suggest that space related radiation significantly modulates the mineralization process and effectively modulates the gene expression levels of Runx2 involved in the differentiation of osteoblasts.

In conclusion, the presented data allow the suggestion that exposure to ionizing radiation interferes with bone formation at the level of cellular differentiation.

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PP187

Implantation of octacalcium phosphate enhances long bone’s repair in rats

Mohammad Reza Arab, Forogh Sargolzaei Aval & Forugh Sargolzaei Aval

Zahedan University of Medical Sciences, Zahedan-Sistan and Baluchestan, Zahedan, Iran.

Background

This study was designed to investigate the process of bone formation caused by implantation of octacalcium phosphate (OCP) in rat tibiae.

Methods

We used 25 young male Sprague–Dawley rats. A full thickness standardized trephine defect, 3-mm in diameter, was surgically created on the superior end of right and left tibia. Amount of 6-µg synthetic octacalcium phosphate was implanted into a bony defect on the right tibia as an experimental group. No OCP particles were implanted in the left tibia as a control group that was otherwise treated identically. Bone formation was examined histologically on 7th, 10th, 14th, 21st, and 28th days after implantation.

Results

In the experimental, on the 7th day after implantation, a few clusters of cartilage cells were observed between the OCP particles. By 10th day, osteoblasts were observed in the interstices of the OCP particles and osteoid seams were formed. Osteogenesis was initiated locally between the OCP particles in central position of the defects on 10th day after implantation. By 14th day after implantation,
Alician blue staining showed hypertrophic chondrocytes that replaced by new bone was observed near the defects margin on 14th and 21st days after implantation. At the end of study implanted OCP was surrounded by newly formed bone.

In the control group, at the end of study, bone formation was observed only along and near the defects margin.

Conclusion
These results demonstrate that octacalcium phosphate could be used in the repair of the long bone defects.

Keyword
Octacalcium phosphate, osteogenesis, tibia, rat. DOI: 10.1530/boneabs.1.PP187

PP188
Intracellular calcium fluxes in human bone cells in osteoporotic and osteoarthritic patients
Monica celli1, Elena Gasbarra2, Claudio Frank3, Alessandro Cutrelli1, Giulio Fioravanti Cinci1 & Umberto Tarantini1
1University of Rome Tor Vergata, Rome, Italy; 2Istituto Superiore di Sanità, NCRI, Rome, Italy.

We studied changes in intracellular Ca\(^{2+}\) concentration in bone cell cultures obtained from human subjects with osteoporosis and osteoarthritis, to differences between these patients and healthy subjects. We enrolled 36 patients: 12 undergoing primary total hip arthroplasty for osteoarthropic femoral fractures (group A, mean age range 57–80), 12 for hip osteoarthritis (group B, mean age range 57–80), and 12 healthy subjects who suffered a high-energy trauma fracture (group C, mean age range 18–30) as controls. All patients gave informed consent for using bone samples as a source of bone cells. Lumbar spine and femoral DXA were performed. Microfluidic techniques of the intracellular calcium concentration was done by fura-2. Imaging was performed with the Argus 50 system (Hamamatsu) with excitation wavelengths of 340 and 380 nm for acquiring ratio images of fura-2. ATP and thapsigargin, inhibitor of the calcium-ATPase of the endoplasmic reticulum, were added during the experiments. Group A reported BMD value of 0.673 ± 0.196 g/cm\(^2\), group B 1.005 ± 0.194 g/cm\(^2\), and group C 1.179 ± 0.259 g/cm\(^2\). Application of ATP (1 mM) induced in group C a fast and transient increase (ratio value from 0.67 ± 0.01 to 1.74 ± 0.13) in intracellular calcium concentration [Ca\(^{2+}\)]. Addition of thapsigargin (100 mM) to the cells induced an additional increase of [Ca\(^{2+}\)]. (ratio value 0.85 ± 0.03) due to release from intracellular calcium stores. In osteoblastic cultures (B) ATP significantly increased [Ca\(^{2+}\)] (ratio value from 0.67 ± 0.01 to 1.53 ± 0.19) but in lower amount than control cells. In this experimental group thapsigargin induced a [Ca\(^{2+}\)] increase (ratio value 0.98 ± 0.05) slightly stronger than the control. In group A, ATP stimulation exhibited a significantly lower increase in [Ca\(^{2+}\)] (ratio value from 0.67 ± 0.01 to 1.21 ± 0.09), while the effect of thapsigargin was similar to control (ratio value 0.87 ± 0.06). Osteoporotic cultures indicate an impairment of intracellular calcium influx. P2 receptors may be important drug targets for bone turnover modulation.

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PP190
Role of vitamin D and K on human osteoblasts in vitro on primary cultures derived from osteoporotic and normal patients
Gianna Rossetti1,2, Mario Marin2, Emiliano Arango1 & Umberto Tarantini1
1Orthopaedic Department, University of Rome Tor Vergata, Rome, Italy; 2Department of Chemistry, University of Bari, Bari, Italy.

This study is focused on the effects of the synergic use of vitamins D and K on human osteoblasts primary cultures derived from osteoporotic and normal patients. The aim of this work is the evaluation of the different cellular behaviour in response to the lipophilic vitamins stimulation. We included 20 osteoporotic and 20 control patients in age between 35 and 50 and in age between 55 and 85. All patients gave informed consent for using bone samples as a source of bone cells. DXA at lumbar spine and femur, in terms of BMD, were performed. Changes in osteocalcin and alkaline phosphatase production were also evaluated in cells cultures. The response of osteoblasts to Vitamin D appears to depend on the stage of osteoblast maturation, with preferential induction of the catabolic factor, receptor activator of nuclear factor eB ligand (RANKL) and had pro-anabolic activity by enhancing the production of matrix vesicles and mineral deposition. Vitamin K is an essential cofactor for the formation of GLA (\(\gamma\)-carboxyglutamic acid) residues in proteins, important not only for blood coagulation but also for calcified tissues; on the other hand vitamin D (1,25-dihydroxy-vitamin D3), is critical for the regulation of serum calcium and phosphorus levels that in turn support bone mineralization and neuromuscular activity. It is well known that vitamin D can stimulate the metabolic activity of human osteoblasts in vitro therefore it is used in osteoporotic patients. We are evaluating the results on vitamins D and K cross action treatment over these subjects.

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PP191
Nuclear translocation of oxytocin receptor mediates increased gene expression in osteoblasts
Adriana Di Benedetto1, Concetta Cuscito1, Graziana Colaianni2, Roberto Tamma1, Beatrice Nico1, Damiana Calvano2, Carlo Zambonin2, Michangelo Corcelli1 & Alberta Zallone1
1Department of Basic Medical Sciences, Neurosciences and Organs of Senses, University of Bari, Bari, Italy; 2Department of Chemistry, University of Bari, Bari, Italy.

The neuro-hypophyseal hormone oxytocin (OT) is a novel anabolic regulator of bone mass (Tamma et al. PNAS, 2009), upregulating expression of critical osteoblast transcription factors. These effects are mediated by oxytocin receptor, a GPCR expressed by osteoblasts. Recently an increasing number of reports indicates that GPCRs could be targeted to the nuclear membrane; prostaglandin receptors, endothelin receptors and \(\beta\)-adrenergic receptors among others (Boivin et al. 2008). Accordingly we found OTRs in osteoblast nuclear extracts after OT stimulation (15–30 min). Confocal microscopy performed on intact cells either transfected with OTR-GFP or stimulated with OT stimulation indicated a nuclear localization after OT stimulation, data confirmed by immunogold staining. Exogenous OTR-GFP, transfected in primary osteoblasts, colocализed makers (CD73, CD90, CD146, CD44, CD105, and HLA-I) while were negative for CD45. Moreover DFSCs differentiated into osteoblast-like cells, produced mineralized matrix nodules and expressed typical osteoblastic markers. Then, DFSCs were characterized for in vitro expression of adhesion molecules integrins and cadherins, in basal and osteoinductive conditions. Our preliminary data showed that, DFSCs express Integrins alpha V, beta 3, alpha 5 and beta 1 in basal undifferentiated conditions; after 1 week of osteogenic trigger, the expression of alpha V, beta 3 and alpha 5 increased, while beta 1 decreased. DFSCs were also tested for the expression of Cadherins, and we found N-Cadherin to be very high expressed in basal conditions, while E-Cadherin was low expressed and P-Cadherin very poor expressed. Furthermore N-cadherin expression increased during the first step of osteogenic differentiation, while catherin in the later times. Such adhesion molecules regulate stem cell maintenance, division and expansion and are involved in cell–cell and cell–matrix interaction. The homing and engraftment of MSCs, in the host tissues are important tools of the regenerative medicine and require cells to interact and recognize each others. Surface molecules as integrins and cadherins could be important key regulators of the differentiation processes; therefore further insights in this field will contribute to the successful generation or repair of damaged tissues.

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with β-arrestin1/2 within 2–3 min after OT treatment, thereafter was found in RAB5-positive endosomal vesicles and then colocalized with transportin-1. Eventually, at least a part of the receptors was sorted to the nucleus where OTR-GFP was evident by confocal microscopy both in intact cells and in isolated nuclei. MALDI-TOF analysis of nuclear proteins immunoprecipitated with anti-OT confirmed this finding; the spectra analyzed with FINDPept database revealed the presence of four peptides corresponding to OTR intracellular loops. We hypothesized as possible role for OTR in nucleus the regulation of transcription and/or translation factors. Indeed, in response to OT stimulus, a physical interaction of native OTRs with the osteoblast transcription factor Runx2 and with the transcription co-activator Snurri-2 was found by immunoprecipitation. The blockade of OTR endocytosis by β-arrestins silencing, prevented OT induced up-regulation of Oxs, ATF-4, and osteocalcin. Similarly by transportin-1 silencing, OTR nuclear localization as well as the up-regulation of Oxs, ATF-4, osteocalcin, and BIP were impaired. Taken together these data suggest that OT anabolic effect on bone could be dependent upon a novel mechanism initiated by β-arrestin-mediated OTR internalization and followed by transportin-1 dependent nuclear translocation.

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**PP192**

**Moderate hypothermia induces growth arrest in normal human osteoblast cells but retained mitochondrial metabolism in vitro**

Mohd Din Aisha, Mohamed Noor Khan Nor-Ashkini, Ab. Rahim Sharaniza, Hapizah Nawawi, Marina Kapitonova, and Gabriele Ruth Anish Froemming

1Institute of Medical Molecular Biotechnology, Jalan Hospital, University Teknologi MARA, Sungai Buloh, Selangor, Malaysia; 2Center for Pathology Diagnostic and Research Laboratories, Clinical Training Centre, Jalan Hospital, University Teknologi MARA, Sungai Buloh, Selangor, Malaysia; 3Faculty of Medicine, Jalan Hospital, University Teknologi MARA, Sungai Buloh, Selangor, Malaysia.

Ablation of osteosarcoma cells by sublethal hypothermia before radiation may increase sarcoma tissue sensitivity by inducing growth arrest. Normal cells that are not lethally damaged by hypothermia and radiation can undergo DNA repair thus promoting cell survival. Nevertheless, understanding of the response of normal bone forming osteoblast cells towards hypothermia is necessary before administering on osteosarcoma cells. In this study we evaluated the response of short-term moderate and severe hypothermia on normal human osteoblast (NHOS) cell metabolism and growth markers. NHOS cells were exposed to moderate (35°C) and severe (27°C) hypothermia, and control at 37°C for 12 h. NHOS cell metabolism was measured with MTS assay while rate of cell proliferation was calculated with Trypan blue staining. Meanwhile changes in NHOS growth gene expression for Cdk1, Cdk2, Cdk4, and p21 (cell cycle progression), and Caspase 3, 8, 9, Bcl-2, and Bax (apoptosis) was quantitated using the RT-PCR. Flow cytometry further confirmed the rate of cell survival while phosphorylation of histone variant (H2AX Ser 139) as a marker for DNA damage was measured at 24 h. The mRNA fold change was statistically analyzed using Student’s t-test. NHOS cells remained metabolically active at 35°C (103 ± 0.32%) conversely at 27°C (56.9 ± 0.12%) cell metabolism was markedly inhibited (P < 0.001). Results showed that NHOS proliferation was insignificantly reduced at 35°C (0.76%) relative to control. Up-regulation of Cdk4 may suggest that hypothermia permits cell cycle re-entry (M/G1 phase). However, overexpression of p21, Cdk1, and Cdk2 mRNA tends to inhibit cyclin-dependent kinase activity signifying that cells are arrested at GI/S and G2/M transitions. Both gene expression and flow cytometry results showed an increase in apoptosis at 27°C. Although Caspase 3 was activated at 35°C, the mRNA expression ratio between Bax and Bcl-2 was low (1.5±5.3). Flow cytometry data for 35°C showed an increase of apoptosis by 3.54% relative to control. Interestingly, hypothermia did not induce DNA damage after 24 h. Our studies on moderate hypothermia (35°C) at 12 h demonstrated a sublethal response towards normal bone cells by temporary arresting NHOS cells. Transient administration of moderate hypothermia on osteosarcoma cells before radiation may enhance radiosensitivity with minimal damage to normal cells.

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**PP193**

**Normal human osteoblast cells exerts an adaptive effect towards moderate hypothermia by retaining bone metabolism and cellular function in vitro**

Mohd Din Aisha, Mohamed Noor Khan Nor-Ashkini, Ab. Rahim Sharaniza, Hapizah Nawawi, Marina Kapitonova, and Gabriele Ruth Anish Froemming

1Institute of Medical Molecular Biotechnology, Jalan Hospital, University Teknologi MARA, Sungai Buloh, Selangor, Malaysia; 2Center for Pathology Diagnostic and Research Laboratories, Clinical Training Centre, Jalan Hospital, University Teknologi MARA, Sungai Buloh, Selangor, Malaysia; 3Faculty of Medicine, Jalan Hospital, University Teknologi MARA, Sungai Buloh, Selangor, Malaysia.

Over the years, it has been demonstrated that the ability to maintain body core temperature in older adult’s declines with age. Temperature is a vital physical factor for cell growth and a downshift in core body temperature (<37°C) might have a direct affect on maintaining bone density or repair fractures. Disruption in any of the cellular processes involved in bone remodelling leads to a net loss of bone mineral density and bone loss. Therefore our study looked at the changes in normal human osteoblast (NHOS) cell cytoskeleton, motility, and viability after short-term hypothermia. The expression of chaperone proteins, bone transcription factors and maturation proteins were also examined. NHOS cells were exposed to moderate (35°C) and severe (27°C) hypothermia and control (37°C) at 1, 12, 24, and 72 h in a water-jacketed incubator. Changes in cell cytoskeleton were calculated based on fluorescence intensity. NHOS viability and motility was measured using MTS assay and CD44 ELISA respectively. Meanwhile, expression level of osteoblast transcription factors (Runx2 and osteonectin), cold (Rbm3), and heat shock (Hsp70) chaperone mRNA was quantitated using RT-PCR. Measurement of bone forming proteins; alkaline phosphatase (ALP), and osteocalcin (OCN) was done by ELISA. Hypothermic conditioning showed noticeable perturbation of the NHOS cytoskeleton compared to control. At 27°C tubulin fibres were seen localized around the cell nucleus while actin was distributed throughout the cytoplasm. Increase in actin fluorescence intensity showed almost a similar trend in production of cell surface CD44 marker protein as both are involved in cell motility. Although cytoskeleton components were altered, NHOS remained metabolically viable at 35°C. Response towards hypothermia constitutively enhanced expression of Rbm3 possibly to facilitate mRNA translation. Meanwhile, Runx2 and osteonectin were shown to co-regulate. Up-regulation of Runx2 induced ostein mRNA under 35°C treatment indicating osteoblast differentiation was retained. Hypothermia increased ALP activity while OCN protein was expressed except at 72 h. Moderate hypothermia exerted an adaptive effect by retaining NHOS cell metabolism and bone function particularly at 12 h. We speculate that decline in core body temperature is not the reason for bone loss seen in elderly since it appears to stimulate bone mineralization.

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**PP194**

**Black tea polyphenols suppress adverse effects of TNF-α-induced inflammation in osteoblast cells**

Haja Zulkilip, Aisha Mohd Din, Norita Salim, Gabriele Anish Froemming, Aleza Mohd Ismail, and Hapizah Nawawi

1Faculty of Medicine, Centre for Pathology Diagnostic and Research Laboratories (CPDRL), University Teknologi Mara, Selangor, Malaysia; 2Faculty of Medicine, Institute of Medical Molecular Biotechnology (IMMB), University Teknologi Mara, Selangor, Malaysia.

Introduction Most chronic inflammatory bone diseases are characterized by loss of bone density due to an increase of osteoclastic activity without equally increased osteoblastic activity which in turn is leading to an imbalance in bone repair and remodelling. Several studies have reported that green tea rich in polyphenols especially catechins could improve bone mass and structure and eventually inhibit bone remodelling. Several studies have reported that green tea rich in polyphenols especially catechins could improve bone mass and structure and eventually inhibit bone remodelling. Several studies have reported that green tea rich in polyphenols especially catechins could improve bone mass and structure and eventually inhibit bone remodelling. Several studies have reported that green tea rich in polyphenols especially catechins could improve bone mass and structure and eventually inhibit bone remodelling. Several studies have reported that green tea rich in polyphenols especially catechins could improve bone mass and structure and eventually inhibit bone remodelling. Several studies have reported that green tea rich in polyphenols especially catechins could improve bone mass and structure and eventually inhibit bone remodelling. Several studies have reported that green tea rich in polyphenols especially catechins could improve bone mass and structure and eventually inhibit bone remodelling. Several studies have reported that green tea rich in polyphenols especially catechins could improve bone mass and structure and eventually inhibit bone remodelling. Several studies have reported that green tea rich in polyphenols especially catechins could improve bone mass and structure and eventually inhibit bone remodelling. Several studies have reported that green tea rich in polyphenols especially catechins could improve bone mass and structure and eventually inhibit bone remodelling. Several studies have reported that green tea rich in polyphenols especially catechins could improve bone mass and structure and eventually inhibit bone remodelling. Several studies have reported that green tea rich in polyphenols especially catechins could improve bone mass and structure and eventually inhibit bone remodelling. Several studies have reported that green tea rich in polyphenols especially catechins could improve bone mass and structure and eventually inhibit bone remodelling. Several studies have reported that green tea rich in polyphenols especially catechins could improve bone mass and structure and eventually inhibit bone remodelling. Several studies have reported that green tea rich in polyphenols especially catechins could improve bone mass and structure and eventually inhibit bone remodelling. Several studies have reported that green tea rich in polyphenols especially catechins could improve bone mass and structure and eventually inhibit bone remodelling. Several studies have reported that green tea rich in polyphenols especially catechins could improve bone mass and structure and eventually inhibit bone remodelling. Several studies have reported that green tea rich in polyphenols especially catechins could improve bone mass and structure and eventually inhibit bone remodelling. Several studies have reported that green tea rich in polyphenols especially catechins could improve bone mass and structure and eventually inhibit bone remodelling. Several studies have reported that green tea rich in polyphenols especially catechins could improve bone mass and structure and eventually inhibit bone remodelling. Several studies have reported that green tea rich in polyphenols especially catechins could improve bone mass and structure and eventually inhibit bone remodelling. Several studies have reported that green tea rich in polyphenols especially catechins could improve bone mass and structure and eventually inhibit bone remodelling. Several studies have reported that green tea rich in polyphenols especially catechins could improve bone mass and structure and eventually inhibit bone remodelling. Several studies have reported that black tea polyphenols treatment with regards to their influence on normal human osteoblast (NHOS) alkaline phosphatase (ALP) activity and bone matrix mineralization under normal and inflammatory conditions.

Methods Total phenolic content (TPC) of green and black tea hot water extracts were determined by Folin-Ciocalteau method. NHOS cells were plated, incubated with 0 and 1 mg/ml of TNF-α and treated with 5, 10, 50, and 100 μg/ml of GTP and BTP respectively at 2, 5 and 10 days. ALP activity was measured colorimetrically.
using an ALP reagent of p-nitrophenylphosphate (PNPP). Determination and quantification of mineralized bone nodules were assessed by alizarin red staining (ARS) technique. The dye was extracted from stained cells and quantitatively confirmed using a spectrophotometer.

Results

TPC measured for GTP and BTP were 77.1 and 83.13 mg GAEE/g respectively. All BTP doses stimulated ALP activity in normal condition at each of the treatment days (P<0.05) except for 100 µg/ml dose at day 2. BTP managed to reverse ALP activity suppressed by TNF-α at all time points except for 50 µg/ml dose at day 2. Results demonstrated that ALP activity was increased with lower doses of GTP (5 µg/ml) at day 2 and 5 while in TNF-α presence, same dose exhibited same effect at day 2 (P<0.05) and day 5. ARS affirmed the presence of calcific deposits by cells. Increase in mineralized areas was observed by the presence of bright coloured bone nodules. Under normal conditions GTP and BTP enhanced mineralization with all tested doses significantly at all time points (P<0.05) implying that these polyphenols elevated osteogenesis in NHOst. In inflammation, all GTP and BTP doses exhibited a significant increase in ARS intensity (P<0.05) on day 2.5 µg/ml of both polyphenols induced mineralization on day 5, evident by a significant increase of ARS intensity (P<0.05). Likewise 50 (P<0.01) and 100 µg/ml (P<0.05) GTP significantly induced mineralization on day 5. All results were compared to control.

Conclusion

BTP exerted comparable anabolic effect to GTP on TNF-α stimulated NHOst by elevating ALP activity and mineralization thereby enhancing bone formation. DOI: 10.1530/boneabs.1.PP194

PP196

The effect of enamel matrix derivative on human gingival fibroblasts cultured on zirconium disc surfaces

Heesu Lee1, Ahran Pae2, Yong-Dae Kwon2 & Seonghee Ko1
1Gangneung-Wonju National University, Gangneung, Republic of Korea; 2KyungHee University, Seoul, Republic of Korea.

Purpose

To investigate the effect of enamel matrix derivative (Emdogain) on the attachment, growth behavior and the genetic effect of human gingival fibroblasts (HGF) cultured on zirconium disc surfaces.

Materials and methods

HGF cells were cultured on i) zirconium discs without enamel matrix derivative (EMD), ii) zirconium discs with EMD 25 µg/ml, and iii) zirconium discs with EMD 100 µg/ml. The cell proliferation activity was evaluated through a MTT assay at 4, 24, and 48 h and the cell morphology was examined by SEM. The mRNA expression of collagen type I, fibronectin, integrin-α, laminin, osteopontin and TGFβ1 in HGF were evaluated by RT-PCR after 24 h culture.

Results

From MTT assay, HGF proliferation was a little higher in EMD 25 µg/ml group than control and EMD 100 µg/ml group. SEM images showed that HGF cells were more flattened on the test groups than control group after 4 h culture and more cellular attachment were observed on EMD 25 µg/ml group than control and EMD 100 µg/ml group after 24 h culture. After 48 h culture, More cellular attachment were similar in all groups. Cellular process in E MD 25 µg/ml group were thin and long. The result of RT-PCR suggest that the mRNA expression of type I collagen increased with dose dependent manner. Enamel matrix derivative decreased the mRNA expression of other protein associated with cellular attachment or nearly affected.

Conclusions

Through this short term culture of HGF on zirconium discs we conclude that enamel matrix derivative may affect the proliferation and attachment of HGF cells and the cell morphology. And enamel matrix derivative stimulates production of extracellular matrix collagen and osteopontin. But more investigation is required to determine appropriate concentration of enamel matrix derivative for utmost cell proliferation and attachment.

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PP197

EGF suppresses BMP2-induced osteogenic differentiation through the up-regulation of Smurf1 expression

Hye-Lim Lee, Kyung Mi Woo, Hyun-Mo Ryoo, Gwan-Shik Kim & Jeong-Hwa Baek
Seoul National University School of Dentistry, Seoul, Republic of Korea.

Although EGF has been known to inhibit osteoblast differentiation, its molecular mechanism has not been clearly elucidated. Smurf1 acts as a negative regulator of BMP signaling by inducing ubiquitination and proteasomal degradation of BMP type I receptor and R-Smads. In this study, we investigated the effect of EGF on the expression of Smurf1 and the role of Smurf1 in EGF-induced inhibition of BMP2-induced osteogenesis. EGF increased Smurf1 expression which was blocked by treatment with a specific inhibitor of EGFR tyrosine kinase, JNK or ERK. Reporter assay using the constructs containing the sequence of Smurf1 promoter, demonstrated that Ap-1 and Runx2 are the transcription factors activated by JNK and ERK, respectively. EGF treatment or Smurf1 over-expression suppressed BMP2-induced expression of osteogenic marker genes, whereas knockdown of Smurf1 partially rescued the expression of these genes in EGF-treated cells. Taken together, these results suggest that the JNK-c-Jun and the ERK-Runx2 signaling pathways play an important role in the regulation of Smurf1 expression by EGF and that Smurf1 partially mediates the inhibitory effect of EGF on osteogenic differentiation.

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**PP198**

Estrogen effect on the sclerostin induction by BMP-2 in human mesenchymal stromal cells

In Sook Kim1, Hoon Joo Yang2, Yun Mi Song1, Soo Jin Ryu3, Ri Yong Kim1 & Seon Jung Hwang1,2,3

1Dental Research Institute, Seoul National University, Seoul, Republic of Korea; 2Department of Oral and Maxillofacial Surgery, Brain Korea 21 2nd Program for Cranio-maxillofacial Life Science, School of Dentistry, Seoul National University, Seoul, Republic of Korea; 3Department of Maxillofacial Cell and Developmental Biology, Brain Korea 21 2nd Program for Cranio-maxillofacial Life Science, School of Dentistry, Seoul National University, Seoul, Republic of Korea.

**Introduction**

Estrogen therapy decreases circulating levels of sclerostin, a protein product of SOST which increase in postmenopausal women. However, the mechanisms of estrogen on the expression of SOST remain unclear. This study was hypothesized that estrogen modulates SOST expression by interfering bone morphogenic protein (BMP) signaling on the basis that BMP is an inducer of SOST in osteoblasts.

**Description of methods**

We investigated the expression of SOST and other BMP-2 responsive genes in the treatment either with BMP-2 (200 ng/ml), estrogen (100 nM), or combination of both using female-originated human mesenchymal stromal cells (BMSCs) by real-time RT-PCR or ELISA. Molecular mechanism was examined using the inhibitor of Wnt (ICL 182, 780: 100 nM) and Smad pathway (AMPK: 10 μM).

**Results**

There was no direct effect of estrogen on SOST expression, but estrogen significantly down-regulated SOST expression which was induced by BMP-2. Treatment with Wnt signaling inhibitor did not affect SOST induction by BMP-2, but counteracted the suppressive effect of estrogen on SOST induction by BMP-2. On the contrary, Smad inhibitor completely blocked SOST induction by BMP-2. This tendency repeated in the expression of other BMP-2 responsive genes such as alkaline phosphatase, BMP-2, or IGFI.

**Conclusions**

Current findings suggest that estrogen regulated SOST expression by cross-talk with BMP-2 signaling. Estrogen suppressed SOST induction by BMP-2 through Wnt signaling.

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**PP199**

Effect of β-cryptoxanthin on the differentiation of human bone-marrow stromal stem-cells treated with pioglitazone

Antonio Casado-Díaz1,2, Raquel Santiago-Mora1, Gabriel Dorado3 & José Manuel Quesada-Gómez1,2

1Hospital Universitario Reina Sofia – IMIBIC, Córdoba, Spain; 2Querper R&D, Córdoba, Spain; 3Dpto. Bioquímica y Biología Molecular .Univ. Córdoba, Córdoba, Spain.

Pioglitazone is a drug of the thiazolidinedione (TZD) class used to treat type 2 diabetes mellitus. TZD is an agonist of peroxisome proliferator-activated receptor γ (PPAR-γ) that improves insulin sensitivity, glucose and lipid metabolism and inflammation. However, TZD induces bone marrow adiposity with suppression of osteogenesis, that could contribute to bone loss and osteoporotic fractures.

β-Cryptoxanthin is a carotenoid with antioxidant properties abundant in fruits and vegetables, with stimulatory effects on osteoblastogenesis and inhibitory effects on adipogenesis. In this study it was investigated whether the β-cryptoxanthin may reduce the effect of pioglitazone on bone marrow stromal cell (BMSC) differentiation into osteoblasts. BMSC were induced to differentiate into osteoblasts. When the cells were treated with pioglitazone in presence of β-cryptoxanthin, the osteoblastogenesis was not decreased. However, the β-cryptoxanthin had no a significant effect on the expression of the adipogenic genes. As a conclusion, the β-cryptoxanthin has a partial positive effect on the differentiation of BMSC into osteoblasts treated with pioglitazone. Therefore, the β-cryptoxanthin could be used to minimize the antosteoblastic effects of the pioglitazone in type 2 diabetes mellitus patients treated with pioglitazone.

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**PP200**

Premixed acidic calcium phosphate cement as a local delivery system for simvastatin

Maryam Montazerolghaem, Håkan Engqvist & Marjam Ott

Division of Applied Materials Science, Department of Engineering Sciences, Uppsala University, Uppsala, Sweden.

In 1999 Mundy et al. showed that simvastatin, a drug administered for high cholesterol levels, had a profound stimulatory effect on osteoblasts. Since then other studies have also confirmed that simvastatin enhances bone formation; however, the lack of a local delivery system has restricted its clinical use. We have used premixed acidic calcium phosphate cement as a local delivery system for simvastatin. To confirm that the simvastatin released retained its activity, in vitro studies were performed. We measured how cell proliferation and differentiation was affected by different doses of simvastatin as well as their ability to quench reactive oxygen species (ROS) production.

Different doses of simvastatin were added to the liquid phase of calcium phosphate cement consisting of β-tricalcium phosphate, monocalcium phosphate anhydrous, and glycerol. The cements were moulded and soaked in PBS. At specified time points PBS was collected and used for cell studies. Saos-2 (human osteoblastic cell-line), and THP-1 (human monocytic cell-line) were seeded in tissue culture plates after which the cement extracts were added to the cells at different time points. The proliferation, measured by Alamarblue, and differentiation, measured by alkaline phosphatase activity (ALP), was quantified for Saos-2 cells after 3, 5, and 7 days. The total ROS production for THP-1 was measured after 24 h by means of luminol amplified chemiluminescence.

The in vitro studies revealed that the simvastatin released from the cements was still active and able to stimulate osteoblast differentiation. It also had the capability to quench ROS production. In conclusion simvastatin can be added to acidic calcium phosphate cements to increase the osteogenic properties and decrease the inflammatory response.

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**PP201**

Primary Human Bone Cells treated with Parathyroid Hormone or Dexamethasone show Effects on micro-RNA Expression Patterns Assessed by Second Generation Sequencing

Navya Laxman1, Carl-Johan Rubin2, Hans Mallmin3, Olle Nilsson3, Maryam Montazerolghaem, Hakan Engqvist & Marjam Ott

1Department of Medical Sciences, Uppsala University, Uppsala, Sweden; 2Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden; 3Department of Surgical Sciences, Uppsala University, Uppsala, Sweden; 4Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden.

**Introduction**

Micro-RNAs (miRNAs) are important post-transcriptional regulators. By binding to complementary RNA strands, they affect mRNA levels and/or mRNA translation. We have previously identified ~90 miRNAs with significant expression levels, with a subset of miRNAs exhibiting interindividual and/or gender differences in expression. In the present project, we have investigated the impact of treatment with parathyroid hormone (PTH) and dexamethasone (DEXA) on global miRNA expression in primary human bone (HOB) cells by second generation sequencing.

**Method**

HOB cells were isolated from human trabecular bone collected from donors undergoing total hip replacement, and treated with either PTH or DEXA or left untreated for 2 and 24 h. Small RNA was isolated from these cells and cDNA synthesized. Second generation sequencing was performed using SOLiD4 on barcoded library constructs. Sequence reads were aligned to a scaffold consisting of all known miRNA sequences. Number of sequence reads mapping uniquely to each miRNA were counted. The value used for each miRNA was the number of reads per miRNA normalized to per million total reads.

**Results**

207 million reads were obtained, and normalized absolute expression was retrieved for the 500 most abundant miRNAs. The 75 miRNAs that exhibited the
highest mean expression across the four experiments per individual were taken forward. Results show significant differences in miRNA expression after 2 h, and even more differences after 24 h. Several miRNAs exhibiting significant differences in expression have predicted mRNA targets involved in bone metabolism, e.g. miR-197 targeting IGF and Wnt pathway members. Conclusion miRNA absolute expression data from second generation sequencing show that PTH and DEXA affect miRNA expression in HOB cells, and that these miRNAs in turn are correlated to expression levels of mRNAs known to affect bone metabolism.

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### PP202

**Expression and function of glutamate transporters in mouse primary osteoblasts**

Wenjie Xie, Silvia Dolder, Mark Siegrist, Antoinette Wetterwald & Willy Hofstetter

Bone Biology and Orthopaedic Research, Department of Clinical Research, University of Bern, Bern, Switzerland.

**Introduction**

Osteoblast lineage cells express glutamate receptors and secrete glutamate, which acts as an autocrine factor to promote cellular differentiation and activation. However, the mechanisms by which glutamate regulates these functions, remain unclear.

**Methods**

Primary osteoblasts were isolated from calvaria of 2-3 days old mice. The cells were treated with inhibitors of glutamate transporters, namely the Scl1a1 and Scl1a3 inhibitor -serine-O-sulfate (SOS, 0.1 mM, 0.2 mM, 0.4 mM) and the Scl1a1a-4 inhibitor -threo-hydroxypo-aspartate (THA, 0.25 mM, 0.5 mM, 1.0 mM) for 3 and 5 days. 1.25(OH)2D3 (10 nM) and TNF- (5 ng/ml) were also used. Gene expression was analyzed by real-time PCR. ALP activity and the number of viable cells were assessed. Glutamate concentrations were determined by the glutamate oxidase method.

**Results**

Transcripts encoding glutamate transporters (Scl1a1-5) and glutamate receptors (APAM3, Grina, Grinlaa and NMDA2D) were expressed in primary mouse osteoblasts. Expression levels of Scl1a1, Scl1a3 and Grina were upregulated by 1.25(OH)2D3 and TNF-α. Inhibition of Scl1a by SOS and THA led to an increase in the concentration of extracellular glutamate in a dose and time dependent manner. SOS and THA decreased osteoblast proliferation, but stimulated osteoblast differentiation in a dose dependent manner. The expression of osteocalcin and type I collagen, two markers of osteoblast differentiation was also upregulated upon inhibition of glutamate transporters. Scl1a block by SOS and THA acts synergistically with 1.25(OH)2D3 to stimulate osteoblast differentiation.

**Conclusion**

Our study demonstrates that glutamate transport is involved in osteoblast differentiation. Inhibition of Scl1a promotes osteoblast differentiation by increasing the concentration of extracellular glutamate. Scl1a transporters control the extracellular glutamate concentrations, and by this mechanism contribute to the stimulation of osteoblast differentiation and activation. This suggests that members of the Scl1a family of glutamate transporters can serve as potential therapeutic targets to modulate the differentiation of osteoblast lineage cells.

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### PP203

**Connectivity Map-based discovery of novel compounds that induce osteoblast differentiation**

A M Brunn1, J van de Peppel1, A van Kerkwijk2, M Janssen1, M Schreuders-Koedam1, T Strijl1, M Eijken1, J P T M van Leeuwen1 & B C J van der Eerden1

1Internal Medicine, Erasmus MC, Rotterdam, The Netherlands; 2Arcarios BV, Rotterdam, The Netherlands.

Osteoporosis is a common skeletal disorder characterized by low bone mass leading to increased bone fragility and fracture susceptibility. Little is currently known about what specific factors stimulate osteoblast differentiation from human mesenchymal stem cells (hMSCs). Therefore, the aim for this project is to determine novel factors and mechanisms involved in human bone production which can be targeted to treat osteoporosis, using gene expression profiling and bioinformatic analyses, including the Connectivity Map, as an in silico approach. Gene expression profiling was performed on hMSCs differentiated towards osteoblasts using Illumina microarrays. Osteogenic hMSC differentiation was assessed by analyses of alkaline phosphate activity (ALP) and mineralization by calcium assay and alizarin red staining. Gene expression was determined by qPCR. Immunofluorescent analysis was performed to examine changes in the cytoskeleton. Kegg analysis was performed to determine enriched pathways. The gene signature of osteogenic hMSCs (top significantly regulated genes 6 h after induction by dexamethasone) was uploaded into Connectivity Map (www.broadinstitute.org/cmap). This identified parbendazole as a compound with a statistically significant correlation gene signature to osteogenic hMSCs. Parbendazole stimulated osteogenic hMSC differentiation as indicated by increased ALP and mineralization, which interestingly occurs independent of the presence of glucocorticoids. Moreover, strong upregulation of glucocorticoid receptor target genes by glucocorticoids, is absent in parbendazole-treated cells. Parbendazole caused profound cell morphological and cytoskeletal changes including strong inhibition of microtubules. Kegg analysis of the gene signature indicated TGF-β signalling, mineral absorption, and MAPK signalling pathways were enriched. By combining genomic and bioinformatic tools against the backdrop of highly characterized human osteogenic differentiating hMSCs we have identified a novel bone anabolic candidate that induces osteoblast differentiation independent of glucocorticoid stimulation. In combination with the Kegg analysis we will identify important cellular processes and signalling cascades that can be manipulated to stimulate bone formation.

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### PP204

**Non-canonical BMP signaling in bone healing**

Gonzalo Sánchez-Duffhues1, Amaya Garcia de Vinuesa1, Peter Kloen2, Marié-Jose Goumans3 & Peter ten Dijke1

1Leiden University Medical Center, Leiden, The Netherlands; 2Academic Medical Center, Amsterdam, The Netherlands.

The healing of bone fractures is a tightly regulated process where released growth factors and cytokines interplay within an inflammatory environment in order to reestablish the functional bone. Recent studies have suggested that endothelial cells may dedifferentiate into mesenchymal multipotent cells via a mechanism called endothelial-to-mesenchymal-transition (EndoMT). Transforming growth factor-β (TGF-β) plays a critical role inducing EndoMT. Subsequent differentiation into mineralizing osteoblast-like is triggered by bone morphogenetic proteins (BMPs). TGF-β and BMPs are part of the TGF-β family of cytokines binding to type I and type II serine/threonine kinase receptors at the plasma membrane, which upon activation signal via Smad and non-Smad signaling pathways, including MAP kinases. Interestingly, they are targeted by the pro-inflammatory cytokines released upon a fracture as well, suggesting a convergence between BMPs and inflammation. Hereby we investigate how BMPs trigger osteoblast trans-differentiation under inflammatory conditions in endothelial cells, therefore uncovering their contribution to bone healing. We demonstrate that BMP-6 and BMP-9 induce very potently the trans-differentiation of endothelial cells into osteoblast-like cells. Noteworthy, the activity of BMP-9 was dramatically enhanced in combination with the pro-inflammatory cytokine TNF-α. Among different pro-inflammatory cascades activated in endothelial cells, down-regulation of the JNK MAP-kinase increased the mineralization of human and murine endothelial cells. Whereas BMP-6 potentiates TNF-α-induced-JNK activation, BMP-9 showed no effect. Finally, we compared the activation of JNK in endothelial cells from the capillaries in bone sections from normal versus delayed-healing patients. JNK and its downstream target c-jun were significantly more activated in fractures with delayed-healing, which also were weakly stained for BMP-9, in comparison to normal-healing fractures. The results presented here suggest a key role for non-canonical BMP pathways, and in particular JNK, on the differentiation of endothelial into osteoblast-like mineralizing cells. Furthermore, they may have application for bone tissue engineering and healing of bone fractures.

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### PP205

**Thrombin receptor deficiency leads to osteopetrosis by decreasing the RANKL/OPG ratio**

B CJ van der Eerden1, K Tudpor2, P Jongwattapaisan3, TE Woudenberg-Vreken1, RJM Bindels1, IJG Hoenderop2 & JPTM van Leeuwen1

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Communication between osteoblasts and osteoclasts is crucial for bone remodeling. Thrombin and its thrombin receptor (TR, PAR-1) are expressed in osteoclasts and osteoblasts, respectively. To date, the physiological roles of thrombin and TR in bone metabolism have not been elucidated. Therefore, we fully characterized the bone phenotype of mice lacking the thrombin receptor. We performed bone microarchitectural analyses of the femurs of 10–12 week old wild type (WT) and TR knockout (KO) mice, using three-dimensional micro-computed tomography (CT). Serum analyses were done for RANKL and OPG levels and in the urine of these mice, we measured the bone resorption marker deoxypyridinoline crosslinks (Dpd). Marine osteoblasts (MC-3T3) were cultured to study the effect of thrombin on RANKL and OPG production as well as on osteoblast signaling pathways, including the p42/44 ERK, PLC-β and PKC, using U0126, U73122 and chelerythrine, respectively, as specific inhibitors. Using CT, we found increased trabecular and cortical bone mass in TR KO mouse femurs compared to WT littermates. Trabecular bone thickness and connectivity were significantly enhanced. Increased bone mineral density (BMD) and decreased urinary DPD concentration in TR KO mice indicated a role for TR on both inorganic and organic phases of bone. Moreover, TR KO cortical bone expands and has a higher moment of inertia, implying stronger bone. Preliminary histological analyses did not reveal any abnormalities in the morphology of the femurs. Serum analysis showed a decrease in RANKL and an increase in OPG levels. In vitro experiments demonstrated a TR-dependent stimulatory effect of thrombin on RANKL mRNA expression and subsequent RANKL secretion into osteoblast culture medium. This effect was blocked by a p42/p44-ERK inhibitor. We conclude that the thrombin/TR system maintains normal bone remodeling by activating RANKL and limiting OPG synthesis by osteoblasts through the p42/44-ERK signaling pathway. TR deficiency inhibits osteoclastogenesis, resulting in osteoporosis.

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PP206

Serum sclerostin does not reflect its expression in bone, but is related to bone mineral density

Nathalie Bravenboer, Ruben Visser, Martin den Heijer & Annemieke Heijboer

VU University Medical Center, Amsterdam, The Netherlands.

Sclerostin is a major negative regulator of osteoblastic activity. Serum sclerostin has a weak positive association with BMD but contradictory results have been described concerning associations with fractures. These contradictions could be explained by the fact that serum sclerostin does not reflect its action in bone. In this study we question whether serum sclerostin is associated with its expression in bone. In addition we aimed to detect associations between sclerostin in serum or in bone and bone density.

Twenty six patients with Crohn’s disease and osteopenia were included. These patients were a subgroup from a large randomized clinical trial, investigating treatment with risodronate (Crohn and Bone study registered as NTR 163 Dutch Trial Register). Serum sclerostin was measured with two different ELISA’s, Mesoscale diagnostics (MD) and Biomedica Gruppe (BG). Sclerostin expression in bone was detected on iliac crest bone biopsies, using immunohistochemistry (mouse-human antibody by R&D systems) and measured as sclerostin positive area (Scl positive area) (NIS elements, Nikon). DEXA, using WHO standardizations, was used to obtain total hip (THP-BMD) and lower lumbar spine bone mineral density (LS-BMD).

Scl positive area and serum sclerostin correlated poorly. Scl positive area and SclRm were r=0.118 (95% CI -0.300-0.498). Interassay correlation between SclRm and SclRp was weak, r=0.062 (95% CI 0.326-0.432).

Scl positive area had a positive correlation with bone mineral density. Respectively for LS-BMD and THP-BMD, r=0.58(95% CI 0.000-0.664) and r=0.55 (95% CI 0.265-0.746). SclRm had a positive correlation only with LS-BMD, r=0.55 (95% CI 0.25-0.75).

Serum sclerostin does not reflect sclerostin status in bone tissue in this study, indicating care should be taken when interpreting serum sclerostin values. A positive correlation was detected between sclerostin expression in bone and bone mineral density. This correlation was reported in serum previously, but this was confirmed in only one of the two assays used in this study.

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PP207

In vitro 3D osteoblast-osteocyte co-culture mechanical loading model

Marisol Vazquez, Bronwen Evans, Sam Evans, Jim Ralphs, Daniela Riccardi & Deborah Mason

Cardiff University, Cardiff, UK.

Introduction

Normal mechanical loading potentially induces bone formation via effects on osteocytes. Current investigations of mechanical loading of bone do not reflect the interactions of the cells within it, mostly focusing on mechanical loading of osteoblasts in monolayers. Existing 3D models do not elucidate the osteoblast-osteocyte interactions that regulate mechanically-induced bone formation. We developed a novel in vitro 3D co-culture model of bone1 to investigate osteoblast-osteocyte interactions.

Methods

MLO-Y4 cells (1.5x106 cells per ml) were incorporated into acid-soluble rat tail tendon type 1 collagen (2 mg/ml in MEM, pH7.4) gels and MC3T3-E1 (1.5x105 cells/well) layered on top and cultured at 37 °C (DMEM 5% dialysed FBS) for 1 week. Co-cultures were fixed with 1% paraformaldehyde, infiltrated with OCT, cryosectioned and labelled with 1) phallolidin and DAPI to assess cell morphology, 2) ethidium homodimer and DAPI to assess cell viability, 3) immunostained using anti-connexin 43 antibody to assess cell connectivity, or 4) immunostained with anti-E11 antibody. Cell phenotype was determined by RT-qPCR of RNA extracted (TriZol) separately from surface osteoblasts (surface zone) and encased osteocytes (deep zone).

Results

Data show co-cultures survive, for at least one week, with osteocyte cell death, within gels, averaging 18.86±3.56% at day 1 and 14.11±2.60% at day 7 comparable to monolayer cultures. MC3T3-E1 and MLO-Y4 cells maintain their morphology, express Runx2, osteocalcin, Coll, ALP mRNA and E11 and connexin 43 protein. 3D MLO-Y4 monolayers released PGE2 after mechanical loading (preliminary data).

Conclusion

We have established a mouse osteoblast-osteocyte 3D co-culture system where MLO-Y4 cells form a network throughout the gel and respond to loading, overlaid with surface osteoblasts that express type I collagen. We are using this system to investigate mechanically-induced signals in osteocytes and osteoblasts.

References


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PP208

The positional origins of human osteoblasts dictate growth and differentiation potential and capacity for paracrine vascular cell cross-talk via VEGF

Mittal Shah1, Valentina Gburcik1, Andrew Sankey3, Peter Reilly5, Roger Emery2, Claire Clarkin1 & Andrew Pittsillides1

1Royal Veterinary College, London, UK; 2University of Southampton, Southampton, UK; 3Imperial College London, London, UK.

Successful long-term, cementless fixation of human shoulder components in osteoporotic (OP) and osteoarthritic (OA) patients poses major challenges. The possibility that enhanced osseointegration may rely on both the region of bone targeted and its relationship with the vasculature remains unexplored. We hypothesise that bone cells derived from subchondral (SC), cortical (C) and trabecular (Trb) bone regions exhibit differing osteogenic potential, which will be diminished in bones from OP patients. Primary osteoblasts from SC, Trb, C explants were obtained from OP (n=3) and OA (n=4) human patients undergoing shoulder arthroplasty and cell growth and gene/protein expression levels determined. Cell proliferation studies consistently illustrated that osteoblasts from all sites in OA patients exhibited 20% (P<0.05) greater growth rates than from OP. Furthermore, osteoblasts from SC and C showed enhanced rates of proliferation, compared to Trb sites (P<0.05) in both OA and OP. Induction of osteogenic differentiation was found to promote greater increases in ALP activity and Ostein and Runx2 mRNA levels in Trb and SC, than in C bone osteoblasts (P<0.05) in OA patients; all OP sites exhibited significantly smaller increases in ALP activity (P<0.05). Vascular endothelial growth factor (VEGF) is an osteoblast-derived signal which couples osteogenesis and angiogenesis. We found that media conditioned by Trb osteoblasts from OA contain highest (21%) increases in ALP activity, compared to Trb sites (P<0.05). Our data indicated: i) that osteoblasts from all osteoporoitic bone sites are likely to be compromised in their osteogenic potential, with limited growth, differentiation
and VEGF production and ii) that osteoblasts from trabecular bone exhibit least proliferation, but greatest differentiation and pro-angiogenic potential, suggesting that they may provide for superior osteointegration. Together, these findings suggest that human osteoblasts with distinct positional origins exhibit divergent osteogenic potential.

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PP209
Bone marrow stromal cells of female BAG-1 heterozygous mice exhibit reduced osteogenic potential
Joanna Greenhough, Paul Townsend, Richard Oreffo & Rahul Tare
University of Southampton, Southampton, UK.

The co-chaperone protein, Bcl-2-associated athanogene 1 (BAG-1) is expressed ubiquitously in bone including cells of the osteoestroid-lineage and, plays an important role in cell proliferation, apoptosis and differentiation by regulating signalling and transcription. The study aims to elucidate the function of BAG-1 in osteoblast development by examining differences in osteogenic differentiation of bone marrow stromal cells (BMSCs) from Bag-1+/− (heterozygous) and wild-type mice. BMSCs isolated from femora and tibiae of 14-week-old Bag-1+/− and wild-type mice were cultured for 28 days in basal and osteogenic (100 ng/ml rhBMP-2) media. Cells were harvested for analysis of proliferation by DNA assay, apoptosis by TUNEL staining, expression of differentiation stage-specific osteogenic genes by qPCR, Alkaline phosphatase (ALP) specific activity and Osteocalcin (OCN) production by ELISA.

BMSCs from Bag-1+/− female mice failed to undergo osteogenic differentiation in response to BMP-2, unlike BMSCs from wild-type female mice that responded to BMP-2 by significantly upregulating ALP and OCN expression in day 28 cultures. Interestingly, in osteogenic cultures, BMSCs from Bag-1+/− female mice proliferated at a significantly higher rate throughout 28 days of culture compared to their wild-type counterparts. In contrast, BMSCs from male Bag-1+/− mice exhibited robust osteogenic differentiation, comparable to the osteogenic response by BMSCs from male wild-type mice. In osteogenic cultures, BMSCs from Bag-1+/− male mice proliferated at a significantly higher rate than their wild-type counterparts between days 1 and 14, while proliferation of BMSCs from both groups decreased between days 14 and 28 of culture. In both female and male mice, no differences in apoptosis were observed between the wild-type and heterozygous groups.

Thus, in female mice heterozygous for Bag-1, proliferation of BMSCs was enhanced at the expense of osteogenic differentiation. These studies indicate an important role for Bag-1 in osteoblast development and the need to understand the role of interacting factors modulating gender differences.

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PP211
Rab27a is involved in bone formation by osteoblasts
Fraser Coxon1, Angela Douglas1,3, Alun Hughes1, Miep Helfrich1, Miguel Seabra2 & Tanya Tolmachova2
1University of Aberdeen, Aberdeen, UK; 2Imperial College, London, UK.

The Rab family GTPases Rab27a and Rab27b play an important role in the trafficking of lysosome-related organelles in specialised cells, such as melanocytes. Since secretory lysosomes, also considered a lysosome-related organelle, are important for osteoclast and osteoblast function, we hypothesised that Rab27 plays a role in bone physiology. In support of this, a recent study demonstrated impaired transport of RANK ligand to the plasma membrane in osteoblasts from mice lacking the Rab27 effector Munc13-4. To assess the potential role of Rab27 in bone cells, we analysed the bone phenotype of mice lacking RAB27a and RAB27b (Rab27a−/− Rab27b−/−; DKO). MicroCT analysis of 8-week-old DKO mice revealed 25% lower bone volume than WT mice. This is likely due to impaired osteoblast function, since Rab27a was detected in mouse calvarial osteoblasts, and increased during differentiation, whereas expression of Rab27a markedly decreased during osteoclast differentiation. Rab27b could not be detected in either cell type, suggesting that Rab27b, but not Rab27a, is important for osteoblast function. Surprisingly, overexpressed GFP-Rab27a localised mainly to the Golgi apparatus rather than lysosomes in osteoblasts. Nevertheless, in support of a role for Rab27 in osteoblasts, mineralisation by differentiated DKO osteoblasts in vitro was slightly reduced compared to WT osteoblasts, whereas there were no differences in the formation or activity of osteoclasts generated from WT or DKO mice. However, the formation of osteoclasts in co-cultures of bone marrow cells with osteoblasts from DKO mice was not impaired, indicating normal RANK ligand trafficking in osteoblasts lacking Rab27. These data therefore suggest that Rab27a is important for bone formation by osteoblasts, but not for stimulation of osteoclastogenesis, where other Rab, that also use Munc13-4 as an effector, may be involved.

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PP212
Bone-forming cultures of rat and mouse calvarial osteoblasts: key differences in protocols
Isabel Orriss1, Mark Hajjawi1, Carmen Huesa2, Vicky MacRae2 & Timothy Arnett1
1University College London, London, UK; 2The Roslin Institute and Royal (Dick) School of Veterinary Studies, Edinburgh, UK.

The in vitro culture of calvarial osteoblasts from neonatal rodents remains an important method for studying the regulation of osteoblast function. Widespread use of transgensics has created a particular need for a reliable, simple method that allows the differentiation and bone-forming activity of mouse osteoblasts to be studied directly. We have established such a method and have identified key differences in optimal culture conditions between mouse and rat osteoblasts. Cells, isolated from neonatal rat or mouse calvariae by bacterial collagenase digestion, were cultured for 14–28 days before staining for alkaline phosphatase (TNAP) and bone mineralisation (Alizarin Red). Rat cells typically required ~14 days in culture, whilst mouse osteoblasts had to be grown for 21–28 days. We found that reliable differentiation of mouse osteoblasts, resulting in abundant TNAP expression and the formation of discretely mineralised collagenous ‘trabecular’ bone elements, occurred only in αMEM culture medium with 10% heat-inactivated FCS (HI-FCS). Dexamethasone had no effect on bone mineralisation or TNAP expression in mouse osteoblasts. In contrast, TNAP expression and bone formation by rat osteoblasts occurred in both αMEM and DMEM culture media (although ~4-fold more efficiently in αMEM), supplemented with either FCS or HI-FCS, and was strongly dependent on dexamethasone (10 nM). Both mouse and rat osteoblasts required β-glycerophosphate (2 mM) and ascorbate (50 μg/ml) for osteogenic
differentiation, and both showed similar sensitivity to exogenous ATP (10 μM), a well-established inhibitor of mineralisation. The high efficiency of osteogenic differentiation observed in zMEM, compared with DMEM (which we have previously used for rat osteoblast cultures) probably reflects the much richer formulation of the former; zMEM contains many additional amino acids (including proline), vitamins and other supplements. These findings should prove useful not only to those wishing to culture mouse osteoblasts successfully but also for laboratories requiring more efficient routine culture of bone-forming rat osteoblasts.

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**Cell biology: osteoclasts and bone resorption**

**PP213**

**Anti-dementia acetylcholine esterase inhibitor inhibits osteoclastogenesis**

Charles Inderjeeth1,2, Jiake Xu1, Bay Si Lim1 & Dian Teguh1

1University of Western Australia, Perth, Western Australia, Australia; 2North Metropolitan Health Service, Perth, Western Australia, Australia.

Background

Alzheimer’s dementia (AD) and osteoporosis (OP) are common and parallel ageing and frequently coexist in an ageing population. Low BMD appears related to increased risk of AD. Various clinical conditions have been shown to alter acetylcholine (Ach) signalling and affect bone. Ach receptor (AChR) subunits and Ach esterase (AChE) are expressed in bone. Presynaptic Botox inhibit Ach release and impair bone healing and decrease bone mineral content. Poliomyelitis destroys motor neurons that use Ach. Patients have impairecd bone growth and a larger proportion develop OP. Smoking; high nicotin e; interact and desensitize nAChR affecting bone remodelling negatively. Anti-dementia drugs inhibit AChE. Donepezil and Rivastigmine bind nAChER and mAChER. Galantamine binds mAChER only. We hypothesised that acetylcholine esterase inhibitors (AChEIs) as used in dementia management would reduce osteoclastogenesis if this was the mechanism of bone effect of Ach.

Methods

BMMs are seeded at 6 × 10^3 cells/well in 96-well plate. Before RANKL stimulation, cultures were incubated with the drugs Donepezil and Galantamine at equimolar concentrations of 0.1–5.0 μM. Control plates were incubated with and without RANKL only for comparison.

Results

At equimolar concentrations Donepezil inhibit, whereas Galantamine enhances osteoestrogenesis. Donepezil inhibited osteoclastogenesis at the lowest concentration of 0.1 μM.

Conclusion

This data suggests Ach may be important in bone biology. AChEIs that bind to nAChR may have the additional benefit of reducing osteoclastogenesis. Donepezil and Galantamine both bind nAChER. This finding is consisten t with a recently published case control study confirming the protective effect of Donepezil and Rivastigmine but not Galantamine in hip fracture reduction. This may have important implications for osteoporosis management in older and dementia populations.

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**PP214**

**Is a network of collagen fibers and blood vessels supporting pre-osteoclast trafficking from the bone marrow to the bone surface?**

Thomas Levin Andersen, Helene Bjørg Kristensen & Jean-Marie Delaissé

Clinical Cell Biology (KCB), Institute of Regional Health Science, University of Southern Denmark, Vejle Hospital, Vejle, Denmark.

Differentiation of osteoclast progenitor cells into mononucleated TRAcP+ pre-osteoclasts occurs in the bone marrow. But how are these cells dispatched to the future bone resorption sites? We hypothesized that the collagen type III/I-rich reticulin network of the bone marrow might provide a structural framework for localization and migration of differentiating pre-osteoclasts towards the bone surface. Therefore, adjacent sections from decalcified paraffin-embedded iliac crest biopsies from 11 human controls were either stained for reticular fibers, or double-stained-immunostained for collagens and cell markers. The association between mononuclear cells positive for osteoclast markers and capillaries or collagen fibers was quantified through histomorphometry, and further analyzed by 3D-reconstructions. Numerous mononuclear TRAcP+ cells were identified within the bone marrow. These cells stained also for other osteoclast markers such as OSCAR and cathepsin K, demonstrating that they are pre-osteoclasts. Staining for reticulin, collagen type I, III, and CD34, combined with 3D-reconstructions, revealed collagen III/I-rich reticulin fibers forming a network throughout the bone marrow. These fibers were connected to the blood vessel network and to bone remodeling compartment canyens, forming a continuum with the collagen present in these structures. Interestingly, double-immunosstainings revealed that 93% of the TRAcP+ or OSCAR+ pre-osteoclasts were associated with these collagen fibers and with the collagen of the vascular wall. In conclusion, the close association of pre-osteoclasts with the collagen III/I-rich reticulin and blood vessel networks supports the hypothesis that these linear structures provide a physical support for trafficking of differentiating pre-osteoclast towards the bone remodeling compartment canyens covering resorptive surfaces.

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**PP215**

**The F-actin modulator SWAP-70 is required for proper podosome dynamics in osteoclasts**

Anne Roscher1, Martin Glössmann2, Reinhold G Erben3, Anne-Helen Lutter1, Michael Chopin1, Lorenz C Hofbauer3, Rolf Jessberger3 & Annette Garbe1

1Institute of Physiological Chemistry, Dresden University of Technology, Dresden, Germany; 2Department of Biomedical Sciences, University of Veterinary Medicine, Vienna, Austria; 3Division of Endocrinology and Bone Diseases, Dresden University Medical Center, Dresden, Germany.

Bone remodeling is a crucial process to maintain a healthy bone structure in order to avoid diseases like osteoporosis or osteoepetrosis. Osteoclasts contribute to this process by resorbing old and brittle bone allowing osteoblasts to renew the bone substance. During resorption osteoclasts rearrange their actin cytoskeleton by forming an F-actin ring generating a resorptive cavity on the bone surface. Recently, we reported that the F-actin binding protein SWAP-70 regulates osteoclast function by modulating the actin cytoskeleton. Swap-70−/− osteoclasts fail to form an F-actin ring resulting in diminished resorptive function, while cytokine induced osteoclast differentiation markers remain unchanged. Swap-70−/− mice develop osteoporosis characterized by increased bone mineral density and impaired osteoclast function. SWAP-70-proficient bone marrow transplantation into Swap-70−/− mice restores osteoclast resorption capacity in vivo suggesting that the osteoclast defect is intrinsic. Ectopic expression of SWAP-70 in Swap-70−/− osteoclasts in vitro restores F-actin ring formation and osteoclast function. By expression of SWAP-70 variants we show that a functional pleckstrin homology domain as well as the acidic part of the coiled-coil region of SWAP-70 are indispensable for osteoclast function. We now have evidence that Swap-70−/− osteoclasts fail to cluster podosomes during osteoclastogenesis and are impaired in organizing podosome-based F-actin rings and an F-actin belt. These data identify SWAP-70 as a crucial player of podosome dynamics in osteoclasts.

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**PP216**

**Glycosaminoglycan sulfation is a key regulator of osteoclast biology and osteogenic bone cell signaling**

Juliane Salbach-Hirsch1, Elena Tsouri1, Nicole Ziegler1, Vera Hintze2, Dieter Schambrauch3,1, Matthias Schnabelrauch4,5, Martina Rau8 & Lorenz Hofbauer1,2

1Division of Endocrinology, Diabetes and Bone Diseases, Dresden University Medical Center, Dresden, Germany; 2Max Bergmann Center of Regenerative Therapies Dresden, Technische Universität Dresden, Dresden, Germany; 3Biomaterials Department, INNOVENT e.V., Jena, Germany; 4Center for Soft Matter, Jena, Germany; 5Center for Regenerative Therapies Dresden, Technische Universität Dresden, Dresden, Germany.

In light of prolonged life expectancy, the need for biomaterials that govern bone regeneration increases. Improved bone regeneration and osseointegration can be achieved by functionalizing implant materials. The extracellular matrix (ECM) affects differentiation of bone cells and is critical for bone regeneration. Here we assessed the role of the natural occurring bone ECM glycosaminoglycans (GAGs) hyaluronan (HA) and chondroitin sulfate (CS), and their sulfated derivatives, on osteoclast directed effects for implant functionalization. The impact of native and sulfate-modified GAGs on viability, morphology, differentiation, gene expression and cell signaling was studied using murine
primary cells, the murine RAW264.7 and MLO-Y4 cell lines as models for osteoblasts, osteoclasts and osteocytes respectively. In response to a direct stimulation with 200 μg/ml native and high-sulfated GAGs profound effects on all stages of osteoclast differentiation were observed. GAG sulfate modification increased the viability of osteoclasts (P < 0.05). However, tartrate resistant acid phosphatase (TRAP)-staining and immunofluorescence of regular sealing zone structures in osteoclasts were profoundly decreased (P < 0.05). This was accompanied by a loss of resorptive activity up to 40% compared to cells exposed to native GAG (P < 0.01) and decreased mRNA levels of osteoclastic marker genes, such as cathepsin K, osteoclast-associated receptor, TRAP (P < 0.05). The viability of osteoblasts and osteocyte-like cells treated with equal concentration of GAGs was not affected. These cells showed increased RANKL/OPG ratios (P < 0.05) and decreased SOST expression levels (P < 0.05). Correspondingly, supernatants collected from these cells suppressed osteoclastogenesis (P < 0.05) but did not affect adhesion and viability. Using surface plasmon resonance, we demonstrated that GAGs can directly bind to OPG, but not RANKL, in a sulfation degree dependent manner resulting in modified OPG bioactivity.

In summary, sulfation of GAGs reduces osteoclastogenesis and pro-osteoclastogenic signaling from osteogenic cells and may represent a useful tool to control enhanced osteoclastic activity and bone loss adjacent to implant surfaces.

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PP217
Collagen-induced arthritis reduces osteoclast differentiation potential and activity and impairs reactivity to neurotransmitter stimuli in an experimental arthritis rat model

Dominique Muschter1, Nicole Schäfer1, Rainer H Straub1, Joachim Grifka2 & Susanne Grüssel1

1Department of Experimental Orthopedics, University Hospital Regensburg, Regensburg, Germany; 2Department of Orthopaedic Surgery, University Hospital Regensburg, Regensburg, Germany.

Osteoclast (OC)-mediated bone destruction is a key feature of rheumatoid arthritis (RA). In RA synovial tissue a reduced density of catecholaminergic nerve fibres has been observed. Studies on sweat gland innervation proved that catecholaminergic fibres have the ability to undergo a phenotypic transition to cholinergic nerves. The sympathetic neurotransmitters norepinephrine (NE), acetylcholine (ACH), and vasoactive intestinal peptide (VIP) affect osteoclastogenesis oppositely and in this context we wanted to study osteoclastogenesis at different phases of collagen-induced arthritis (CIA) in DA rats. The influence of NE, ACH, and VIP on differentiation and activity of bone marrow macrophage-derived osteoclasts from CIA and control animals are compared at various time-points post immunization (pI). The expression profile for NE, ACH, and VIP neurotransmitter receptors is analyzed on mRNA and protein level. OC numbers were tendentially lower in arthritic animals. ACH stimulation markedly elevated OC formation in controls (15 and 40 days pI). NE decreased osteoclastogenesis via β2-adrenoceptors and enhanced via α-adrenoceptor stimulation. VIP time-point dependently inhibited (10 and 15 days pI) or stimulated (20 and 40 days pI) osteoclastogenesis. Cells from arthritic animals were less affected. By trend, osteoclasts from arthritic animals showed decreased activity in a catepsin K activity and in a matrix degradation assay. Reduced gene expression changed in the time course of arthritis. 20 days past immunization muscarinic ACh receptors M3 and M5 were significantly upregulated, whereas VIP receptor PACR1 was significantly downregulated. After 40 days adrenoreceptors α1D and α2B were significantly downregulated. So far, on protein level we analyzed β2 adrenoceptor expression and localization and could not find any CIA-induced changes.

We conclude that CIA suppresses OC differentiation and activity as well as reactivity to neurotransmitter stimulation but the underlying processes remain unknown as yet. NE, ACH, and VIP receptor gene expression was affected time-point dependently but the physiological impact needs further investigation.

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PP218
Protective effect of polyphenols from berries of Aronia melanocarpa against low exposure to cadmium-induced imbalance in the RANKL/-RANKL/OPG system in the bone tissue of rats

Joanna Rogalska & Malgorzata M Brzóka

1Department of Experimental Orthopedics, University Hospital Regensburg, Regensburg, Germany; 2Department of Orthopedic Surgery, University Hospital Regensburg, Regensburg, Germany; 3Department of Internal Medicine I, Experimental Rheumatology and Neuroendocrinology, University Hospital Regensburg, Regensburg, Germany.

Bone is constantly being formed and shaped by the action of osteoclasts and osteoblasts. A proper equilibrium between both cell types metabolic activities is required to ensure an adequate skeletal tissue structure, and it involves resorption of old bone and formation of new tissue. It is reported that treatment with anti-epileptic drugs (AEDs) can elicit alterations in skeletal structure, in particular in bone mineral density. Nevertheless, the knowledge regarding the effects of AEDs on bone cells are still scarce, particularly on osteoclastic behavior. In this context, the aim of this study was to investigate the effects of five different AEDs on human osteoclastic cells. Osteoclastic cell cultures were established from precursor cells isolated from human peripheral blood, and were maintained in the absence (control) or in the presence of 10–3–10–6 M of different AEDs (valproate, carbamazepine, gabapentin, lamotrigine, and topiramate). Cell cultures were characterized throughout a 21-day period for tartrate-resistant acid phosphatase (TRAP) activity, number of TRAP+ multinucleated cells, presence of cells with actin rings and expressing vimentin and calcitonin receptors, and apoptosis rate. Also, the involvement of several signaling pathways on the cellular response was addressed.

All the tested drugs were able to affect osteoclastic cell development, although with different profiles on their osteoclastogenic modulation properties. Globally, the tendency was to inhibit the process. Furthermore, the signaling pathways involved in the process also seemed to be differentially affected by the AEDs, suggesting that the different drugs may affect osteoclastogenesis through different mechanisms.

In conclusion, the present study showed that the different AEDs had the ability to negatively modulate the osteoclastogenesis process, shedding new light towards a better understanding of how these drugs can affect bone tissue.

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Hydroxyapatite (HA) has been widely used as a biocompatible ceramic in many areas of medicine, mainly for contact with bone tissue, due to its resemblance to mineral bone. Owing to the nanostructures of bone tissue, new nano-HA-based materials are among the most promising challenges in bioactive ceramics. Recently, it was observed that fluoroquinolones have the ability to interfere with osteoclastogenesis, in standard polystyrene cell culture plates. The aim of this work is to assess the osteoclastogenic-modulation properties of different fluoroquinolones in cell cultures performed over HA surfaces with nano- and micro-structured topography (nHA and mHA respectively). The sinterization temperature used was 830°C (nHA) and 1300°C (mHA). The HA disks were analysed by scanning electron microscopy (SEM). Osteoclastic precursor cells were isolated from the peripheral blood. Cells were seeded over HA disks. Cell cultures, maintained for 21 days in the presence of M-CSF and RANKL and treated with $0.3 \times 10^{-5}$–$0.3 \times 10^{-7}$ M norfloxacin, ciprofloxacin and levofloxacin, were characterized for total protein content, cellular morphology, presence of cells with actin rings and expressing vitronectin and calcium receptor, TRAP activity and HA resorption ability. In addition, the involvement of some osteoclastogenic-associated signalling pathways on the observed cellular response was also addressed.

Osteoclastogenic behaviour of cell cultures was modulated by the HA surface, with a high osteoclast differentiation degree being observed over nHA disks. The presence of the tested fluoroquinolones was able to elicit changes in the cellular response. Namely, in all tested conditions, the osteoclastic response was either increased or not affected by these molecules. The relative contribution of the analysed signalling pathways was also modulated by fluoroquinolones. In conclusion, the present work may contribute to a better understanding of the potential effects of fluoroquinolones on bone tissue, particularly in contexts where it is important to ensure proper bone tissue regeneration.

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**PP221**

Modulation of osteoclastogenesis by antihypertensive drugs
Teresa Oliveira1,2, João Costa-Rodrigues1, Ricardo Ferraz1,2, Cristina Prudêncio1–4 & Maria Fernandes1
1Laboratory for Bone Metabolism and Regeneration, Faculdade de Medicina Dentária, Universidade do Porto, Porto, Portugal; 2CISA/COB, Escola Superior de Tecnologia da Saúde do Porto, Instituto Politécnico do Porto, Porto, Portugal; 3REQUIMTE-CQFB, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Lisboa, Portugal; 4Centro de Farmacologia e Biologia Química (U28-FCT), Faculdade de Medicina, Universidade do Porto, Porto, Portugal.

Despite its rigid structure, bone is a dynamic tissue that is in constant remodeling. This process requires the action of the bone-resorbing osteoclasts and the bone-synthesizing osteoblasts. One of the adverse effects attributed to some antihypertensive agents is the ability to alter normal bone metabolism. However, their effective actions on human bone cells remain to be clarified. In this work, the effects of five calcium channel blockers, a class of antihypertensive drugs (AHDs), were investigated on osteoclastic differentiation.

Osteoclastic cell cultures were established from precursor cells isolated from human peripheral blood, and were maintained in the absence (control) or in the presence of $10^{-7}–10^{-4}$ M of different AHDs (amlodipine, felodipine, diltiazem, lercanidipine, and nifedipine). Cell cultures were characterized throughout a 21-day period for tartrate-resistant acid phosphatase (TRAP) activity, number of multinucleated cells, presence of cells with actin rings and expressing vitronectin and calcium receptors, and apoptosis rate. Also, the involvement of several signaling pathways on the cellular response was addressed. It was observed that the tested AHDs had the ability to differentially affect osteoclastogenesis. At low doses, amlodipine and felodipine caused an increase on osteoclastic differentiation, while the other drugs inhibited it. At higher doses, all the molecules caused a decrease on the process. The tested AHDs also showed different effects on the analysed signaling pathways. In conclusion, AHDs appeared to have a direct effect on human osteoclast precursor cells, affecting their differentiation. Interestingly, some of them increased while others inhibited the process. Unraveling the mechanisms beneath these observations might help to explain the adverse effects on bone tissue described for this drug class.

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**PP222**

Mitogen- and stress-activated protein kinase 1 activates osteoclastogenesis in vitro and plays critical roles in bone destruction in vivo
Seong-Hee Kim & Jeong-Ok Kwon
Seoul National University, Seoul, Republic of Korea.

Osteoclasts are cells specialized for resorption of calcified tissue. Osteoclasts are formed from precursor cells of monocyte lineage under the control of receptor activator of nuclear factor kappaB ligand (RANKL). Mitogen- and stress-activated protein kinase 1 (MSK1) has been reported to be an important regulator of immune response and mitogenic signaling. In this study, we for the first time found that MSK1 was activated by RANKL in osteoclast precursor, bone marrow macrophages. The inhibition of upstream kinases, ERK1/2 and p38, but not JNK, could suppress the MSK activation upon RANKL stimulation. An MSK1 inhibitor efficiently repressed the induction of c-Fos and NFATc1 and the phosphorylation of CREB by RANKL. Besides, the inhibition of MSK1 successfully blocked RANKL-induced osteoclastogenesis. In addition, knock-down of MSK1 using siRNA significantly inhibited osteoclastic differentiation. The induction of c-Fos and NFATc1 and the phosphorylation of CREB and ATF2 were also inhibited by siRNA. Moreover, the knockdown of MSK1 could significantly block the recruitment of c-Fos to the NFATc1 promoter upon RANKL stimulation. NFATc1 retrovirus transduction almost completely rescued the defect in the differentiation of MSK1-silenced BMMs. Furthermore, in vivo knockdown of MSK1 could protect RANKL-induced osteoclastogenesis and bone erosion. Therefore, MSK1 is an important novel molecule involved in RANKL signaling and osteoclast differentiation.

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**PP223**

Methylation is not involved in repression of ADRA2A in osteocytes
Vid Mlakar, Janja Zupan, Janja Marc & Simona Jurkovic Mlakar
Faculty of Pharmacy, University of Ljubljana, Ljubljana, Slovenia.

Osteoporosis is an age related disease characterised by a progressive decrease of bone mineral density and loss of bone quality. Twin studies show that genetic component contributes up to 85% of the BMD variability of population. Surprisingly little variability of BMD can be explained by genetic polymorphisms (~2.9%). This highlights the complex genetic architecture and suggests that many other molecular processes and genes have to be involved. Our previous research showed that ADRA2A is deregulated in osteoporotic bone osteoblasts in comparison to osteoarthritic bone osteoblasts. In addition, it has been known for some time that adrenergic stimulation results in osteoclast differentiation leading to bone resorption. There are numerous evidences that this happens through adrenergic receptors β2 (AR2β) signalling. The same role is apparently performed by two other AR (ADRA2A and ADRA2C). Double AR knock-out female mice have a high bone mass phenotype. Additionally, it has been shown that such mice also exhibit lower tartrate-resistant acid phosphatase (TRACP) and receptor activator of NF-κB (RANK). In order to show that ADRA2A could be involved in bone turnover in men its expression and methylation in bone cells were investigated. Expression of ADRA2A was investigated using immunohistochemistry. Methylation analysis of ADRA2A promoter region was performed on DNA samples isolated from 65 individuals using high resolution melting (HRM) curve analysis, single strand conformation analysis (SSCA) and automatic sequencing. The results show that ADRA2A is expressed by osteoblasts and lining cells but not osteocytes. Methylation analysis did not reveal methylation of ADRA2A promoter DNA. The study showed that ADRA2A may play important role in adrenergic signalling in osteoblasts and lining cells. Although ADRA2A promoter is rich with CpGs and therefore a good target for repression by methylation, other mechanisms must be responsible for ADRA2A repression in osteocytes.

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Polyunsaturated fatty acids and phytoestrogens modulate osteoclastogenesis and bone resorption in raw 264.7 macrophages

Natalie Shepherd1, Cassandre De Jager2, Abe Kasonja1, Sumari Marais1, Yakko Touwen1, Marlena Kuker3 & Magdalena Coetzee1
1Department of Physiology, Faculty of Health Sciences, University of Pretoria, Pretoria, South Africa; 2Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, New Zealand.

Osteoclasts are produced by fusion of pre-osteoclasts derived from stem cells of the monocyte/macrophage lineage in the presence of receptor activator of NF-κB ligand (RANKL) produced by osteoblasts. The phytoestrogens; genistein and daidzein, which are isoflavones found in Leguminosae such as soybeans, are currently being investigated for prevention of postmenopausal osteoporosis. Some polyunsaturated fatty acids (PUFAs) have been shown to exert a bone protective effect in certain communities. The aim of this study was to determine the in vitro effects of genistein and daidzein together with the PUFAs, arachidonic acid (AA) and docosahexaenoic acid (DHA) on osteoclastogenesis and bone resorption.

For osteoclast formation, RAW 264.7 murine macrophages were grown in the presence of RANKL (15 μg/ml), 0.02% ethanol (vehicle control), PUFAs (20 μM–80 μM) and phytoestrogens (10−7 M–10−5 M) for 5–7 days, stained for tartrate resistant acid phosphatase and the number of multiloculated osteoclasts quantified. Bone resorption assays were conducted on osteotest plates coated with an inorganic synthetic bone surface. After 7 days of incubation, cells were washed off and after performing a von-Kossa stain, resorption was observed with a microscope. Three experiments were conducted in quadruplicate.

Results show that the formation of multiloculated osteoclasts was dose-dependently inhibited by exposure to either PUFAs or phytoestrogens. When the cells were exposed to these compounds, resorption pits on the bone analogue plates were smaller compared to the vehicle control. This observation may be attributed to a decrease in the number or activity of mature resorbing osteoclasts. Combination of PUFAs and phytoestrogens resulted in major inhibition of osteoclastogenesis and resorption pit formation suggesting an additive effect of these compounds in this model. PUFAs and phytoestrogens may have a protective effect on bone in vitro and combining these agents may exacerbate the inhibition. More work is required to confirm these findings and to clarify the possible mechanisms involved.

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Inhibition of lipopolysaccharide induced osteoclast formation and bone resorption in vitro and in vivo in mice by cystin C

1Molecular Periodontology, Umeå, Sweden; 2Center for Bone and Arthritis Research (CBAR), Gothenburg, Sweden; 3Institute of Chemistry, Gdańsk, Poland; 4Clinical Chemistry and Pharmacology, Lund, Sweden; 5Pharmacology and Molecular Sciences, Medivir, Stockholm, Sweden; 6Institute of Biochemistry (CAU), Kiel, Germany.

RANKL induced osteoclastogenesis is mediated by several transcription factors such as NF-κB, AP-1 and Nfatc1. We have found that also cysteine proteinases are involved in the signaling pathway downstream RANK. Thus, cystatin C, Z-RVLG-CHN2 (the sequence of which is based upon one of the enzyme inhibitory domains in cystatin C) and the fungal molecule E-64 – inhibit RANKL induced mouse and human osteoclast formation in vitro (Strässberg et al. in revision). Here, we demonstrate that osteoclastogenesis stimulated by lipopolysaccharide (LPS) E.coli (10 μg/ml) in RANKL-primed (4 ng/ml RANKL for 24 h) mouse bone marrow macrophages (BMM) is inhibited by cystatin C, Z-RVLG-CHN2, and E-64. The effect was associated with decreased LPS-induced mRNA expression of Cox2, Csk, Csrc, Cfos, and Nfatc1, and protein expression of Nfatc1 and c-Fos. Using fluorescent tagged cystatin C, we found that cystatin C was taken up by BMM, but only in LPS stimulated cells. Inhibition of osteoclastogenesis by cystatin C was observed also using LPS stimulated BMM on bone slices. Cystatin C inhibited LPS induced upregulation of JunB, Fra-2, p52, RelB, and IκBα mRNA. All three cysteine proteinase inhibitors using BMM from cathespin K deficient mice also inhibited osteoclast formation. Similarly, the cathespin K inhibitor L-908235 did not inhibit osteoclast formation. The data suggest that cystatin C inhibits osteoclast formation by inhibiting LPS-induced differentiation of osteoclast progenitors and that the effect is due to inhibition of signaling pathways downstream TLR-4 known to be important in RANKL-induced osteoclastogenesis. Most importantly, LPS-stimulated osteoclast formation in skull bones of adult mouse was inhibited by E-64, as assessed by counting the number of cathespin K+ osteoclasts. These data indicate that i) cysteine proteinases are important in LPS- and RANKL-induced osteoclastogenesis both in vitro and in vivo, and ii) inhibition of osteoclast formation is not explained by inhibition of cathespin K activity.

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Regulation of osteoclastogenesis by toll-like receptor 5

Ali Kassem1, Pernilla Lundberg3, Catharina Lindholm1, Pedro C. Souza1 & Ulf H. Lerner1,2
1Molecular Periodontology, Umeå University, Umeå, Sweden; 2Centre for Bone and Arthritis Research at Institute for Medicine, Sahlgrenska Academy at University of Gothenburg, Gothenberg, Sweden, 3Department of Physiology and Pathology, University of São Paulo State, Araraquara, Brazil.

Infections within or in the vicinity of the skeleton induce osteolytic diseases such as periodontitis, septic arthritis, osteomyelitis. Although host production of osteotropic cytokines is crucial, the precise mechanism by which pathogen associated molecular patterns induce osteoclastogenesis and bone loss is not fully understood. Recognition of these molecules by pattern recognition receptors is highly preserved through evolution with trans-membrane Toll-like receptor (TLR) family as the key component. Many infectious bacteria express flagella for motility and sensing with flagellin as the principal substituent recognized by TLR5. We have studied how activation of TLR 5 affects bone resorption, osteoblasts and osteoclast progenitors.

TLR5 activation by flagellin from S. Typhimurium and B. Subtilis increased Ca-release from mouse calvarial bones. This was associated with increased expression of the osteoclastic genes Cpsf5, Csk, Ocsar, and C-Fos and enhanced bone matrix degradation, as assessed by release of the collagen fragment CTX. The TLR5 agonists increased the mRNA and protein expression of RANKL and reduced OPG mRNA and protein. Recombinant OPG inhibited Ca-release triggered by TLR5 activation. TLR5 activation also increased mRNA expression of Cox2, but flagellin induced bone resorption was independent of prostaglandins. Osteoblasts isolated from the peristeme of calvarial bones expressed Tlr5 mRNA and stimulation of these cells with flagellin induced RANKl mRNA and suppressed Opg mRNA. In contrast to activation of TLR4, which results in robust inhibition of RANKL stimulated osteoclast formation in mouse bone marrow macrophage cultures (BMM), TLR5 stimulation did not inhibit RANKL signaling in early osteoclast progenitors. Similar to activation of TLR4, TLR5 agonists stimulated osteoclast formation in RANKL-primed BMM. These data show that TLR5-signaling stimulates periosteal osteoclast formation and bone resorption by enhancing RANKL/OPG ratio in osteoblasts and enhances osteoclastogenesis in RANKL-primed BMM.

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RANKL immobilized on β-TCP induces and maintains osteoclast formation

John Chou1,2, Wilhelm Hofstetter1 & Frank M Klenke1,2
1Group for Bone Biology and Orthopaedic Research, Department of Clinical Research, University of Bern, Bern, Switzerland; 2Department of Orthopedic Surgery, Inselspital, Bern University Hospital, Bern, Switzerland.

β-tricalcium phosphate (β-TCP) biomaterials have been approved for the repair of osseous defects. However, in large defects, the substitution of biomaterial by autogenic bone is inadequate to provide sufficient long-term mechanical stability. We aimed to develop composites of β-TCP ceramics and receptor activator of nuclear factor-κ B ligand (RANKL) to enhance the formation of osteoclasts thereby stimulating material resorption. RANKL was immobilized on β-TCP ceramics by i) superficial adsorption (passive short-term release) and ii) co-precipitated together with calcium phosphate, resulting in an incorporation of the protein into a crystalline layer of calcium phosphate and a cell-mediated long-term release. Murine osteoclasts precursors were seeded onto the ceramics. After 15 days, the formation of osteoclasts was evaluated with tartrate-resistant acidic phosphatase (TRAP) staining and quantified with TRAP-activity. Additionally, the expression of the osteoclast markers calcitonin receptor and cathespin K were quantified by real-time PCR. Superficially adsorbed RANKL
did not induce the formation of osteoclasts on β-TCP ceramics. When co-precipitated onto β-TCP ceramics RANKL induced the formation of osteoclasts as demonstrated by positive TRAP-stainings and a 2-fold increase of TRAP+ area which was similar to the controls. Development of osteoclast lineage cells was further confirmed by an increased expression of cathepsin K and calcitonin receptor. Our study shows for the first time that RANKL immobilized on β-TCP ceramics induces the formation of osteoclasts. However, osteoclast formation requires a long-term release system of RANKL. RANKL co-precipitation may induce osteoclast differentiation due to a residual passive release of the protein. Subsequently, matured osteoclasts mediate the release of RANKL by resorbing the protein containing calcium phosphate layer, thereby perpetuating their differentiation and activation. It remains to be proven whether the formation of osteoclast leads to a stimulation of biomaterial resorption. Experiments focusing on the resorptive activity of osteoclasts formed on β-TCP ceramics are ongoing.

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PP228

Bisphosphonates differently affect jaw and long-bone marrow cells

Jenny A F Vermaat1, Anke C C Jansen2, Greetje A P Renders1, Teun J de Vries1,2, Vincent Everts1,2

1Department of Oral Cell Biology/Functional Anatomy, Academic Centre for Dentistry Amsterdam (ACTA), University of Amsterdam and VU University Amsterdam, MOVE Research Institute Amsterdam, Amsterdam, The Netherlands; 2Department of Periodontology, Academic Centre for Dentistry Amsterdam (ACTA), Amsterdam, The Netherlands.

Bisphosphonates (BPs) such as zoledronic acid (ZA) are widely used to treat bone diseases. The use of BPs can lead to osteonecrosis of the jaw (ONJ), but it is not clear why in particular the jaw bone is affected. Previously, it was shown that osteoclasts derived from different bone sites have different properties. We hypothesize that BPs have distinct effects on bone-site specific osteoclasts or precursors. To investigate this, female C57BL/6J mice were injected intraperitoneally with 0.5 mg/kg zoledronic acid (ZA) or saline once a week. At baseline and after 1, 3, and 6 months, jaw and long-bone marrow cells were isolated and osteoclastogenesis was induced in vitro. The number of multinucleated TRACP-positive cells was assessed. Bone volume and the degree of mineralization of bone (DMB) of the humeri and mandibles were assessed with microCT. After 6 months of treatment, fewer jaw bone marrow cells were isolated from ZA-treated mice than from controls. This effect was not seen for long bones. ZA treatment did not affect the osteoclastogenic potential of long-bone and jaw osteoclast precursors. ZA treatment significantly increased the bone volume and the DMB of both humeri and mandibles. In conclusion, these results indicate that ZA reduces the number of jaw bone marrow cells without affecting long-bone marrow cells. Our findings support the hypothesis that BPs have distinct effects on different osteoclast precursors and may help to gain more insight into the pathogenesis of BP-related ONJ.

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PP229

The D477N mutation in OPTN leads to increased bone turnover and enhanced osteoclast formation in OptnD477N/D477N mice

Sachin Wani1, Rami Obaid1, Ruth Jones2, Stuart Ralston1 & Omar Albagha1

1University of Edinburgh, Edinburgh, UK; 2University of Dundee, Dundee, UK.

Recent GWAS have identified variants in the OPTN gene that predispose to Paget’s disease of bone (PDB), a disease characterised by focal areas of increased bone turnover. The aim of this study was to investigate the role of OPTN in bone metabolism using in vitro knock-down experiments in primary osteoclast precursor cells derived from mouse bone marrow. We used lentiviral particles expressing either shRNA targeted against the OPTN gene or a non-targeting shRNA (-ve control) and Optn knock-down was confirmed (>70%) using western blot analysis. Optn was expressed during osteoclast formation and its expression significantly increased during later stages of osteoclast development in WT mice. The number of osteoclasts formed from Optn-depleted bone marrow cells was significantly higher compared to non-targeted cells (253 ± 39 vs 139 ± 41; P < 0.001). We also found that the number of large osteoclasts (> 10 nuclei) was higher in Optn-depleted cells (92 ± 56) compared to non-targeted cells (37 ± 18; P < 0.001). Furthermore, osteoclast survival after withdrawal of RANKL was 45% higher in Optn-depleted cells (P < 0.05).

Quantitative assessment of NF-κB activation by reporter assays showed significantly increased NF-κB activity in the Optn-depleted cells at the basal level and 72 hrs after stimulation with RANKL compared to non-depleted cells (P < 0.05). In conclusion, Optn depletion is associated with increased NF-κB activity leading to enhanced osteoclast formation, size and survival. Our data suggest that OPTN may act as a negative regulator of osteoclast differentiation. This provides a possible mechanism by which variants in OPTN increase susceptibility to Paget’s disease of bone but further studies will be required to investigate the role of OPTN in osteoclast biology.

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PP231

Osteoclasts activity is affected by adenosivirus infection

Ana Isabel Espirito Santo1, Lynnett Danks2, David Mahoney3, Yourudies Vattakuzhi1, Afsaneh Sabokbar1 & Nicole Horwood1

1Kennedy Institute of Rheumatology, London, UK; 2Tokyo Medical and Dental University, Yushima, Japan; 3Botnar Research Centre, Oxford, UK.

Osteoclast resorption depends on their ability to reorganise their actin cytoskeleton and form the sealing zone. In order to resorb bone, osteoclasts become polarised by condensing their podosomes into a highly dynamic podosomal belt. The podosome turnover is regulated by several factors such as non-receptor tyrosine kinases, small GTPases and actin-binding proteins. The innate immune system responds to viral pathogens. Cytoplasmic double-stranded DNA activates the innate immune system inducing IFN (interferon) production, inflammasome activation, and cell death. We studied whether transfecting osteoclasts with DNA affected their differentiation and resorption ability. The differentiation and activity of adenosivirus infected human osteoclasts was determined relative to non-infected cells. By analysing the formation of TRAP

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positive cells, no effect on osteoclasts differentiation was observed however, a reduction in resorption was found. Early infection significantly inhibited osteoclasts resorption compared to late infection. MTT cell viability assay determined no effect on osteoclast cell viability following transfection. Interestingly, an increased in TSG-6 (tumor necrosis factor stimulated gene-6) expression was observed in infected osteoclasts. TSG-6 expression is known to be induced in response to inflammatory cytokines and to downregulate osteoclasts activity.

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PP232
The use of photo-activatable fluorophores to study the turnover of the receptor activator of NFκB receptor in health and disease
David Mellis, Angela Duthie, Susan Clark & Julie Crockett
University of Aberdeen, Aberdeen, UK.

Familial expansile ostesloysis (PEO) is characterised by focal areas of increased bone turnover driven by bone-resorbing osteoclasts. The syndrome is caused by a heterozygous tandem insertion duplication mutation within the signal peptide region of TNFRSF11a (encoding receptor activator of NFκB; RANK). Our recent research has demonstrated that heterotrimetric receptor formation may hold the key to the disease phenotype. We have shown previously that, whilst homogenous overexpression of FEO-RANK leads to accumulation of the protein within an ER compartment, heterozygous overexpression results in FEO-RANK expression at the plasma membrane likely as a result of interaction with wildtype-RANK. In this study we investigated whether turnover of the RANK receptor is affected by carriage of the mutation using live-cell confocal microscopy.

We generated several photoactivatable expression constructs containing either wildtype (WT) or mutant RANK (FEO) and tagged with either photoactivatable GFP or mCherry. The constructs either alone or in combination were over-expressed in HeLa cells and the fluorescence activated by exposure to ultraviolet (UV) light. Only proteins expressed at the time of UV exposure were activated and produced a fluorescent signal. The rate of receptor turnover was measured indirectly by monitoring the loss of fluorescent signal in live cells, where any newly synthesised proteins produced post-UV activation did not fluoresce. Using a live-cell imaging LSM710 confocal microscope, we analysed the rate at which the WT and FEO-RANK proteins were degraded over a period of 40 minutes. We consistently found that FEO-RANK (GFP or mCherry tagged) was degraded more slowly than WT-RANK (GFP or mCherry tagged) when expressed alone or in combination. This is an exciting observation since it may provide an explanation for the increased osteoclast activity in this syndrome and increase our understanding of the control of RANK signalling in osteoclasts.

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PP233
Investigating homozygous vs heterozygous expression of disease-associated receptor activator of NFκB mutations in vitro
David Mellis, Angela Duthie, Susan Clark & Julie Crockett
Musculoskeletal Research Programme, University of Aberdeen, Aberdeen, UK.

Early-onset Paget’s disease of bone (ePDB), familial expansile osteolysis (FEO) and expansile skeletal hyperophosphatasa (ESH) are related syndromes caused by heterozygous tandem insertion duplication mutations within the signal peptide region of TNFRSF11a (encoding receptor activator of NFκB; RANK). Given that patients are always heterozygous for these mutations we have generated thirteen cell lines to investigate the molecular consequences of these mutations in vitro. Bidirectional expression constructs (in pBI-CMV vector) were generated containing cDNAs for: myc-tagged and FLAG-tagged wildtype RANK (WTF-RANK); myc-tagged mutant RANK (WFMut; homozygous mutation); myc-tagged and FLAG-tagged mutant RANK (WFMut-Mut; heterozygous mutant); myc-tagged and FLAG-tagged mutant RANK (WFMut-Mut; homozygous mutant). The entire expression cassette was then excised and ligation into the pcDNA-FRT vector (Invitrogen). We used the Flp-In system (Invitrogen) to generate Hela cell lines expressing single copies of the bidirectional expression cassettes. Specific regions of genomic RANK DNA from each cell line sequence verified to confirm the presence of each RANK construct. PCR confirmed that the RANK transcript is single copies of the bidirectional expression cassettes. Specific regions of genomic RANK DNA from each cell line sequence verified to confirm the presence of each RANK construct. PCR confirmed that the RANK transcript is

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PP235
Differential effects of nitrogen-containing bisphosphonates on human PBMCs and MUTZ-3 cells
Aaron Kwaasi1, Guillaume Mabilleau1,2, James Dunford1, Frank Ebetino1,3, Ali Zarei1, Michael Paziunas1, Asia SabokCur1 & Graham Russell1,2
1Oxford University, Oxford, UK; 2University of Angers, Angers, France; 3Sanford-Burnham Medical Research Institute, La Jolla, California, USA.

Extracellular nucleotides stimulate both the formation and resorptive activity of osteoclasts. Ecto-nucleotide pyrophosphatase/phosphodiesterases (NPPs) hydrolyse extracellular nucleotide triphosphates to their corresponding monophosphate and orthophosphate (PP). We investigated if osteoclasts express functional NPPs and whether Npp1 gene deletion influenced osteoclast formation and activity. Osteoclasts were formed from the bone marrow of 8 and 15 week old knockout (Npp1-/-) or wild-type (Npp1 +/+) mice. RT-PCR demonstrated expression of Npp1, Npp3, Entpd1, Entpd3 and the PP transport molecule, Ank in early and mature osteoclasts. Npp1 expression was increased in mature, resorbing osteoclasts relative to precursor cells, whilst Entpd3 expression was decreased. Significant total NPP activity indicated the expression of functional enzymes in wild-type osteoclasts. Cultured osteoclasts were activated to resorb by medium acidification to pH6.9. Npp1 mRNA expression was upregulated in mature, resorbing osteoclasts compared to mature, inactive osteoclasts; total NPP activity was also increased 2-fold in acidified cells. Culture of cells from 8 and 15-week old Npp1-/- mice indicated that both osteoclast formation and resorptive function were unaffected by gene deletion. Analysis of the cortical bone from 8, 15 and 22-week old Npp1-/- mice showed that whilst the preisosteo diameter was unchanged, the endosteal diameter was increased ~20% at 15 and 22-weeks, suggesting increased endosteal osteoclast activity in vivo, particularly in older animals. ATP, a key substrate for NPPs, is constitutively released from osteoclasts. We found that baseline ATP release was unaffected in Entpd1-/- osteoclasts; however, ATP release in response to fluid flow was 2-fold lower in Entpd1-/- osteoclasts. The rate of ATP breakdown was unaffected in Entpd1-/- osteoclasts. However, Entpd3-/- osteoclasts displayed increased expression of other ecto-nucleotidases, such as Entpd1, Entpd3 and Entpd3 as well as ANK. These results suggest the possibility that NPPs may play a role in the regulation of osteoclast function.

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Cell biology: osteocytes

**PP237**

**Nucleotide and mechanically induced ATP release pathways in osteocytes**

Tina M Kringlebø 1, Ivana Novak 1, Peter Schwarz 1,3 & Niklas Rye Jørgensen 3

1Department of Diagnostics and Medicine, Research Center of Ageing and Osteoporosis, Glostrup Hospital, Glostrup, Denmark; 2Department of Biology, Copenhagen University, Copenhagen, Denmark; 3Faculty of Health Science, Copenhagen University, Copenhagen, Denmark.

**Background**

We have previously shown that MLO-Y4 osteocytes express a number of P2 receptors, respond to a broad range of nucleotides (e.g. UTP) by increasing intracellular calcium concentration and release ATP upon both mechanical and UTP stimulation. The aim of this study therefore is to investigate how the osteocytes release ATP and whether there is a difference in release pathway depending on the type of stimulus.

**Methods**

ATP release was investigated in vitro in MLO-Y4 osteocytes by measuring real-time luciferase-generated luminescence using the ATP Bioluminescence Kit HSII (Roche) and a NOVOSTAR luminometer (BMG Labtech). Mechanical stimulation was applied on the cells by injecting liquid into the wells at 310 μl/s. UTP stimulation was applied by injecting 10 μM UTP into the wells using the lowest speed level, 100 μl/s. The involvement of hemi-channels and vesicles in ATP release was studied by adding pharmacological inhibitors.

**Results**

It was found that mechanically-induced ATP release was significantly reduced by up to 50% when hemi-channels were blocked by 35–75 μM carbonoxolone (P < 0.05). In contrast, UTP-stimulated ATP release was significantly increased more than fivefold by 75 μM carbonoxolone (P < 0.05) and this effect was confirmed when using 10–200 μM pannexin-1 blocking peptide (P < 0.05). In addition to this, both mechanically and UTP-induced ATP release could be reduced by up to 50% when blocking the vacuolar H^+ pump using 0.5–1 μM bafilomycin A1 (P < 0.05).

**Conclusion**

Results indicate that ATP signalling in the osteocyte network can be induced by both mechanical stimulation and P2 receptor activation. Mechanically-induced ATP release is indicated to occur via both hemi-channel and vesicular pathways, while UTP-induced ATP release at least in part occurs via a vesicular release pathway. The elevating effect of carbonoxolone and pannexin-1 blocking peptide on UTP evoked ATP release should be studied further.

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**PP238**

**Calcium Sensing Receptor is expressed on/in osteocyte-like MLO-Y4 culture and modulated by strontium ranelate**

Priscilla C Aveline 1, Hecimi Toumi 2, Eric Lespessailles 1, Cédric Boudot 2, Romuald Montavert 2, Gaël Y Rochefort 1 & Claude-Laurent Benhamou 1

"EA 4708 I3MTO, Orléans, France; "INSERM U1088, Amiens, France.

**Introduction**

The calcium sensing receptor presence (CaSR) at the surface of the osteocytes has never been clearly investigated. The CaSR are known to be present on osteoblasts. Osteocytes being old osteoblasts embedded in the matrix, the expression of CaSR is likely, and could constitute a key role to calcium signalling. Strontium ranelate (SrRan) has shown to activate osteoblasts by fixation on CaSR (Chattopadhyay N 2007, Biochem Pharmacol; Hurtel-Lemaire AS 2009, J Biol Chem). Our aim has been to investigate this expression of CaSR on the osteocyte, and its modulation by SrRan.

**Materials and methods**

We used MLO-Y4 cell cultures (osteocytes) and the RAW cells, not treated with SrRan, constitute the controls and as known to express CaSR. Several SrRan concentrations (0, 0.1, 1, and 5 mM) have been tested at different times (0, 1, 2, 3, 5, 7, and 14 days). To assess CaSR expression on the membrane and inside cell, we used a CaSR mouse primary antibody (ThermoScientific). After, we revealed the primary antibody by a fluorescent secondary antibody (phycocerythrin) via a flow cytometry.

**Results**

First, CaSR were present on the MLO-Y4 membrane and still with a higher level in the total cell (membrane and inside cell) which confirmed its presence both on the membrane and inside the cell. CaSR expression levels of MLO-Y4 were approximately twice the RAW cell expression with 1 mM SrRan and three times with 5 mM SrRan. Second, a dose-effect of SrRan was identified. CaSR levels increased significantly with 1 and 5 mM concentrations, and at 5 and 7 days of SrRan exposure (5 days: 1 mM=+30% and 5 mM=+70% compared to 0 mM, and 7 days: 1 mM=+175% and 5 mM=+385% compared to 0 mM).

In conclusion, our data have shown that: i) CaSR is expressed both on and inside the osteocytes and ii) its expression is modulated by SrRan, with a dose-dependent increase.

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Collagen and glycosaminoglycans (GAGs) such as hyaluronan (HA) and chondroitin sulfate (CS) are basic elements of bone structure and collagen-GAG composites are currently developed for a wide range of applications. Here, we report on the molecular and cellular effects of GAGs and their sulfated derivatives on osteocytes, which are fundamental orchestrators of bone remodeling.

Materials and methods

The effects of native and sulfate-modified GAGs on viability, necrosis, apoptosis, and gene expression were determined using ELISA photometric immunoassays of DNA fragmentation, and viability was evaluated with a fluorometric assay. The gene expression profile was examined by real-time PCR.

Results

Native and sulfated GAGs were stable and non-cytotoxic. At a concentration of 200 μg/ml, unsulfated HA did not reduce apoptosis compared to control, whereas highly sulfated HA led to a significant reduction of apoptosis both in comparison to control and unsulfated HA (P < 0.05). Moreover, highly sulfated CS decreased apoptosis by 30% compared to control and to its native form (P < 0.05). Similar results were observed for cell necrosis. Both forms of HA significantly decreased cell viability when compared to control (P < 0.05), whereas CS did not affect cell viability. At concentrations ranging from 10 to 200 μg/ml, unsulfated HA dose-dependently increased the RANKL/OPG ratio compared to control, whereas highly sulfated HA significantly downregulated the RANKL/OPG ratio when compared to its native form (both P < 0.05). The expression of SOST, the gene encoding sclerostin, was also reduced by 38% by highly sulfated HA when compared to control (P < 0.05). Native HA and CS did not alter SOST expression.

Conclusion

Highly sulfated HA may maintain the phenotype of healthy and functional osteocytes but the clinical significance of these findings needs to be validated in vivo.

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PP240

RANKL subcellular trafficking in osteocytes

Masashi Honma, Yuki Ikebuchi, Yoshiaki Kariya, Madoka Hayashi, Naoki Hayashi, Shigeki Aoki & Hiroshi Suzuki

Department of Pharmacy, The University of Tokyo Hospital, Tokyo, Japan.

RANKL is the central player in the regulation of osteoclastogenesis and the quantity of RANKL presented to osteoclast precursors is an important factor determining the magnitude of osteoclast formation. It had been believed for years that osteoblastic cells are the major source of RANKL presented to osteoclast precursors, and we have previously focused on RANKL intracellular behavior in that osteoblastic cells are the major source of RANKL presented to osteoclast determining the magnitude of osteoclast formation. It had been believed for years that osteocytic and osteocytes embedded in collagen gel to analyze how osteocytes support osteoblastic cells. However, recent two reports controverted this traditional concept and showed that the osteocyte is a central player in regulating osteoblastic cells. Nevertheless, making the study of osteocyte functions in vitro difficult. In the present study, we developed a novel co-culture system of osteoclast precursors and osteocytes embedded in collagen gel to analyze how osteocytes support osteoclastogenesis. Experiments using this model revealed that osteocytic RANKL is provided as a membrane-bound form to osteoclast precursors through osteoclastogenesis processes and that the contribution of soluble RANKL to the osteoclastogenesis supported by osteocytes is minor. Moreover, the regulation of RANKL subcellular trafficking, such as OPG-mediated transport of newly synthesized RANKL molecules to lysosomal storage compartments, the release of RANKL to the cell surface upon stimulation with RANK, are confirmed to be functional in osteocytes. These results provide a novel understanding of the regulation of osteoclastogenesis.

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PP241

Inhibition of osteocyte-induced osteoclast precursor proliferation and migration by mechanical strain

Seong-Hee Ko1 & Heesu Lee2

1Department of Pharmacology, Gananeung-Wonju National University, Gangneung City, Republic of Korea; 2Department of Oral Anatomy, Gananeung-Wonju National University, Gangneung City, Republic of Korea.

The osteocyte most likely plays a role in bone remodeling by instructing osteoclasts to remove bone at specific sites. This entire process includes recruitment, proliferation and differentiation of osteoclast precursors. And osteocytes are responsible for detecting and responding to mechanical strain and may send signal to other cells. Therefore, to determine the role for osteocytes and mechanical strain in bone remodeling, we examined the effect of steady or pulsatile shear stress of osteocytes on osteoclast precursor migration and proliferation. We used the MLO-Y4 cells as in vitro model for osteocytes, RAW 264.7 cells as osteoclast precursors. For fluid flow experiments, MLO-Y4 cells were exposed to 2 h of pulsatile fluid flow (PFF) at 2, 4, 8, 16±0.6 dynes/cm² or steady fluid flow (SFF) using Flexcell Streamer system. Y4 CM was collected during 24 h cultures after fluid flow experiment (1st – 24 h Y4 CM) or after collecting 1st – 24 h Y4 CM (2nd – 24 h Y4 CM). We did proliferation assay of RAW 264.7 cells with control media or 10% Y4-CM at specific time. The migration of RAW 264.7 cells was assayed using transwells with control media or Y4-CM. MLO-Y4-CM increased osteoclast precursor proliferation and migration. And the increase of RAW 264.7 cell migration induced by MLO-Y4 cells was partially blocked by M-CSF antibody. After MLO-Y4 cells were exposed to SFF, 1st – 24 h Y4 CM had no effect on RAW 264.7 cell proliferation and migration, but, 2nd – 24 h Y4 CM decreased RAW 264.7 cell migration compared to control CM (Y4-CM without strain). After MLO-Y4 cells were exposed to PFF, 1st – 24 h Y4 CM decreased RAW 264.7 cell migration and proliferation to control CM. These results suggest that osteocytes can regulate the bone remodeling by communication with osteoclast precursors and that mechanical strain may inhibit the bone resorption which is induced by osteocytes.

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PP242

Activation of the parathyroid hormone-receptor is involved in the pro-survival effect of hypoxic shock in osteocyte-like MLO-Y4 Cells

Marta Maycas1, Juan A Ardura1, Luis Fernández de Castro2, Arancha Gortázar1 & Pedro Esbrí1

1IIS-Fundacion Jimenez Diaz, Madrid, Spain; 2IMMA-Universidad San Pablo CEU, Madrid, Spain.

The PTH type 1 receptor (PTH1R) is an important modulator of bone remodeling. In mice, PTH1R ablation in osteocytes produces trabecular bone reduction and impaired calcium homeostasis; meanwhile, its overexpression in these cells promotes periosteal and endocortical bone formation. Osteocytes can translate mechanical stimuli into bone-forming signals. Skeletal unloading induces osteocyte apoptosis and bone loss, whereas mechanical stimuli prevent osteocyte apoptosis through inducing β-catenin accumulation and ERK nuclear translocation. PTH1R activation stimulates the canonical Wnt/β-catenin pathway and promotes osteoblast survival. Here, we aimed to explore the possible involvement of PTH1R activation in cell protection conferred to osteocyte-like MLO-Y4 cells by mechanical stimulation. Cells were subjected to hypoxic shock (240 mOmm-2) for a short time (≤ 10 min) in the presence or absence of PTH1R antagonists or after transfection with a PTH1R siRNA. Cell viability was assessed by Trypan blue exclusion after incubation with the proapoptotic agent etoposide (50 μM) for 6 h. Changes in cell localization of β-catenin and nuclear ERK were examined by western blot. Calcium signaling measurement was assessed by confocal microscopy in cells preloaded with the fluorochrome Fluo-4 AM. Hypoxic shock stimulated a rapid (< 1 min) and transient Cai²⁺ response in MLO-Y4 cells. In addition, this mechanical stimulus induced β-catenin stabilization, related to an increased nuclear and membranous localization, and increased nuclear ERK at 10 min. These changes were associated with an enhanced MLO-Y4 cell survival. These events were all inhibited by cell pre-treatment with two PTH1R antagonists, PTHrP (7–34) and JB2420 (1 μM), or transfection with PTH1R siRNA. Moreover, both the calcium antagonist verapamil (1 μM) and Rp-cAMPS (25 μM), which prevents protein kinase A activation (another PTH1R signaling pathway) also prevented these changes triggered by mechanical stimulus. Collectively, these findings indicate that a rapid activation of the PTH1R occurs after mechanical stimulus in osteocytes, leading to an increased survival.

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In summary, both SrRan and CM from mechanically-stimulated osteocytes enhanced bone nodule formation by ASCs, regardless whether the primary bone nodule formation by ASCs. CM from PFF-treated primary bone cells cultured in absence of mechanical stimulation did not affect enhanced ki67 expression and bone nodule formation by AT-MSCs. CM from and VEGFA. SrRan did not alter this response to PFF. SrRan (3 mM) alone Wnt5a and reduced Wnt10b and IGF1 expression by human primary bone cells. SrRan alone, in the absence of PFF, enhanced gene expression of BMP2 and osteoclast formation at days 7 and 10 (Alizarin Red Staining).

Conditioned medium (CM) from SrRan and/or PFF-treated osteocytes was added towards mesenchymal stem cells. This study assessed the effect of SrRan on paracrine signaling from mechanically-stimulated human osteocytes towards mesenchymal stem cells.

Human primary bone cells, used as a model for osteocytes, were cultured for 24 h in the presence of SrRan (0–3 mM), and treated with/without pulsating fluid flow (PFF) for 60 min. Treatment effects were assessed by quantification of nitric oxide (NO; Griess assay) in the culture medium, and by quantification of mRNA expression of Wnt5a, Wnt10b, BMP2, IGF1, PTN, and VEGFA (Taqman PCR).

SrRan alone, in the absence of PFF, enhanced gene expression of BMP2 and Wnt5a and reduced Wnt10b and IGF1 expression by human primary bone cells. PFF enhanced NO production as well as gene expression of Wnt5a, BMP2, PTN, and VEGFA. SrRan did not alter this response to PFF. SrRan (3 mM) alone enhanced klotho expression and bone nodule formation by AT-SCs. CM from primary bone cells cultured in absence of mechanical stimulation did not affect bone nodule formation by ASCs. CM from PFF-treated primary bone cells enhanced bone nodule formation by ASCs, regardless whether the primary bone cells were cultured in the presence or absence of SrRan. In summary, both SrRan and CM from mechanically-stimulated osteocytes enhanced osteogenic differentiation of ASCs, but not in a synergistic manner. Thus, SrRan did not appear to affect paracrine signaling from mechanically-stimulated human osteocytes towards cultured ASCs.

PP245
IGF1 regulates MC-3T3 and human primary osteoblast to osteocyte differentiation in 3D culture
Nicole E E Scully1,2, Deborah J Mason1,4 & Bronwen A J Evans1,3
1School of Medicine, Institute of Molecular and Experimental Medicine, Cardiff University, Cardiff, UK; 2Division of Pathophysiology and Repair, School of Biosciences, Cardiff University, Cardiff, UK; 3Arthritis Research UK Bioinformatics and Bioengineering Centre, Cardiff University, Cardiff, UK.

Osteocytes differentiate from osteoblasts, are embedded in mineralised matrix and are critical regulators of bone remodelling. In vitro osteocyte models are limited to cell lines in monolayer, which do not represent their 3D environment in vivo. We have shown that osteoblasts in 3D gels differentiate along the osteocyte pathway. Since IGF1 regulates osteoblasts, and is involved in osteocyte response to mechanical loading, we hypothesised that IGF1 modulates osteocyte differentiation and function.

We maintained osteoblasts (MC-3T3; HOBS) in 3D type I collagen gels (250 µl; 48-well plates; 15 days) in α-MEM, IGF1 (5 µg/ml). Cell number, viability and phenotype (IHC, confocal), IL6, VEGF, and FGF23 secretion (ELISA), and the expression of osteocyte-related genes (DMP1, RANKL, E-11, and FGF23; qRTPCR) were measured.

Cells with IGF1 appeared more dendritic than untreated cells. Cell viability was high both with IGF1 (>85% MC-3T3 s < >90% HOBs) but MC-3T3 numbers were decreased with IGF1. From day 11 onwards, the expression of DMP-1, Cx43, RANKL (P < 0.01), and FGF23 (P < 0.001) were significantly increased in MC-3T3 s with IGF1, compared to untreated, also confirming differentiation to osteocytes. There was no change in MC-3T3 IL6 secretion, but VEGF was higher with IGF1 on all days (P < 0.01), and FGF23 secretion only detected with IGF1 treatment from day 11. In HOBs, and with IGF1, VEGF, and IL6 (P < 0.05) secretion on all days were significantly reduced when compared to untreated controls.

IGF1 modulated cell number and function, with, generally similar results obtained with both cell types tested. Modulation of VEGF secretion, however, was different between the two cell types. FGF23 production with IGF1 treatment highlighted the possible role of IGF1 in osteocyte differentiation and function. This novel 3D in vitro system provides a tool to further study the role of IGF1 in osteocyte differentiation and function, especially those related to the mechanosensing signaling pathways.

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PP246
Development of a novel 3D mineralising culture system to investigate the differentiation of osteoblasts to osteocytes
Nicole E E Scully1,4, Sam L Evans3,4, Deborah J Mason2,4 & Bronwen A J Evans1
1School of Medicine, Institute of Molecular and Experimental Medicine, Cardiff University, Cardiff, UK; 2Division of Pathophysiology and Repair, School of Biosciences, Cardiff University, Cardiff, UK; 3School of Engineering, Institute of Mechanical and Manufacturing Engineering, Cardiff, UK; 4Arthritis Research UK Bioinformatics and Bioengineering Centre, Cardiff University, Cardiff, UK.

Osteocytes make up >90% of bone cells, are embedded in mineralised matrix where they form a communication network. Osteocytes differentiate from osteoblasts, and are mechanosensitive. They are very difficult to isolate with cell lines in monolayer, which do not represent their 3D environment. Recent publications indicate that osteoblasts maintained in in vitro 3D collagen gels may differentiate to osteocytes.

We maintained osteoblasts (MC-3T3; human primary) in 3D type I collagen gels (250 µl; 48-well plates; 15 days) in either α-MEM (basal medium) or mineralising medium (basal medium, dexamethasone, and 10 nM 1,25-dihydroxyvitamin D3). Cell number, viability, and phenotype (IHC, qRT-PCR, and confocal microscopy), gel stiffness (Losenhausen machine), and VEGF and IL6 secretion (ELISA) were quantified.

Cells appeared more dendritic over time and formed connecting cellular networks (H&E, Phalloidin). Cell viability was similar in both media (>85% MC-3T3 s >95% human primary), but cell numbers were significantly higher (P < 0.001) in mineralising conditions. Mineralisation was confirmed from day 7 (calcium). DMP-1 was not expressed (IHC) at day 3 but then gradually increased in expression (days 7–14). E11 was low at day 3 (IHC; qRT-PCR), peaked at day 10 (P < 0.001), and returned to lower levels by day 14. Gel stiffness significantly increased over 11 days (P < 0.01) and the mineralised gels were stiffer than those of control.

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in basal medium (P<0.01). VEGF and IL6 secretion also changed significantly with time and culture conditions. Mechanical loading conditions for these 3D osteocyte cultures are currently being optimized. Osteoblasts maintained in 3D gels differentiate along the osteogenic pathway. It is possible to mineralise these cultures thus mimicking further their in vivo environment. This methodology provides a novel model to study osteocyte differentiation and function, and will enable important studies relating to bone loading, repair and regeneration.

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**Chondrocytes and cartilage**

**PP247**

Expression of novel cartilage genes during maturation of cultured chondrocytes

Babatunde Awodele, Michiko Mirans, Charles Pagel & Eleanor Mackie

Faculty of Veterinary Science, University of Melbourne, Parkville, Victoria 3010, Australia.

Formation and growth of long bones occur through the process of endochondral ossification, which depends on proliferation and hypertrophy of chondrocytes in growth cartilage. In a subtractive hybridization study of equine cartilage, we recently identified a number of genes, the roles of which in growth cartilage have not been characterized. A subset of these genes was found to be differentially expressed between the zones of equine growth cartilage. The genes encoding ATPase H+ transporting lysosomal d2 subunit (Atp6v0d2), DEAD box polypeptide-5 (Ddx5), triose phosphate isomerase-1 (Tpi1) and thymosin β 4 (Tmsb4) were more highly expressed in the hypertrophic zone than in the reserve and proliferation zones of equine growth cartilage, while Foxa3 was more highly expressed in the reserve zone than in the hypertrophic zone. We examined the expression of these genes during maturation of ATDC5 cells (a murine teratocarcinoma-derived chondrocyte-like cell line), with the aim of using this cell line to identify the roles of the genes of interest in chondrocyte hypertrophy. ATDC5 cells were cultured to confluence, then changed to medium containing insulin-transferrin-sodium selenite, triiodothyronine and ascorbate-2-phosphate, with the aim of using this cell line to study the functions of these genes in chondrocyte hypertrophy. Expression of novel cartilage genes during maturation of ATDC5 cells will provide an appropriate vehicle for manipulation of gene expression to investigate the functions of these genes in chondrocyte hypertrophy.

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**PP248**

Intracellular calcium is influenced by the nuclear magnetic resonance therapy in Cal-78 chondrosarcoma cells

Bibiane Steinecker-Frohnwieser1, Lukas Weig1 & Werner Kullich1

1Ludwig Boltzmann Institute for Rehabilitation of Internal Diseases, Saalfelden, Austria; 2Department of Special Anaesthesia and Pain Management, Medical University, Vienna, Austria.

Calcium represents one of the most versatile and universal signalling particles regulating many different cellular processes. Changes in [Ca2+]i give rise to a vast diversity of modulatory events, amongst others, influencing activities of kinases and ion channels.

It was demonstrated that nuclear magnetic resonance therapy (NMRT) treatment in osteoarthritis led to reduced pain and improved function followed by increase in quality of life. Less is known about how NMRT influences cellular processes, a vast diversity of modulatory events, amongst others, influencing activities of kinases and ion channels. NMRT might transmit its modulation of ion channels is discussed. Likewise, NMRT might influence activities of kinases. The role of these events in the context of endochondral ossification is currently being investigated.

Investigation of NMRT influencing the cellular messenger [Ca2+]i, cells were stimulated with NMRT for 1 h ± IL1β. Induction of calcium release was initiated by histamine application (1–100 μM). Fura-2 functioned as indicator for calcium imaging. [Ca2+]i concentration was calculated by determining the 340:380 nm ratio. A functional involvement of MAPKs was investigated by applying U0126 (MAPK/ERK inhibitor) and SB203580 (p38/MAPK inhibitor) prior to calcium measurements. The influence of NMRT on protein kinase activity was further investigated via a phospho-MAPK array.

Our preliminary results demonstrate that NMRT treatment induced calcium release in general and the basal calcium under NMRT in particular. A first brief screening regarding the activity of kinases revealed an apparent up-regulation of MAPK/ERK and p38 MAPK in NMRT stimulated cells. Obviously under inflammatory conditions NMRT influences [Ca2+], by modulating cell’s calcium influx and/or calcium release leading to increased MAPK activity, both possibly playing a role in the game of observed pain reduction.

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**PP249**

Impairment of endochondral ossification by Hoxa2 overexpression: a plausible molecular explanation of idiopathic proportionate short stature

Pierre M L Deprez1, Miloud G Nichane1,2, Benoît Lengle1, René Rezsohazy1,2 & Catherine Nyssen-Behets1

1Pôle de Morphologie, Institut de Recherche Expérimentale et Clinique, Université catholique de Louvain, Brussels, Belgium; 2Institut des Sciences de la vie, Université Catholique de Louvain/Vrije-Neuve, Belgium.

Introduction

Using transgenic mice ectopically expressing Hoxa2 all along chondrogenesis, we previously associated the resulting animal phenotype to human idiopathic proportionate short stature. Our analysis showed that this overall size reduction was due to a negative influence of Hoxa2 in the very first step of endochondral ossification. As the molecular pathways underlying this pathogenesis are still unknown, we here tried to identify the impact of Hoxa2 overexpression on the main factors involved in endochondral ossification.

Materials and methods

In our transgenic mice Col2a1/Hoxa2-lacZ, Hoxa2 expression was induced in Col2a1 expressing territories and maintained thereafter, i.e. all over the endochondral bone pieces. Mice bearing the hsp-actin-STOP-fox-Hoxa2-lacZ transgene only (β3-Hoxa2-lacZ) were considered controls. Using immunohistochemistry and western blotting, we compared the expression of Baxp1, Runx2, Sox5, Sox6, Bmp1a, Foxc2, β3-integrin, Bmp7, Gdf10, Gdf5, Ihh, Wnt5a, Bmp4, Fgf3, Gdf6, Mecoc1, Meox2, Pax1, Pth/P, Msx1, Msx2, osteropontin, Pax9, S-100, and Sox9 in El13.5 transgenic and control mice.

Results

Persistent expression of Hoxa2 in chondrogenic territories provokes a general down-regulation of the main factors controlling the endochondral differentiation cascade, i.e. Sox5, Baxp1, Bmp7, Ihh, Mox1, Pax1 and Wnt5a. As a consequence, Hoxa2 misregulation in mice induces a proportionate short stature phenotype mimicking human idiopathic conditions.

Conclusions

Together, our results give insights for understanding proportionate short stature pathogenesis and reveal molecular mechanisms linking the activity of a Hox protein, Hoxa2, and its negative impact on endochondral skeleton development.

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**PP250**

In vitro effects of caffeine on the proliferation, apoptosis, and gene transcripts expression of chondrogenic differentiation in growth cartilage of rats

Amanda Maria Sena Reis, Raquel Viana Raad, Natália de Melo Ocarino & Rogéria Serakides

Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Minas Gerais, Brazil.

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. But despite changing the postnatal bone growth, there are few studies about its effect on growth cartilage. The objective of this study was to evaluate the *In vitro* effects of caffeine on proliferation, apoptosis and gene transcripts expression of chondrogenic differentiation in growth cartilage. There had been used the femoral epiphysal cartilage of 80 newborn rats which were
PP251
Endochondral bone growth of rats
Amanda Maria Sena Reis, Ana Cláudia Moura Batista, Natália de Melo ocarino & Rogéria Serakides
Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.

Despite the presence of skeletal anomalies in fetuses of female rats treated with caffeine, their effect on bone’s formation and growth have not yet been elucidated. The objective of this study was to evaluate the effects of caffeine on the formation and endochondral bone growth in rats. There had been used 36 Wistar rats distributed among the control group and others treated with caffeine at doses of 25, 50, and 100 mg/kg. Treated groups received caffeine daily throughout pregnancy and lactation. There was assessed, through histomorphometry, the formation and endochondral bone growth of offspring with 3 and 21 days of age. Among the progeny of rats treated with higher doses of caffeine, malformations were observed, including syndactyly and brachydactyly. In the vertebral and/or long bones from newborn rats, there have been found significant reduction in the length of the limbs and vertebral bodies, in the thickness of the epiphyseal plate and in the percentage of trabecular bone tissue of the primary spongiosa. In all groups treated with caffeine, epiphysis of long bone cartilage also presented chondrocytes with pyknotic nuclei and empty lacunae of chondrocytes, characteristic of cell death, as well as, glycosaminoglycans deficiency in the matrix. The 21-day of age offspring of mothers treated with caffeine presented considerably increased level of Sox9 expression. These findings suggest a possible signalling connection between these two proteins. Furthermore, the results indicate a negative feedback loop during chondrogenesis. Further experiments will apply explant mouse mandibles and limbs to investigate impact of c-Myb overexpression and downregulation, respectively, on tooth and bone phenotype in the intact organs and to compare endochondral- and intramembranous-types of ossification.

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PP252
Modulation of c-Myb during chondrogenesis
Veronika Oralova1,*, Marcela Buchtova1,9, Eva Janeckova1,2, Abigail Tucker1 & Eva Mataiova1,3
1Institute of Animal Physiology and Genetics AV CR, v.v.i., Brno, Czech Republic; 2Faculty of Science, Masaryk University, Brno, Czech Republic; 3University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic; 4Department of Craniofacial Development and Stem Cell Research, King’s College, London, UK.

The c-Myb transcription factor is associated with proliferation of undifferentiated cells in number of tissues, but recent data suggests its role also in differentiation. c-Myb is important in formation of the cartilage, bone and apparently also in hard tissue mineralization (Matalova et al. 2011). Embryonic micromasses were established from mouse front limbs at the embryonic day E12. Micromass cultures represent an effective tool for experimental biology and they are routinely used in molecular studies of embryogenesis. Moreover, micromass technology approach enables investigators to follow tissue formation from a single organ to organized spheres in a controlled environment (Meyer et al. 2007).

Techniques of electroporation and lipofection were applied in the gain-in-function experiments, siRNA approach for loss-of-function investigation. Sox9 was investigated as a marker of chondrogenesis, gene expression was followed using qPCR. Transient transfection by constructs carrying Sox9 overexpressing vectors markedly decreased c-Myb expression in cultured micromasses, whereas Sox9 level was enhanced. Transient transfection using constructs carrying c-Myb overexpressing vectors enhanced markedly both, c-Myb and Sox9 expression. Along with overexpression, siRNA c-Myb and siRNA Sox9, respectively, were transfected, representing downregulation impact. siRNA, c-Myb treated cultures expressed significantly lower level of Sox9, whereas siRNA Sox9 treated cells showed considerably increased level of c-Myb expression. These findings suggest a possible signalling connection between these two proteins. Furthermore, the results indicate a negative feedback loop during chondrogenesis.

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PP253
Can adrenomedullin be a potential osteoarthritis treatment?
Aurore Chatron-Colillet, Frédéric Velard, Dominique Côme, Hélène Lin, Hang Kong Ea & Frédéric Liote
INSERM UMR-606, Lariboisière Teaching Hospital, Paris, France.

Objective
Chondrolysis, chondrocyte apoptosis, and local inflammation are described to exacerbate osteoarthritis development. We therefore aimed to investigate the effect of adrenomedullin (ADM) and its truncated peptide (22–52ADM) on in vitro and in vivo models. Both have exhibited anti-apoptotic and anti-inflammatory properties in collagen-induced arthritis (CIA) in mice. Methods
In normoxia or hypoxia (physiological condition), ADM and its receptor complex (CLR, RAMPs) expression was investigated in bovine articular chondrocytes (BAC) at the mRNA (RT-qPCR) and protein levels (IEA, immunofluorescence). ADM and 22–52ADM anti-apoptotic effect was assessed on Fas-ligand (FasL)-mediated apoptosis using caspase-specific fluorogenic substrates. To assess the ADM anti-inflammatory effect on IL1β-stimulated chondrocytes, RT-qPCR analyses were performed to assess production of pro-inflammatory factors. Secondly, meniscectomized mice were injected i.p. three times a week during 8 weeks with PBS, ADM or 22–52ADM (1.2 μg/g). Joints were then prepared for histological analysis to quantify chondrocyte apoptosis (TUNEL) and cartilage degradation (Safranin-O).

Results
Using immunofluorescence, we have demonstrated CLR and RAMPs were more colocalized when chondrocytes were cultured in hypoxia, and especially in inflammatory environment. Coupled with AMPs measurements, those data suggest that the receptor is functional. Moreover, in such conditions, ADM secretion was significantly increased and exogenous ADM (10−7 M) demonstrated anti-apoptotic activity. Nevertheless, ADM failed to modulate mRNA production of pro-inflammatory factors. Regarding joint degradation rate of meniscectomized mice, neither ADM nor the 22–52ADM have had a protective effect on apoptosis and chondrolysis. Conclusion
In «physiological environment», BAC were able to produce both ADM and functional receptor components. In addition, ADM treatment prevented Fas-L-induced apoptosis in hypoxia although its anti-inflammatory effect was not confirmed in these cells. Contrary to our expectations based on the CIA model, ADM or its derived peptide 22–52ADM administered systemically did not disclose any effect on OA progression. Direct intra-articular effects of ADM might be investigated.

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Oxygen tension-mediated regulation of chondrogenic differentiation: application to stem cells based osteochondral repair

Sophie Portron1,2, Vincent Hivernaud1,2, Christophe Merceron1,2, Julie Lesouer1,2, Martial Masson1,2, Olivier Gauthier1,3, Claire Vinatier1,2, Laurent Beck1,2 & Jerome Guichard1,2
1INSERM, UMR5791, Center for Osteoarticual and Dental Tissue Engineering, Group STEP 2Skeletal Tissue Engineering and Physiopathology, Nantes, France; 3University of Nantes, UFR Odontology, Nantes, France; 2Center for Preclinical Research and Investigation of the ONIRIS Nantes-Atlantic College of Veterinary Medicine, Food Science and Engineering (CRIP), Nantes, France.

Purpose
Multipotent stromal cells (MSC) have been considered promising for the regenerative strategies of articular cartilage. However, the MSC chondrogenic differentiation can ultimately lead to the formation of hypertrophic chondrocytes responsible for the calcification of cartilage. To prevent this MSC-dependent production of a calcified matrix in articular site, MSC chondrogenic differentiation has to be carefully controlled. Given that articular cartilage is avascular, we questioned whether in addition to its stimulatory role in the early differentiation of chondrogenic cells, hypoxia may prevent their hypertrophic differentiation.

Materials and Methods
Human adipose MSC and ATDC5 murine cells were used. Cells were cultured in normoxia (21% O2) or hypoxia (5% O2). The effects of hypoxia on the hypertrophic differentiation was evaluated by i) the production of GAGs by Alcian Blue Staining, ii) the expressions of hypertrophic differentiation markers (Mmp13, Col10a1, Runx2, and Alpl) by RT-PCR and TaqMan low density array, and iii) the measurement of alkaline phosphatase and MMP13 activities. Cell viability was assessed by cell counting and total protein production. The transcriptional activity of hypoxia inducible factor-1 alpha (HIF-1α) and HIF-2α was evaluated by No-Shift DNA binding assay.

Results
Our data indicate that a 5% O2 promoted the transcriptional activity of HIF-1α and HIF-2α. A 5% O2 decreased the production of a calcified matrix, down-regulated the expression of hypertrophic markers and reduces Alkaline phosphatase and MMP13 activities as compared to 21% O2, without affecting cell viability and protein production.

Conclusions
Our data suggest that a 5% O2, in addition of being able to chondrogenically commit MSC, inhibits the hypertrophic differentiation of chondrogenic cells. These results make hypoxia an instrumental tool to prevent the formation of a calcified matrix in MSC-based cartilage tissue engineering. On the contrary, 21% O2 was found to up regulate the terminal differentiation of chondrogenic cells. These data make normoxia a potent factor useful for bone repair through endochondral strategy.

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Reference

PP255
Effects of an in vitro low-oxygen-tension preconditioning of adipose stem cells on their in vitro chondrogenic potential: application in cartilage tissue repair

Sophie Portron1,2, Christophe Merceron1,2, Olivier Gauthier1,3, Julie Lesouer1,2, Sophie Source1,2, Martial Masson1,2, Borhane Fellah1,3, Olivier Geoffroy1,4, Elodie Lallemand1,4, Pierre Weiss1,2, Jerome Guichard1,2 & Claire Vinatier1,2
1INSERM, UMR5791, Center for Osteoarticual and Dental Tissue Engineering, Group STEP 2Skeletal Tissue Engineering and Physiopathology, Nantes, France; 3University of Nantes, UFR Odontology, Nantes, France; 2Center for Preclinical Research and Investigation of the ONIRIS Nantes-Atlantic College of Veterinary Medicine, Food Science and Engineering (CRIP), Nantes, France; 4Department of Equine Surgery, College of Veterinary Medicine of Nantes (ONIRIS), Nantes, France.

Purpose
Multipotent stromal cells (MSC)-based regenerative strategy is promising for the repair of cartilage, which is an avascular tissue in which cells experience hypoxia. Hypoxia is known to promote the early chondrogenic differentiation of MSC. Therefore, the aim of our study was to determine whether low oxygen tension could be used to enhance the regenerative potential of MSC for cartilage repair.

Methods
MSC from rabbits or human adipose tissues (ASC) were preconditioned in vitro in control or chondrogenic (ITS and TGFβ3) medium and in 21 or 5% O2. Chondrogenic commitment was monitored by measuring COL2A1 and ACAN expression level by real-time PCR. Preconditioned rabbit and human ASC were then incorporated in a Si-HPMC hydrogel and respectively injected i) in rabbit articular cartilage defects for 18 weeks or ii) subcutaneously in nude mice for 5 weeks. The newly formed tissue was qualitatively and quantitatively evaluated by cartilage-specific immunohistological staining (Acanthinic Blue, type II collagen) and scoring (O'Driscoll score). The phenotype of ASC cultured in a monolayer or within Si-HPMC in control or chondrogenic medium and in 21 or 5% O2 was finally evaluated using real-time PCR.

Results/Conclusions
5% O2 increased the in vitro expression of chondrogenic markers in ASC cultured in induction medium. The cells implanted within the Si-HPMC hydrogel and preconditioned in chondrogenic medium formed a cartilaginous tissue, regardless of the level of oxygen. In addition, the 3D in vitro culture of ASC within the Si-HPMC hydrogel was found to reinforce the prochondrogenic effects of the induction medium and 5% O2. Altogether, these data indicate that although 5% O2 enhances the in vitro chondrogenic differentiation of ASC, it does not enhance their in vivo chondrogenesis. These results also highlight the in vitro chondrogenic potential of ASC and their potential value in cartilage repair.

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Reference
Background
Cartilage matrix breakdown in osteoarthritis (OA) is due to mechanical stress and inflammation leading to increased metalloproteinases (MMPs) production. Currently, IL1β is thought to have a major role in this process. IL1β is synthesized as an inactive precursor, which is cleaved into the secreted active form. This maturation process mainly occurs in the inflammasome complex. Inflammasome is constituted by initiators (including NALP3) and adaptor molecules (ASC) which oligomerize to recruit and activate caspase-1, which in turn processes IL1β precursor. We aimed to clarify the role of both inflammasome and IL1β in cartilage breakdown.

Methods
IL1β release from cartilage explants of OA patients were assessed (ELISA). LPS, IL1α and TNFα treatments were used to induce MMP (−3, −8, −13) gene expression (real-time PCR) and protein release (ELISA, zymography, and western blot) in primary mouse articular chondrocytes cultures. Effects of NALP3 deficiency (using NALP3−/− mice), caspase-1 inhibition (using Z-YVAD-FMK) and IL1 blockade (using IL1RA) were investigated. Finally, excessive dynamic compression (0.5 Hz and 1 MPa for 6 h) led to increased MMP activity was applied on mouse cartilage explants from WT, NLRP3−/− or IL1R1−/− mice and load-induced GAG release were assessed.

Results
Despite NLRP3, ASC, and caspase-1 expression in OA chondrocytes, no IL1β production was found. In mouse articular chondrocytes, LPS, IL1α, and TNFα dose-dependently increased MMP-3, MMP-9 and MMP-13 both at gene and production was found. In mouse articular chondrocytes, LPS, IL1β and TNFα treatments were used to induce MMP (−3, −8, −13) gene expression (real-time PCR) and protein release (ELISA, zymography, and western blot) in primary mouse articular chondrocytes cultures. Effects of NALP3 deficiency (using NALP3−/− mice), caspase-1 inhibition (using Z-YVAD-FMK) and IL1 blockade (using IL1RA) were investigated. Finally, excessive dynamic compression (0.5 Hz and 1 MPa for 6 h) led to increased MMP activity was applied on mouse cartilage explants from WT, NLRP3−/− or IL1R1−/− mice and load-induced GAG release were assessed.

Conclusion
This study suggests that OA cartilage can be degraded independently of NLRP3-inflammasome activity.

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PP258
Transcription factor Nkx3.2 plays crucial role in primary chondrogenesis by up-regulating type II collagen a1 transcription activity
Kosuke Ebina1, Yoshitaka Kawato1, Makoto Hirao2, Yui Honjo3, Tomimitsu Morimoto1, Jun Hashimoto1, Kenrin Shi1 & Hideki Yoshikawa1
1Department of Orthopedic Surgery, Graduate School of Medicine, Osaka University, Osaka, Japan; 2Department of Orthopaedic Surgery, Osaka Minami Medical Center, National Hospital Organization, Osaka, Japan; 3Department of Rheumatology, Osaka Minami Medical Center, National Hospital Organization, Osaka, Japan.

Objectives
Sox9 is a dominant but insufficient transcription factor to induce thorough primary chondrogenesis, so other factors which may induce primary chondrogenesis besides Sox9 have been assumed. The previously reported function of transcription factor Nkx3.2 is to maintain chondrogenic phenotype by suppressing Runx2, while recent studies demonstrated that mouse Nkx3.2 null mice shows severe metaphyseal dysplasia which is similar to that seen in type II collagen a1 (Col2a1) null mice. Therefore, we hypothesized that Nkx3.2 may play a crucial role in primary chondrogenesis besides Sox9.

Methods and results
Mouse multipotent mesenchymal C3H10T1/2 cells and mouse chondrogenic N1511 cells were cultured with bone morphogenetic protein 2 (BMP2) to induce endochondral ossification. Over-expression of Nkx3.2 with wild-type Nkx3.2 (WT-Nkx3.2) plasmid up-regulated glycosaminoglycan (GAG) production and expression of Col2a1 mRNA, and these effects were evident before up-regulation of Sox9. RNAi-mediated inhibition of Nkx3.2 and Sox9 both abolished GAG production and Col2a1 mRNA expression. Interestingly, even when Sox9 is down-regulated, over-expression of WT-Nkx3.2 restored GAG production and Col2a1 mRNA expression to a certain extent. Dual luciferase reporter assays revealed that WT-Nkx3.2 up-regulated Col2a1 enhancer activity in both C3H10T1/2 and N1511 cells in a dose-dependent manner, although it fell short of WT-Sox9. Finally, ChIP assays revealed that Nkx3.2 bounds to the 48 bp Col2a1 enhancer element.

Conclusions
Our results demonstrated that Nkx3.2 is necessary not only in maintaining chondrogenic phenotype, but also in inducing primary chondrogenesis by up-regulating Col2a1 enhancer activity besides Sox9. Further investigation is expected to apply Nkx3.2-targeted treatment to cartilage regeneration.

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PP259
The influence of 2-oxoglutaric acid on articular cartilage of gastrectomised rats
Piotr Dobrowolski1, Ewa Tomaszewska2, Paulina Kurlak1 & Stefan Pierzykowski1,3
1Maria Curie-Sklodowska University, Lublin, Poland; 2University of Life Sciences in Lublin, Lublin, Poland; 3Lund University, Lund, Sweden; 4Institute of Agricultural Medicine in Lublin, Lublin, Poland.

Surgical removal of the stomach (gastrectomy, Gx) leads to osteopenia in animals and in humans. In the rat, Gx causes loss of calvaria and trabecular bone, which can be reduced by 2-oxoglutaric acid (2-Ox), a precursor of hydroxyproline the most abundant amino acid in bone collagen. The purpose of this study was to investigate the effects (if any) of Gx on articular cartilage and if dietary 2-Ox can protects against eventual adverse effects of Gx. Twenty female Sprague–Dawley rats were subjected to Gx and divided between two groups: Gx_2-Ox in the drinking water and Gx_Vehicle (i.e. drinking water without 2-Ox). Another 20 rats were shamoperated and divided between two groups: Sham_2-Ox and Sham_Vehicle. The daily dose of 2-Ox was 0.43 g/100 g rat. All the rats were killed 8 weeks later and the femora and tibiae were collected. The histology and histomorphometry analyses of articular cartilage from knee joints were done. Gx caused significant decrease of total thickness and superficial, intermediate and deep zone thickness of articular cartilage. The effect of Gx was evident in tibia and less pronounced in femur. Gx also affected the structure, decreased density and changed the spatial distribution of thick and thin collagen fibers especially in tibia deep articular layer. 2-Oxoglutareic acid prevented the reduction in the total thickness and superficial as well as intermediate zones thickness of tibia, showing also slight increasing effect in femur articular cartilage which was not significantly affected by Gx. 2-Ox also abolished spatial changes of collagen distribution and structure caused by Gx. Gastrectomy affects articular cartilage quantitatively and qualitatively on the structural level acting selectively on particular bone, however, there are functional foods, namely 2-oxoglutaric acid, that can abolish these effects, most probably, by accelerating of collagen synthesis.

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PP260
Dietary 2-oxoglutarate protects femoral cartilage of 9 months male pigs prenatally treated with dexamethasone
Ewa Tomaszewska1, Piotr Dobrowolski2, Monika Hulas-Stasiak2 & Paulina Kurlak2
1University of Life Sciences in Lublin, Lublin, Poland; 2Maria Curie-Sklodowska University, Lublin, Poland.

Our earlier results indicate that prenatal exposure to dexamethasone, synthetic glucocorticoid, may disturb metabolic processes in skeletal system with long-term consequences. Functional foods show a beneficial action that improve the state of health and reduce the risk of disease. The study was performed to determine whether 2-oxoglutaric acid (2-Ox) can abolish the growth inhibiting effect of prenatally administered dexamethasone (DEX) manifested in the growth plate and articular cartilage. The study was performed on 12 male pigs delivered by the sows administered (i.m) with 3 mg of dexamethasone every second day from day 70 of the pregnancy to the parturition. Half of delivered male piglets were supplemented with 2-oxoglutaric acid during 9 months of postnatal life (0.4 g/kg body weight, daily). The histomorphometry of growth plate and articular cartilage of femur was determined. Immunohistochemical staining with
anti osteocalcin, osteopontin, and osteoprotegerin antibodies was performed. Postnatal administration of 2-Ox to piglets affected by prenatal action of DEX, compared with not-supplemented piglets, improved the thickness of each zone of the growth plate and two zones (superficial and radial) of articular cartilage in the femur. Moreover, 2-Ox increased expression for osteocalcin, osteopontin, and osteoprotegerin in bone tissue. 2-Ox given to piglets during 9 months of postnatal time after prenatal DEX overload significantly reduced the negative action of DEX in articular and growth cartilages connected with higher activity of all cells of bone tissue.

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PP261
Prenatally administered acrylamide programs a gut-bone axis of guinea pig newborns

Ewa Tomaszewska1, Piotr Dobrowolski2, Paulina Kurlak2, Barbara Badzian1, Monika Hulas-Stasialak2, Iwona Puzio1 & Krzysztof Kostro1
1University of Life Sciences in Lublin, Lublin, Poland; 2Maria Curie-Sklodowska University, Lublin, Poland.

Acrylamide is a byproduct that forms when certain carbohydrate and aminoacid rich foods are fried, baked, or roasted at high temperatures (>120 °C). Our earlier study showed that acrylamide altered the morphology and histology of the small intestinal wall damaging the intestinal barrier and reducing absorption surface. The study was performed to determine whether acrylamide influences gut-bone axis in fetus when administered to guinea pig during the last 35 days of pregnancy. The study was carried out on newborns born by guinea pigs receiving clear tap water to drink (the control group, n=6) and by guinea pigs receiving acrylamide in water dose drink in the dose of 3 mg/kg BW per day (the Ac group, n=6). The amount of acrylamide in water was adjusted daily, according to weight increase of pregnant guinea pigs, to achieve appropriate dose. The histomorphometry of growth plate and articular cartilage of tibia as well as small intestine wall was determined. Immunohistochemical staining with anti cadherin antibodies was performed to mark adherent type cell–cell junctions in small intestine epithelium. Lowered expression for cadherin was found in the duodenum and middle part of jejunum in the Ac group of newborn offspring. Acrylamide administration significantly reduced the thickness of the hypertrophy and calcified zones of growth plate, and thickened the radial zone of articular cartilage of tibia. Present study showed that acrylamide might influence development and mineralization of bones during prenatal time by disturbance of gut-bone axis.

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PP262
Establishing an in vitro system to study chondrocyte phenotypes associated to human hereditary hemochromatosis and identify molecular players involved in chondrocyte related iron metabolism

Marcio Simao1,2, Paulo Gavala1, Jorge Pinto1, Ea Kongs3 & M Leonor Canela1,3,4
1Centre of Marine Science (CCMAR), Faro, Algarve, Portugal; 2Department of Biomedical Sciences and Medicine (DCBM-UALG), Faro, Algarve, Portugal; 3Institute for Molecular and Cell Biology (IBMC), Porto, Douro Litoral, Portugal; 4INSERM U606, Hospital Lariboisiere, Paris, Ile-de-France, France.

Background
Bone metabolic disorders, such as osteoarthritis (OA), osteopenia and osteoporosis have been associated to iron overload, both in humans and animal models. In the case of hereditary hemochromatosis (HH), arthropathy represents one of the most prevalent and disabling symptoms. This work aims at investigating the roles of HH-related HFE mutation and iron accumulation on chondrocyte metabolism.

Materials and methods
Primary cultures of articular chondrocytes were developed from WT and hfe KO mice based on the methodology described by Gosset (Nature Protocols, 2008). These cultures were subjected to three iron citrate treatments at several concentrations (0–300 mM) and characterized by analysis of i) gene expression of molecular markers through qPCR and ii) glycosaminoglycan production by Alcian Blue Staining, and presence of collagen II by immunofluorescence assay.

Results
Primary cultures of Hfe KO chondrocytes were established and analysis of cell morphology, alcin blue staining and collagen II protein accumulation were consistent with a chondrocyte phenotype. Expression of genes associated to cartilage metabolism including collagen II and X, aggrecan, and Sox9 were shown to be upregulated in chondrocytes from Hfe KO relatively to WT mice. Expression of Hfe and Ferroportin was strongly upregulated upon iron overload while expression of Transferrin receptor-2 was low and did not respond to iron overload.

Conclusions
We have established for the first time primary cultures of articular chondrocytes from Hfe KO mice and shown that these chondrocytes were significantly upregulated in the mutant cells, indicating changes in normal chondrocyte metabolism. Furthermore, when exposed to iron overdoses, primary chondrocytes showed an absence of response of Transferrin-receptor 2 but a clear response of Hfe and Ferroportin, indicating the presence of a regulatory mechanism in response to iron.

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PP263
Inflammatory effects on knee joint tissue by indoxyl sulfate

Ya-Yun Chen1, Heng-Sheng Lee2,3 & Yu-Juei Hsu3
1Department of Pathology, Tri-Service General Hospital, Taipei, Taiwan; 2Graduate Institute of Pathology and Parasitology, National Defense Medical Center, Taipei, Taiwan; 3Division of Nephrology, Department of Internal Medicine, Tri-Service General Hospital, Taipei, Taiwan.

Indoxyl sulfate (IS) is one of a number of protein-bound uremic toxins that accumulate in patients with chronic kidney disease. Current conventional hemodialysis is ineffective at removing this toxin. Although IS may impair osteoblast function and induce abnormalities of bone turnover or arthropathy, the effects on knee joint tissue by IS has not been investigated yet. The present studies have been carried out to test the IS effects on synovial fibroblasts, mesenchial fibrochondrocytes, and articular chondrocytes.

Our results showed a significant upregulation of cyclooxygenase 2 (COX-2) and interleukin 8 (IL8) in three type cells following IS treatment at a concentration of 100 μg/ml for 24 h. COX-2 was increased 11.5±4.95, 4.21±0.89; and 3.95±0.35-fold in synovial fibroblasts, mesenchial fibrochondrocytes, and articular chondrocytes respectively. IL8 showed 5.87±2.32, 2.98±1.00, and 2.31±0.93-fold increase in synovial fibroblasts, mesenchial fibrochondrocytes, and articular chondrocytes respectively. A dose dependent manner was also identified. IL6 showed no significant change at the same condition examined. The production of nitric oxide (NO) by Griess reaction was 1.62±0.55 and 1.28±0.34-fold increase in synovial fibroblasts and mesenchial fibrochondrocytes respectively.

Uremic toxins have been identified to metabolism by organic anion transporters (OATs) which the roles in joint tissue were unknown. The expression and regulation of OAT1, OAT2, OAT3, OAT4, and URAT1 in three type cells following IS stimulation was then examined. We here first identified that only OAT4, not OAT 1-3 and URAT1, was expressed in three type cells. The novel upregulation of OAT4 by IS stimulation was recognized and showed OAT4 1.83±0.47 and 2.46±0.93-fold increase in synovial fibroblasts and mesenchinal fibrochondrocytes respectively.

Our results showed that IS may induce inflammatory response and oxidative stress in synovial fibroblasts, mesenchial fibrochondrocytes and articular chondrocytes. OAT4 may play an important role in IS metabolism in joint tissue.

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PP264

Upregulation of GAP-43 is linked to the cartilage repair by microarray analysis
Chih-Shan Chang1 & Heng-Sheng Lee1,2
1Graduate Institute of Pathology and Parasitology, National Defense Medical Center, Taipei, Taiwan; 2Department of Pathology, Tri-Services General Hospital, Taipei, Taiwan.

Better quality of cartilage repair in developing skeleton is recognized. The assessed repair factors may be important in osteoarthritics and those factors would be the targets for the management of osteoarthritics. Microarray analysis of cartilage repair in rat knee joint was therefore carried out. Surgical injury on the femoral cartilage of the right patello-femoral joint in the 3- and 8-week-old rats for 2 weeks was first made. The left side of joint cartilage was used as the sham control.

The results showed that cellular proliferation over the surgical injured cartilage in the 3-week-old rat was identified by histology, whereas not in the sham control side and 8-week-old joint cartilage. Primary cultures from the joint cartilage with 1 × 10^6 cells to observe cell proliferation were performed. Fibroblastic morphology with increased growth rate in injured groups was seen. Then, the gene expression level in the sham control and injury groups by microarray analysis demonstrated some novel genes involvement in this process. The top five upregulated genes were asporin (log2 ratio 4.49), growth associated protein 43 (GAP-43) (4.43), tenasin N (4.36), C1q and tumor necrosis factor related protein 3 (4.06), and ADAM metallopeptidase (3.94).

Both asporin and GAP-43 upregulation were confirmed by real time polymerase chain reaction. Further functional verification by cartilage frozen sections in different time courses including 1, 2, 3, and 4 weeks was carried out, especially GAP-43. GAP-43 has been known as a nerve growth associated protein which involves in neurite outgrowth. Here, we newly identified that GAP-43 was expressed strongly on 2 weeks cartilage repair period by immunohistofluorescence.

The GAP-43 expression was correalted with the cyclooxygenase 2 expression during the repair process. On present data, the upregulation of GAP-43 is novelly linked to the cartilage repair process. The target of GAP-43 in osteoarthritis pathogenesis may be value of further investigation.

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PP265

Decrypting TGFβ signaling in age-induced osteoarthritics
Amaya Garcia de Vimiesa1, Esmeralda Blaney-Davidson1, Gonzalo Sanchez-Dufuera1, Arjan van Caam2, Elly Vitters2, Ingrid Meulenbelt1, Marie Jose Goumans1, Peter van der Kraan2 & Peter ten Dijke1
1Leiden University Medical Center, Leiden, The Netherlands; 2Nijmegen Medical Centre, The Radboud University, Nijmegen, The Netherlands.

 Destruction of the articular cartilage is the major feature of Osteoarthritis (OA).

Ageing is the primary risk factor, but how ageing results in OA is still an enigma. In OA, articular chondrocytes degrade their own matrix, while in healthy articular cartilage they preserve it.

Transforming growth factor β (TGFβ) is a central regulator of chondrocyte proliferation, differentiation and extracellular matrix production. Deregelation of TGFβ signaling has been implicated in OA and other cartilage diseases. TGFβ can play both protective and deleterious roles in the articular cartilage, which can be explained by the fact that TGFβ signal via the TGFβ type 1 receptor ALK5, but also via ALK1.

Activated ALK1 induces the phosphorylation of intracellular effectors Smad1/5/8, while ALK5 signals via Smad2/3 resulting in opposite chondrocyte responses. In ageing and OA cartilage the ratio ALK1/ALK5 is increased, leading to preferential activation of the Smad1/5/8 signaling pathway, which mediates the expression of matrix metalloproteinase 13 (MMP13), which is the most potent cartilage-degrading enzyme, contributing to the degradation of the cartilage.

In an attempt to find novel druggable targets that modulate the TGFβ signaling pathway, we have monitored the expression of a number of TGFβ superfamily members and their extracellular regulators in three experimental mouse models: i) C57Bl/6; ii) STR/ort mouse strains, that spontaneously develop OA during ageing; and iii) DMM-inducible OA model, by destabalization of the medial meniscus. Importantly, the mRNA expression of a number of TGFβ family members was strikingly modified towards the onset of OA. Our results point out several members of the TGFβ signaling pathway as important novel candidates that could be implicated in the changes observed on chondrocytes during age-induced OA and their potential use as therapeutic tools and early diagnostic biomarkers.

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PP266

The Rho/ROCK GTPase pathway differentially modulates chondrocyte and osteoblast differentiation from pluripotent stem cells
Dalea M Bukhary, Fraser McDonald & Agamennnon E Grigoriadis
King’s College London, London, UK.

It is well-established that in vitro differentiation of embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) can recapitulate embryonic development through germ layer induction, enrichment and expansion of specific lineages. We have used PSC technology and developed a novel, nESC differentiation system for investigating the mechanisms of chondrocyte and osteoblast lineage commitment and differentiation. This step-wise, serum-free protocol uses specific recombiant factors to investigate i) the mechanisms of PSC commitment to mesoderm and bone/cartilage cell lineages and ii) the role of Rho GTPase signaling in ESC-derived chondrocyte/osteoblast differentiation. Activation of the Nodal/Activin and canonical Wnt pathways together with inhibition of BMP signaling (Noggin) directed ESCs to form a primitive streak-like population expressing Brachyury, which was further enriched to mesodermal subpopulations expressing both lateral plate and paraxial mesoderm markers, which subsequently differentiated efficiently in monolayer culture to chondrocyte and osteoblast lineages. Inhibition of Rho/ROCK signaling using the ROCK inhibitor, Y-27632, at different stages of mesoderm enrichment and differentiation phases modulated chondrogenesis and osteogenesis, showing up to a two-to threefold increase in cartilage and bone nodule formation. This was confirmed by qPCR analysis of osteoblast (Runx2, ALP, and BSP) and chondrocyte (Sox9 and Coll2)-specific genes, as well as by Alcan Blue Staining and Coll2 antibody staining of differentiated chondrocyte monolayers. Preliminary data also suggest that differential exposure to bFGF and BMP4 together with stage-specific addition of Y-27632 enhanced differentiation and/or expansion of hypertrophic chondrocytes and mineralizing osteoblasts. Finally, renal capsule grafting studies showed that the ESC-derived mesodermal populations gave rise to both cartilage and bone in vivo, mimicking endochondral ossification. The ESC model system provides defined, manipulatable and expandable chondro-osteoprogenitor populations that will provide insights into the molecular basis of bone/cartilage development and disease, as well as for generating specific populations for bone and cartilage tissue repair and replacement.

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Genetics

PP267

A genetic determinant of vitamin D and its role in prostate cancer
Olivia Trummer1, Eva Thurner1, Tanja Langsenlehner1, Uwe Langsenlehner2, Sabine Krenn-Piko1, Winfried März1,3, Thomas Pieber1, Barbara Obermayer-Pietsch2 & Wilfried Renner1
1Medical University of Graz, Graz, Austria; 2GKK Outpatient Department, Graz, Austria; 3Syslab Services LLC, Mannheim, Germany.

Preclinical and epidemiologic data suggest that vitamin D deficiency may play a role in the pathogenesis and progression of prostate cancer. Based on recently reported genetic determinants of vitamin D sufficiency we investigated a functional T2-G single nucleotide polymorphism (SNP) in the group-specific component (GC) gene for its association with 25-hydroxy (25-OH) vitamin D and 1.25 dihydroxy (1.25-OH) vitamin D levels and further to test a possible association with metastatic progression and mortality of prostate cancer.

The association of the GC variant with vitamin D levels was analyzed in male participants of the cross-sectional LURIC study comprising 2310 men. The role of the GC variant in prostate cancer outcome was analyzed in the prospective PROCAGENE study comprising 702 prostate cancer patients with a median follow-up of 82 months.

In the LURIC study, the G allele of the GC polymorphism was associated with lower 25-OH-vitamin D levels (TT genotype: 18.6 ng/ml; TG: 17.9 ng/ml; GG: 15.1 ng/ml; P ≤ 0.001) and lower 1.25-OH-vitamin D levels (TT: 35.3 pmol/l; GG: 32.7 pmol/l; P = 0.004). In the PROCAGENE cohort, GC genotypes were not associated with biochemical recurrence (HR 0.89, 95% CI 0.70–1.13; P = 0.32), development of metastases (HR 1.20, 95% CI 0.88–1.63; P = 0.25) or overall survival (HR 1.10; 95% CI 0.84–1.43; P = 0.50).

We conclude that a casual role of vitamin D SNPs in disease progression and mortality in prostate cancer patients is unlikely.

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PP268
No mutations in the serotonin related TPH1 and HTR1B genes in patients with monogenic sclerosing bone disorders
Eveline Boudin1, Karen Jennes3, Fenna de Freitas1, David Tegay2, Qvibe Mortier1 & Wim Van Hul1
1Department of Medical Genetics, University of Antwerp, Antwerp, Edegem, Belgium; 2Department of Medicine, New York Institute of Technology College of Osteopathic Medicine (NYITCOM), Old Westbury, New York, USA.

Since the identification of LRPS as the causative gene for the osteopetrosis pseudoglioma syndrome (OPPG) as well as the high bone mass (HBM) phenotype, LRPS and the Wnt/b-catenin signalling have been extensively studied for their role in the differentiation and proliferation of osteoblasts, in the apoptosis of osteoblasts and osteocytes and in the response of bone to mechanical loading. However, more recently the direct effect of LRPS on osteoblasts and bone formation has been questioned. Gene expression studies showed that mice lacking lrp5 have increased expression of tp1, the rate limiting enzyme for the production of serotonin in the gut. Furthermore mice lacking either tph1 or htr1b, the receptor for serotonin on the osteoblasts, were reported to have an increased bone mass due to increased bone formation. This led to the still controversial hypothesis that LRPS influences bone formation indirectly by regulating the expression of tph1 and as a consequence influencing the production of serotonin in the gut. Based on these data we decided to evaluate the role of TPH1 and HTR1B in the development of craniofibular hyperostoses, a group of monogenic sclerosing bone dysplasias. Using Sanger sequencing, we screened the coding regions of both selected genes in 53 patients with a form of craniofibular hyperostosis who lack a mutation in the known causative genes LRPS, LRPS, and SOST. We found several common and rare coding variants in both studied genes. However, we could not identify disease-causing variants in neither of the tested genes and therefore, we cannot provide support for an important function of serotonin in the pathogenesis of sclerosing bone dysplasias.
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PP269
Expression analysis of mesenchymal KS483 cells during differentiation towards osteoblasts
Igor Fijalkowski, Eveline Boudin, Vere Borra & Wim Van Hul
Department of Medical Genetics, University of Antwerp, Antwerp, Edegem, Belgium.

The murine osteoprogenitor cell line, KS483 (Perucros, The Netherlands) is a well-established model for investigation of osteoblast differentiation and bone formation processes. The mesenchymal characteristics of this cell line allow it to differentiate into either adipocytes or mature, mineralizing osteoblasts. Various phases can be distinguished during osteoblast differentiation and maturation; namely proliferation, matrix formation, matrix maturation, and mineralization. We now performed whole genome expression analysis of mRNA in order to gain additional insight into the molecular mechanisms driving these processes with the focus on Wnt/b-catenin signaling involvement. KS483 cells were differentiated for 28 days in two biological replicates. Total cellular RNA was isolated at eight time points during this process and cRNA was focused on Wnt/b-catenin pathway. Six Wnt genes were expressed throughout the differentiation process, namely Wnt5a, Wnt7a, Wnt7b, Wnt10a, and Wnt10b. Expression of seven members of the LRP family of Wnt co-receptors was detected; namely Lrp1, Lrp4, Lrp5, Lrp6, Lrp10, Lrp11, and Lrp12 with Lrp10 being the most abundant. Predominant role of Lrp6 over Lrp5 can be suggested as it displays, on average, threefold higher expression. Expression of known modulators of Wnt/b-catenin pathway was also investigated. For example for the R-spondin family of Wnt activators, Rspo2 and Rspo3 expression was detected and was accompanied by Lgr5 expression in the late stages of differentiation. In conclusion, we were able to provide a useful and informative tool to investigate the osteoblast differentiation and bone formation. We believe that it grants valuable insight into the molecular mechanisms underlying these processes.
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PP270
SQSTM1/P392L post-zygotic mutations in unrelated patients with Paget's disease of bone
Sabrina Guay-Belanger1,2, Edith Gagnon2, Jean Morissette2, Jacques P Brown1,2 & Lauretta Micheli1
1Department of Medicine, Laval University, Quebec, Quebec, Canada; 2CHU de Quebec Research Centre, Quebec, Quebec, Canada.

Introduction
Paget’s disease of bone (PDB) has an autosomal-dominant mode of inheritance in one-third of cases. The germinal SQSTM1/P392L mutation is the most frequent mutation, present in 40% of patients with a familial form of PDB, and 8% of unrelated patients. Fibrous dysplasia (FD) is a rare bone disorder, mono or polyostotic, caused by post-zygotic mutations in GNAS gene, for which a PCR-clamping method was developed to ease their detection and avoid bone biopsies. Given the focal nature of PDB, this study aimed at optimizing this PCR-clamping method to search for SQSTM1/P392L post-zygotic mutations in peripheral blood of PDB patients.

Methods
We optimized the PCR method in nine FD patients with a locked nucleic acid (LNA) specific for the GNAS/R201L mutation, which blocks the wild-type allele amplification. Thereafter, we optimized the PCR method in PDB patients, using a LNA specific for the SQSTM1/P392L mutation, and we analyzed 210 unrelated PDB patients non carrier of a germinal SQSTM1/P392L mutation. We compared PDB patients with post-zygotic mutation to unrelated patients with germinal mutation and without mutation.

Results
We found, for the reference post-zygotic GNAS/R201L mutation, one carrier among FD patients. Seven (3.3%) unrelated PDB patients carried a SQSTM1/P392L post-zygotic mutation. PDB patients with a SQSTM1/P392L post-zygotic mutation had a Renier’s index lower than patients carrying a germinal mutation (6.9±4.1 vs 15.5±10.4, P=0.046) and had a younger age at diagnosis, sex, number of affected bones, Renier’s index (an anatomical index measuring the PDB extent), and total alkaline phosphatase levels.

Conclusion
This study confirmed that PCR-clamping increases the sensitivity of detection of SQSTM1/P392L post-zygotic mutations which may occur in unrelated patients with PDB. Further analyses are required to understand the functional consequences of this post-zygotic mutation in PDB.

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PP271
A familial case of osteogenesis imperfecta: study of genotype-phenotype correlation.
Alessandra Mihalich, Emanuela Ponti, Francesca Broggi, Anna Maria Di Blasto & Maria Luisa Bianchi
Istituto Auxologico Italiano IRCCS, Milano, Italy.

Osteogenesis imperfecta is a clinically heterogeneous heritable connective tissue disorder. Most OI cases are due to mutations in type I collagen genes, COL1A1 and COL1A2 encoding the pro-alpha1(I) and pro-alpha2(I) chains respectively. However, genotype-phenotype correlation has not been completely elucidated yet. In this study we evaluated a familial case including a mother and a daughter, classified as OI type I. The daughter had more severe clinical features compared to the mother. Both were carrying a 4005 >T mutation in COL1A1 gene, which leads to loss of a splicing site with retention of intron 49 and insertion of a stop codon in the mRNA. Accordingly, both patients had lower levels of COL1A1 transcripts compared to control subjects. Owing to the retention of intron 49, mRNA derived from the mutated allele should be longer than that derived from the wild-type allele. Differential expression analysis of the two alleles was performed on mRNA derived from dermal fibroblasts using RT-PCR. A low amount of transcripts derived from the mutated allele was present only in the daughter. Semi-quantitative determination of allele expression was evaluated by Real-time PCR with primers and probe specific for the mutated and the wild-type mRNA. In the daughter, the levels of the mutated transcripts were 17 times higher than in the mother. In contrast, wild-type mRNA levels were similar in the two patients. Based on these results, it is tempting to speculate that the more severe clinical characteristics of the daughter might be due to the concomitant presence of a quantitative and a qualitative defect. Furthermore, these findings highlight the importance of a detailed molecular characterization of each genetic variant to explain the different phenotypic consequences of the same mutation.

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Polynesian and Caucasian ethnicities

Dorit Naot1, Usha Bava1, Ally Choi1, Karen Callon1, Rocco Pito1-3, Jeromie Bentley2, Greg Gamble1 & Jillian Cornish1
1University of Auckland, Auckland, New Zealand; 2Middlemore Hospital, Auckland, New Zealand.

Polynesians have higher bone mineral density and lower rate of hip fracture compared to age-matched Caucasian in New Zealand, and anecdotal evidence suggests that bones of Polynesian patients heal much faster than those of Caucasians. We compared gene expression in osteoblasts cultured from bone samples taken from patients of Polynesian and Caucasian origin, in order to identify genes and pathways that contribute to the greater density and accelerated healing of Polynesian bones. The study had the approval of the Regional Ethics Committee. RNA was extracted from primary osteoblasts cultured from bone samples obtained during orthopaedic surgery from 30 Polynesian and 30 Caucasian patients. Global gene expression was determined in ten samples from each group using PrimeView GeneChip microarrays (Affymetrix). The samples were age, sex, and BMI matched. Of the >20,000 genes represented on the arrays, 171 genes had a twofold or greater difference in expression levels between the two groups, with about half of the genes showing higher levels of expression in each group. A number of the genes identified by the microarrays were further investigated by real-time PCR in the larger group of samples. So far, the levels of expression of NOV (nephroblastoma overexpressed), EFNB2 (ephrin B2), and EFHD1 (EF-hand domain family, member D1) were found to be significantly lower in the Polynesian group, with approximately twofold difference between the groups for all three genes. Significant differences have been identified between osteoblasts of the two ethnic groups and hypotheses about the contribution of the candidate genes to the accelerated healing of Polynesian bone can be formulated and tested.

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PP273

Functional analysis of the two Runx3 promoters in osseous and non-osseous cells: implications for tissue differentiation specific transcription of distinct isoforms

Natércia Conceic¸a˜n, Brigitte Simo˜es1,2 & M Leonor Cancela1,3
1Centre of Marine Sciences (CCMAR), University of Algarve, Faro, Portugal; 2PhD Program in Biomedical Sciences, Department of Biomedical Sciences and Medicine, University of Algarve, Faro, Portugal; 3Department of Biomedical Sciences and Medicine, University of Algarve, Faro, Portugal.

The Run-domain transcription factors Runx2 and Runx3 are known to drive chondrocyte maturation from prehypertrophic to the terminal stage. The RUNX family proteins form dimers with CBFs, and bind to consensus sequences of 5′-PuACCPuCa-3′ upstream of target genes to activate or repress transcription. To address the role of Runx3 transcription factor in zebrafish, we have isolated the different splice variants encoding distinct runx3 protein isoforms and their corresponding expression patterns were assessed in zebrafish tissues, during development and in bone-derived cells undergoing differentiation, by real-time RT-PCR.

To further understand the molecular mechanism affecting runx3 gene expression, we analyzed the activity of its two promoters, P1 and P2, that drive transcription of the different variants leading to distinct protein isoforms, in osseous and non-osseous cells, using a promoter-derived luciferase reporter system. We performed transient transfection assays with either the full promoters or serial deletion constructs. We observed a reduction in runx3 P1 promoter activity when the complete 5′-UTR was deleted (from −17 to −701 bp), and in P2-promoter activity when two regions (from −1232 to −663 and from −713 to −554 bp) were deleted. These results indicate that the identified regions contain important transcription factors or enhancer binding sites for Runx3 transcription. To identify upstream regulators of the runx3-P1 and P2 promoters, we performed chromatin immunoprecipitation assays in Hek293 cells with runx3-P1 or runx3-P2 luciferase constructs and several transcription factors expression vectors. Runx2 was identified as one regulator of runx3-P2 promoter activity. The runx2-responsive site on the runx3 promoter was identified by in silico analysis and is being confirmed by mutation analysis.

Collectively, our results identify the regions of runx3-P1 and P2 promoters important for runx3 basal transcription and provide a first basis to correlate expression of distinct Runx3 variants with specific transcription factors affecting P1 or P2 promoter activity.

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PP274

Association between polymorphisms in leptin, its receptor and β adrenergic receptors genes and bone mineral density in postmenopausal Korean women

Heon Kim2, Seung-Yup Ku1,2, Soo Hyun Kim1,2, Young Min Choi1,2, Jong Hak Kim3 & Jung Gu Kim1,2
1Department of Obstetrics and Gynecology, Seoul National University College of Medicine, Seoul, Republic of Korea; 2Clinical Research Institute, Seoul, Republic of Korea; 3Department of Anesthesiology and Pain Medicine, School of Medicine, Ewha Womans University, Seoul, Republic of Korea.

Objective

The purpose of this study was to investigate the association between single nucleotide polymorphisms (SNPs) in leptin (LEP), its receptor (LEPR) and β adrenergic receptor (ADRB) genes and bone mineral density (BMD) in postmenopausal Korean women.

Methods

We performed the LEP c.280G>A, LEPR c.326A>G, c.668A>G, c.1986G>C, c.2096G>T, ADRB2 c.46A>G, c.79C>G, c.718T>C, c.741G>T, c.769G>A, ADRB3 c.190T>C polymorphisms were analyzed in 592 postmenopausal Korean women. Serum levels of leptin, soluble leptin receptor (sLR), osteoprotegerin (OPG), soluble receptor activator of the nuclear factor-κB ligand (sRANKL), bone alkaline phosphatase, and carboxy-terminal telopeptide of type I collagen were measured and BMDs at the lumbar spine and femoral neck were also examined.

Results

The LEPR c.1986G>C polymorphism only was found to be related with BMD at the femoral neck, and higher BMD was demonstrated with an increasing number of G allele (P = 0.04). Osteoporosis at femoral neck were 3.27- and 3.89-times more frequently observed in the AG and GG genotypes compared to AA genotype in ADRB2 c.46A>G polymorphism (P = 0.02 and P = 0.02 respectively). However, no significant differences in serum levels of leptin, sLR, OPG, sRANKL, and bone turnover markers were detected among single and haplotype genotypes.

Conclusions

Our results suggest that the LEPR c.1986G>C polymorphism may be one of genetic factors affecting femoral neck BMD in postmenopausal Korean women, and that analysis of ADRB2 c.46A>G polymorphism may be useful in identifying women at risk of osteoporosis.

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PP275

Genetic aspects of bone remodeling disturbance in patients with aggressive periodontitis

Anastasia Zinovyeva1,2, Victoria Atrushkevich1,2 & Alexander Polyakov1,2
1Moscow State University of Medicine and Dentistry, Moscow, Russia; 2Moscow State Scientific Center of the Russian Academy of Medical Sciences, Moscow, Russia.

Introduction

Aggressive periodontitis (AgP) is an inflammatory disease causing rapid loss of teeth in young patients.

Aim

Determine degree of impact of COL1A1 gene on likelihood of AgP development.

Materials and methods

Study included 47 patients with AgP, 40 patients with osteoporosis (OP), and 64 healthy patients (HP). Polymorphic variant c.104-441G>T (COL1A1) was studied. Statistics: Fisher’s, P ≤ 0.05/95% CI.

Results

Genotype distribution in AgP patients and HP showed that likelihood of AgP development increased sevenfold if patient was carrier of T/T genotype (P = 0.002/95% CI 2.26–21.47). Genotype distribution in OP and HP groups showed that the risk of OP development is significantly related to G/G genotype which increases the likelihood of OP development 4.3-fold (P = 0.001/95% CI 1.84–10.11). Moreover, study of OR showed that genotype G/G is protective for AgP patients as its carriers are significantly less likely to develop OP (P = 0.05/95% CI 0.21–0.99).

Discussion

The AgP c.104-441G>T can increase the risk of OP development in G/G genotype subjects and AgP in T/T genotype subjects. So it can be a marker of both diseases. T/T variant in proband genotype can be a predictor of AgP development in close relatives. Thus analysis of this gene is recommended to detect disease at an early stage, and differential diagnosis of AgP from other forms of periodontitis.

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PP276

No association between the CYP1B1/Leu432Val polymorphism and osteoporosis-related traits in Slovak postmenopausal women
Radoslav Omelka1, Vladimira Krajcovicova1, Jana Spankova1, Jana Durisova1, Monika Martinakova1, Drahorim Galbavy2 & Maria Bauera
1Constantine the Philosopher University, Nitra, Slovakia; 2Private Orthopedic Ambulance, Nitra, Slovakia.

It is well known that sex hormone deficiency leads to increased bone turnover and subsequent bone loss. The metabolism of estrogens involves, among others, oxidation (mainly hydroxylation) by CYPs. The aim of this study was to determine whether Leu432Val polymorphism in the CYP1B1 gene is present also in Slovak population and subsequently, if it is associated with femoral and spinal bone mineral density (FBMD and SBMD), bone remodeling markers and fracture incidence in this population. The study sample consisted of 338 postmenopausal women (63.4±7.5 years) including osteoporotic, osteopenic, and healthy individuals. Subjects were selected according to the inclusion and exclusion criteria. Genotypes were detected using PCR-RFLP method. CYP1B1 genotypes and allele frequencies were tested using the chi-square test. The differences between the genotypes were analyzed by GLM procedure and covariance analysis after correction of the measurements for age and BMI. To find the association within the population we calculated the distribution of genotypes of CG (46.7%), CC (34.6%), and GG (18.6%), where C allele disposed higher frequency (0.58). We didn’t find a relationship between Leu432Val polymorphism and femoral and spinal BMD. Within the association study of CYP1B1 genotypes with bone formation (ALP and OC) and bone resorption markers (CTX), we found no overall association among analyzed postmenopausal women, as well as fracture incidence (P>0.05). Our data reveal no association between Leu432Val polymorphism and parameters of bone turnover markers and FBMD, SBMD in a population of Slovak postmenopausal women. The results can contribute to a comprehensive view of the genetics of osteoporosis. All procedures were approved by the Ethical Committee of the Specialized Hospital of St Svorad in Nitra. This work was supported by grants KEGA 025UKF-4/2012; 035UKF-4/2013.

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PP277

A genomic and transcriptomic approach to the high bone mass phenotype: evidences of heterogeneity and of additive effects of TWIST1, IL6R, DLX3, and PPARG
Fernando Sarrion1, Leonardo Mellibovsky2, Roser Ureiziti1, Sergi Civit1, NeusCols1, Natalia Garcia-Giralt1, Guy Yoskovitz1, Alvaro Aranguren1, Jorge Malouf2, Luis del Rio2, Roberto Gueñu2, Xavier Nogues2, Adolfo Díez-Pérez2, Daniel Grinberg1 & Susana Ballecs1.

The aims of the study were to establish the prevalence of the high bone mass (HBM) phenotype in a cohort of Spanish postmenopausal women (BARCOS); to determine whether any of the HBM cases carry LRP5 or DKK1 mutations; to test the hypothesis of an inverse correlation between the number of common variant alleles and HBM; and to characterize the expression of osteoblast-specific and Wnt pathway genes in primary osteoblast RNA samples from two HBM cases. HBM individuals within the BARCOS cohort were identified according to the criterion of a sum score ≥4. Relevant exons of LRP5 and DKK1 were PCR-amplified and sequenced. Fifty-five BMD SNPs from Estrada et al. (NatGenet 44:491–501, 2012) were genotyped in the HBM cases and a weighted score was obtained for each individual. Scores were plotted against Z-score values. Primary osteoblasts from two HBM and five controls were cultured and RNA was extracted. A qPCR Custom Panel was used to analyse the expression of 88 osteoblast-specific and/or Wnt pathway genes. A 0.7% of individuals displayed Z-score values in the HBM range (11±600). No mutations in the LRP5 gene were found in these women and one had a rare missense change in DKK1 (p.Y74F). Regarding risk alleles, results pointed to an inverse correlation between those Z-scores in the HBM group of women, although the woman with the highest Z-score presented with the highest risk score. A frequency penetrant unknown genetic variant may explain this case. Finally, the expression analysis showed that levels of ILAR, DLX3, TWIST1, and PPARG mRNA were inversely related to Z-score and that one HBM case presented with high levels of RUNX2 while the other displayed very low SOX9.

In conclusion, we provide evidences of heterogeneity and of additive effects of TWIST1, ILAR, DLX3, and PPARG for the HBM phenotype.

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PP278

Gene-wide association study of RANK and RANKL genes in the bone context: functional study of BMD-associated SNPs
Natalia Garcia-Giralt1, Guy Yoskovitz1, Maria Rodriguez-Sanz1, Roser Ureiziti1, Sergio Artero-Ballester1, Daniel Prieto-Alhambra1, Leonardo Mellibovsky2, 3Daniel Grinberg2, Xavier Nogues2, 3Susana Ballecs2 & Adolfo Díez-Pérez2, 3

Institut Hospital del Mar d’Investigacions Mèdiques (IMIM), RETICEF, Barcelona, Catalonia, Spain; 1Departament de Genètica, Universitat de Barcelona, IBUB, CIBERER, ISCIII, Barcelona, Catalonia, Spain; 2Servei de Medicina Interna, Hospital del Mar, Universitat Autònoma de Barcelona (UAB), Barcelona, Catalonia, Spain; 3NIHR Biomedical Research Unit, University of Oxford, Oxford, UK.

Over the past decade, many GWAS and meta-analyses were performed to identify genes and regions involved in bone metabolism and in the osteoporotic phenotypes. Nevertheless, the majority of these GWAS results were not tested at any functional level. This study aims to find and study functional regions in the RANK and RANKL genes that encode well-established proteins in the bone remodeling equilibrium. SNPs, chosen for their location in an evolutionary conserved region or replicated from previous studies, were genotyped in the BARCOS cohort of 1098 postmenopausal women. SNP rs9594738, which lies 184 bp upstream of the RANKL gene, was found to be associated with lumbar spine bone mineral density (Log additive model: beta coefficient = -0.021, P = 3.8 × 10⁻⁸). Functional experiments exploring this RANKL distal region (DR) harboring rs9594738 demonstrated the region’s capacity to inhibit the RANKL promoter in reporter gene assays. Moreover, DR was activated in vitamin D presence. In conclusion, our results demonstrate DR functionality in the RANKL gene context, with a vitamin D involvement.

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PP279

Genome-wide association study meta-analysis identifies the SOAT1/AXDND1 locus to be associated with hip and forearm fracture risk
Ulrika Petersson-Kynner1, 2, Andrea Lacroix3, Joel Eriksson4, Ulrica Bergström3, Beatrice Melin3, Carl Wibom4, Lissbeth Vandenput5, Preetha Rajaraman6, Patricia Hartge7, Stephen Chanock8, Göran Hallmans2, David Duggan2, Charles Kooperberg3, Samuel Handelman4, Aaron Aragaki1, Maria Nathaniel1, 9André Utterlinden10, Fernando Rivadeneira1, 2, Rebecca Jackson1 & Claes Ohlsson4

1Pharmacology and Clinical Neurosciences, Umeå University, Umeå, Sweden; 2Public Health and Clinical Medicine, Umeå University, Umeå, Sweden; 3Fred Hutchinson Cancer Research Center, Seattle, Washington, USA; 4Center for Bone and Arthritis Research, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden; 5Surgical and Perioperative Sciences, Umeå University, Umeå, Sweden; 6Department of Radiation Sciences, Oncology, University of Umeå, Umeå, Sweden; 7Radiation Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, Bethesda, Maryland, USA; 8Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, Maryland, USA; 9Translational Genomics Research Institute, Phoenix, Arizona, USA; 10Department of Pharmacogenomics, College of Medicine, Ohio State University, Columbus, Ohio, USA; 11Genomics Core Facility, University of Gothenburg, Gothenburg, Sweden; 12Department of Internal Medicine, Erasmus Medical Center, Rotterdam, The Netherlands; 13Division of Endocrinology, Diabetes and Metabolism, Ohio State University, Columbus, Ohio, USA.

Hip and forearm fractures are the two clinically most important non-vertebral fractures. Twin studies have demonstrated a high heritability of these fractures and the heritable component of fracture risk is largely independent of BMD. To identify common genetic variants associated with hip and forearm fractures, we performed a genome-wide association study (GWAS ~ 2.5 million SNPs) meta-analysis of two large fracture data sets within the well-characterized UFO cohorts: UFO-hip: 1014 hip fractures and 862 controls, and UFO-forearm: 1060 forearm fractures and 1055 controls). All fractures were confirmed through radiographic reports. Replication was performed in the Women’s Health Initiative (WHI) cohort (1845 hip fractures verified by medical records and 2120 controls). We identified one SNP within the SOAT1/AXDND1 locus (1q25.2) that was associated with fracture risk at genome wide significance (OR per allele = 1.33; P=3.1×10⁻⁷) in the UFO discovery meta-analysis. This SNP was associated with fracture risk both in the WHI replication cohort (OR 1.16, P=2.1×10⁻⁵) and in the combined analyses comprising 7956 subjects (3919 cases and 4037...

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controls; OR = 1.24, \( P = 5.6 \times 10^{-10} \). However, it was not associated with BMD or biochemical bone markers, suggesting that its association with fractures is BMD-independent. A genetic score (GS), including information from 63 SNPs earlier shown to be reproducibly associated with BMD, was significantly associated with both hip (\( P = 7.9 \times 10^{-10} \)) and forearm (\( P = 8.6 \times 10^{-10} \)) fractures. Models including both the SNP in the \( SOAT1 \)/\( AXDN1 \) locus and the GS demonstrated that the impact of the SNP in the \( SOAT1 \)/\( AXDN1 \) locus on fracture risk was independent of the BMD-associated GS.

In summary, both a BMD-associated GS and a non-BMD-associated genetic variant in the \( SOAT1 \)/\( AXDN1 \) locus are associated with hip and forearm fractures.

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PP280

Association between dentinogenesis imperfecta and mutations in \( COL1A1 \) and \( COL1A2 \) genes

Kristofer Andersson\(^{1}\), Goran Dahlöf\(^{1}\), Eva Äström\(^{1,2}\), C-J Rubín\(^{4}\), A Kindmark\(^{4}\), Katarina Lindahl\(^{4}\), Östen Ljunggren\(^{4}\) & Barbro Malmgren\(^{4}\)

\(^{1}\)Division of Pediatric Dentistry, Department of Dental Medicine, Karolinska Institutet, Stockholm, Sweden; \(^{2}\)Department of Woman and Child Health, Karolinska Institutet, Stockholm, Sweden; \(^{3}\)BM3, Karolinska University Hospital, Stockholm, Sweden; \(^{4}\)Department of Endocrinology, Medical Sciences, Uppsala University, Uppsala, Sweden.

Introduction

Dentinogenesis imperfecta (DI) is a common dental aberration in patients with osteogenesis imperfecta (OI). Mutations that cause abnormal collagen chains will cause more serious types of OI and it has been claimed that DI should be a marker for qualitative defective collagen. It has also been supposed that normal development of teeth may be more dependent on normal \( \alpha(1) \) chains which are encoded by \( COL1A1 \) and \( COL1A2 \) genes respectively. The purpose of the present study is to investigate the correlation between dentinogenesis imperfecta and mutations in \( COL1A2 \) and \( COL1A1 \) genes.

Subjects and methods

126 families with DI accepted participation. Exons and flanking intron sequences of \( COL1A1 \) and \( COL1A2 \) have been sequenced in 93 of these families; 54 type I, 21 type IV, 15 type III, and 3 with unclear OI type. Only one patient from each family was included in the study. Clinical and radiographic examinations were performed in 85 of these patients regarding DI.

Results

Mutations in the \( COL1A1 \) gene were found in 63 patients. DI was observed in 21 of these patients (33%). Mutations in the \( COL1A2 \) gene were found in 20 patients and DI was present in 14 of these patients (70%; \( P = 0.013 \)).

Conclusion

It seems to be a correlation between DI and mutations in the different \( \alpha \)-chains. Patients with a \( \alpha(2) \) aberration had DI significantly more often compared to those with an \( \alpha(1) \) aberration.

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PP281

Role of the functional Toll-like receptor-3 promoter polymorphism in the increased risk of osteoarthritis

Sui-Lung Su & Hsin-Yi Yang

NMDIC, Taipei, Taiwan.

Toll-like receptors (TLRs) appear to be involved in the pathogenesis of osteoarthritis (OA) and recent studies have suggested that polymorphisms in TLR9, an endosomal TLR are associated with knee OA in at least one population. TLR3 is also found on the surface of endosomes where they respond primarily to nucleic acid based pathogen-associated molecular patterns (PAMPs) from viruses and bacteria. We therefore determined the predictive value of TLR3 gene polymorphisms and further functional study on knee OA in a Han Chinese population. Two separate populations were studied in a two stage case–control study with a total of 823 OA cases and 594 healthy controls. Four single nucleotide polymorphisms (SNPs) of TLR genes were evaluated by PCR–RFLP assays. Real-time PCR were performed to test the functional expression of the identified promoter polymorphism following dexamethasone stimulation. An association with polymorphisms at rs3775296 and rs3775290 of TLR3 and knee OA was identified in both populations. The ATCA haplotype of TLR3 was associated with a decreased risk of OA CTTA and CCTA haplotypes were associated with an increased susceptibility. We also found a significant difference in the expression of TLR3 by dexamethasone treatment among the various genotypes of rs3775296 (\( P < 0.001 \)). Our findings indicate that a SNP in the promoter region of TLR3 is associated with elevated TLR3 mRNA level and with susceptibility to knee OA in the Han Chinese population.

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PP282

Phenotypic dissection of bone mineral density facilitates the identification of skeletal site specificity on the genetic regulation of bone

John P Kemp\(^{1,2}\), Carolina Medina-Gomez\(^{3,4}\), Karol Estrada\(^{3,5}\), Denise Herpe\(^{5}\), Carola Zillikens\(^{1,4}\), Nicholas Timpson\(^{1,2}\), Beate Pourcain\(^{1}\), Susan Ring\(^{1}\), Albert Hofman\(^{1}\), Vincent W V Jaddoe\(^{2}\), George Davey Smith\(^{1,2}\), André G Uitterlinden\(^{1,5}\), Jonathan H Tobias\(^{4}\), Fernando Rivadeneira\(^{1,2}\) & David M Evans\(^{1,2}\)

\(^{1}\)School of Social and Community Medicine, MRC CAiTE Centre, University of Bristol, Bristol, UK; \(^{2}\)School of Social and Community Medicine, University of Bristol, Bristol, UK; \(^{3}\)Department of Internal Medicine, Erasmus University Medical Center, Rotterdam, The Netherlands; \(^{4}\)Department of Epidemiology, Erasmus University Medical Center, Rotterdam, The Netherlands; \(^{5}\)The Generation R Study Group, Erasmus University Medical Center, Rotterdam, The Netherlands; \(^{6}\)School of Clinical Sciences, University of Bristol, Bristol, The Netherlands, UK.

Heredity of bone mineral density (BMD) varies at skeletal sites, possibly reflecting different relative contributions of environmental and genetic factors. To quantify shared genetic influences across different sites, we estimated the genetic correlation of BMD at the upper limb (UL), lower limb (LL), and skull (S) obtained from whole body DXA scans, using bivariate genome-wide complex trait analysis (GCTA). The study (\( n = 9395 \)) combined data from the Avon Longitudinal Study of Parents and their Children (\( n = 5299 \), mean age = 9.9 years) and the Generation R study (\( n = 4096 \), mean age = 6.2 years). GCTA estimates indicated that LL- and UL-BMD shared a high proportion of common genetic architecture (\( r_g = 0.78 \)), compared to UL- and S-BMD (\( r_g = 0.58 \)) and LL and S-BMD (\( r_g = 0.43 \)). To explore the basis for these differences, genome-wide association analyses (GWAS; with meta-analysis) were performed to identify genetic signals associated with specific skeletal regions. A novel variant was identified within the \( RIN3 \) gene, independent of that previously reported in association with BMD, which was specifically associated with LL-BMD (\( P < 5 \times 10^{-8} \)). Several genetic variants previously reported to be associated with BMD differed in their associations with BMD at different sub-regions. Specifically, effect sizes of variants which were independent, but proximal, revealed considerable degrees of site specificity at the WNT16 (\( rs173031 \)) and \( CENPW \) (\( rs2243232 \)) loci. WNT16: \( rs12229306 \) showed stronger associations with S-BMD (\( b = 1.07, P = 1.5 \times 10^{-11} \)) and UL-BMD (\( b = 0.19, P = 3.0 \times 10^{-5} \)) compared to LL-BMD (\( b = 0.02, P = 0.2 \)). \( rs2098004 \) was more strongly associated with UL-BMD (\( b = 0.18, P = 4.1 \times 10^{-6} \)) compared to S-BMD (\( b = 0.09, P = 3.6 \times 10^{-5} \)) and LL-BMD (\( b = 0.10, P = 3 \times 10^{-5} \)). \( CENPW \) rs2130604 was associated with S-BMD (\( b = 0.11, P = 3.3 \times 10^{-5} \)) more strongly than with UL-BMD (\( b = 0.04, P = 0.02 \)) and LL-BMD (\( b = 0.02, P = 0.28 \)). Our results suggest that BMD at different skeletal sites are to a certain extent under distinct genetic influences. Allowing for these differences may help to uncover new genetic influences on BMD, by providing greater power due to stronger site specific genetic effects.

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PP283

Discovery and replication of several loci significantly associated with lean body mass: a large meta-analysis of genome wide association studies (GWAS) from the ‘charge’ and ‘gofors’ consortia

Douglas P Kiel\(^{1}\), Laura M Yerges-Armstrong\(^{2}\), Yi-Hsiang Hsu\(^{1}\), Lizette Stolk\(^{1,5}\), David Karasik\(^{1}\), Judith F Loos\(^{3,6}\), Vilimundur Gudnason\(^{3}\), Albert Smith\(^{7}\), Jeffrey R O’Connell\(^{7}\), Amish Fu\(^{7}\), Mao Fu\(^{7}\), Elizabeth A Streit\(^{7}\), Jane A Cauley\(^{7}\), John A Robbins\(^{1}\), Bruce Psaty\(^{7}\), Toby Johnson\(^{1,7}\), Zohsin Kutilak\(^{1,12}\), Braxton D Mitchell\(^{7}\), Gregory Livshits\(^{13,14}\), Tamara B Harri\(^{15}\), Claes Ohlsson\(^{16}\) & M Carola Zillikens\(^{1,5,6}\)

\(^{1}\)Hebrew SeniorLife and Harvard Medical School, Boston, Massachusetts, USA; \(^{2}\)Division of Endocrinology, Program in Personalized and Genomic Medicine, Department of Medicine, Diabetes and Nutrition, University of Maryland School of Medicine, Baltimore, Maryland, USA; \(^{3}\)Department of Internal Medicine, Erasmus MC, Rotterdam, The Netherlands; \(^{4}\)MRC Epidemiology Unit, Addenbrooke’s Hospital, Institute of Metabolic

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ECTS 2013
The creatine kinase (CK) is a dimeric enzyme, involved in energetic metabolism. It is present in many tissues, but higher concentration in skeletal and cardiac muscle. Therefore, conditions that involve muscle tissue may increase this serum enzyme. Such enzyme elevation is usually observed in inflammatory myopathies and others autoimmune diseases. Sometimes some elevation in CK is not fully understood out of these contexts, especially in absence of characteristic symptoms in muscle: weakness, myalgia, and fatigue.

Objectives
We study patients with or without symptoms having raising CK found in laboratory tests.

Materials and methods
We assessed patients at our Rheumatology Unit, with CK values greater than minimal of three times of the normal value. Diagnostic procedures performed: interrogatory, exhaustive physical examination (muscle strength, muscle tone, and OTR), laboratory tests: CRP, ESR, protein electrophoresis, ANA and others autoantibodies, according to the clinical context (Jo and MI); TSH, transaminases, EMG, muscle biopsy, and immune tagging (dystrophin, sarcoglycans, and calpain) according to appropriate procedure.

Isolated increased of the enzyme, required more intensive investigations to rule out other causes of elevations or situations that raise CK. (heart attack, rabdomiolisis, iatrogenic, toxics, endocrine: hypo/hyperthyroidism, Cushing’s diseases, hypoparathyroidism, infectious myopathy, myotostias, storage diseases, and glycogenosis (Pompe disease and McArdle disease), mitochondrial myopathy, and neuromuscular (ELA, mutations of gen cavn-3).

Results
Of all patients (n 128), of both genders with CK elevations we found a dominant distribution of PM/DM (78%), among others collagen diseases (9.4%), such as RA, SEL, SSc, vasculitis, sarcoidosis, and sudeck. Also significant increases medicated patients with toxic effects (4.7%: statins, zidovudine), endocrine (3.9%): hypo/hyperthyroidism, Cushing’s diseases, hypoparathyroidism, vitamin D deficiency, and muscle dystrophy (2.3%): steinert, dystrophopathpy, Nieman Pick, and amyotrophic lateral sclerosis. Patients in whom was no probable cause was found for the enzyme elevations (1.6% idiopathic).

Conclusions
There are many diseases that can generate elevations of CK, many of which are accompanied by clear symptomatology but others less do so. The challenge is getting to elucidate the cause of the enzymatic elevations not covered in the usual diagnostic or included within the group of idiopathic hyperekplexia.

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Muscle, physical activity and bone

Raising CK: what it means according to different clinical landscape?

Juan Jose Scali, Susana Visentin, Ramiro Berruezo, Daniel Sevilla, Gonzalo Pacheco & Rodrigo Garcia
Carlos G. Durand Htal, Buenos Aires, Argentina.

Introduction
The creatine kinase (CK) is a dimeric enzyme, involved in energetic metabolism. It is present in many tissues, but higher concentration in skeletal and cardiac muscle. Therefore, conditions that involve muscle tissue may increase this serum enzyme. Such enzyme elevation is usually observed in inflammatory myopathies and others autoimmune diseases. Sometimes some elevation in CK is not fully understood out of these contexts, especially in absence of characteristic symptoms in muscle: weakness, myalgia and fatigue.

Objective
We study patients with or without symptoms having raising ck found in laboratory tests. Materials and methods

We assessed patients at our rheumatology unit, with CK values greater than minimal of three times of the normal value. Diagnostic procedures performed: interrogatory, Exhaustive physical examination (muscle strength, muscle tone, and OTR), laboratory tests: CRP, ESR, protein electrophoresis, ANA and others autoantibodies, according to the clinical context (Jo and MI); TSH, transaminases, EMG, muscle biopsy, and immune tagging (dystrophin, sarcoglycans, and calpain), according to appropriate procedure.

Isolated increased of the enzyme, required more intensive investigations to rule out other causes of elevations or situations that raise CK. (heart attack, rabdomiolisis, iatrogenic, toxics, endocrine: hypo/hyperthyroidism, Cushing’s diseases, hypoparathyroidism, infectious myopathy, myotostias, storage diseases, glycogenosis (Pompe disease and McArdle disease), mitochondrial myopathy, and neuromuscular (ELA, mutations of gen cavn-3).

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Conclusions
There are many diseases that can generate elevations of CK, many of which are accompanied by clear symptomatology but others less do so. The challenge is getting to elucidate the cause of the enzymatic elevations not covered in the usual diagnostic or included within the group of idiopathic hyperekplexia.

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PP286

Muscle function and quality of life in primary hyperparathyroidism
Lars Rolighed1, Anne Kristine Amstrup2, Niels Frederik Breum Jakobsen2, Tanja Sikaer2, Leif Mosekilde2, Peer Christiansen2 & Lars Rejnmark2
1Department of Surgery F, Aarhus, Denmark; 2Department of Endocrinology and Metabolism MEA, Aarhus, Denmark.

Introduction
In spite of the frequent encounter of ‘asymptomatic’ primary hyperparathyroidism (PHPT), the patients often describe various relevant improvements postoperatively suggesting a subclinical biological effect of elevated PTH or hypercalcemia.

Materials and methods
To evaluate muscle function, postural stability, and quality of life (QoL) in untreated PHPT, we assessed maximal isometric muscle strength in upper and lower extremities, time of ten-repeated-chair-stands (RCS), time to walk 3 m and back (TTW), balance function, and questionnaires of QoL in 38 untreated PHPT patients and 58 controls matched on age, sex, and menopausal status.

Results
Patients and controls had a mean age of 59 years and 47 (81%) were women. We found marked differences between groups in PTH (13.9 vs 4.8 pmol/l) and ionized calcium levels (1.45 vs 1.24 mmol/l), whereas plasma creatinine and 25(OH)D levels did not differ. In PHPT, the SF-36 questionnaire showed a lower QoL in all eight domains (P<0.05) and the WHO-S index showed a reduced well-being (P<0.001).

Postural stability was impaired in PHPT during normal standing with eyes open (P<0.05) and eyes closed (P<0.001). Female patients spent significantly longer time on performing the RCS- and TTW-tests, and had a lower muscle strength in upper (P<0.01) and lower extremities (P<0.001) compared with female controls. In men, muscle function and strength did not differ between groups.

Conclusions
In PHPT, constantly elevated PTH and calcium levels seem to have deleterious effects on muscle strength, muscle function, postural stability and QoL. The increased risk of fracture in PHPT may therefore both be related to a decreased

PP287

Muscle strength and peripheral fractures in osteoporotic patients
Hans-Christof Schober, Franka Hamann & Johanna Torner
Klinikum Südstadt, Rostock, Germany.

Introduction
Osteoporosis and fractures are multifactorial events. Muscle weakness and changes in bone density are of special importance. The aim of this study was to determine wether clinically applicable physical tests could be used to identify the relation between physical performance and fractures.

Methods
Data of 179 community dwelling female patients (mean age 74±5.4 years) suffering osteoporosis were retrospectively investigated. Peripheral fractures were documented by questionnaire, vertebral fractures were detected using X-ray. Height and weight as well as body composition (% body fat, % body muscle, and visceral fat) were measured. Parameters of physical activity like time in tandem stand, Chair-rising test, and gait speed were measured by stopwatch. Hand grip strength on the right and left side were analyzed using a hand held dynamometer (kg).

Bone turnover markers like PTH and vitamin D, Tartrat-resistant acid phosphatase 5b (TRAP5b), alkaline phosphatase, and bone specific alkaline phosphatase were quantified. The statistical calculation was accomplished using Spearman’s correlation coefficients and Glimmix procedure.

Results
Hand grip strength on left hand (nondominant) correlates negatively (P<0.01) with the number of peripheral fractures. Gait speed correlates negatively (P<0.02) whereas visceral fat mass correlates positively (P<0.04) with the number of vertebral fractures.

Conclusion
Two components of the frailty model were associated with an increased number of peripheral and vertebral fractures. The role of visceral fat has to be determined. Clinically applicable physical tests are able to describe the relation between muscle weakness and the number of fractures. Training might be useful in these patients.

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PP288

Microgravity modulates nitric oxide homeostasis in vascular system
Pradeep Thagaraj & Suvo Chatterjee
AU-KBC Research Center, Chennai, India.

Microgravity causes adverse health problems to astronauts during space flight, especially to bone, heart, and muscles. The vascular system plays a central role in various organs and the skeletal tissues. This new environment makes vascular adaptation difficult. Nitric oxide plays an essential role in the vascular system by modulating basal vascular tone. An alteration of NO metabolism or bioavailability has been thought to be one of the main factors for vascular disorders. Restoring NO equilibrium in the system has been proposed as a promising therapeutic tool in alleviating vascular problems in space or post space travel. Blood vessels are lined with endothelium, an expansive cell layer with total surface area of 4000–7000 m² in an average-sized human. Therefore, alteration in endothelial NO is anticipated to perturb vessel health. Results of this study demonstrated that exposure of the endothelium to limited periods (2–24 h) of microgravity resulted in elevated NO production and faster growth and development of vascular tubes in both in vitro and in vivo models. To understand the elevated NO perturbations in heart under microgravity we investigated the cardiac functions using Chick embryo and zebra fish as models to determine heart rate under microgravity. Results showed that in the presence of NO, the rate of heart beat increased significantly under microgravity. Removal of NO resulted in heart beat returning to normal. Results suggest that administration of NO based therapy to astronauts during space flight could potentially overcome microgravity mediated vascular problems and improve the performance of heart, through which other organs, including bone, could be rescued to a nearly normal condition.

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PP289

The outcomes and costs of falls in elderly women
Inga Tamulaityte-Morozoviene, Vidmantas Alekna, Rimantas Stukas & Marija Tamulaityiene
Faculty of Medicine, Vilnius University, Vilnius, Lithuania.

Objective
To examine the outcomes and costs of direct medical care for falls in elderly women in Lithuania.

Methods
Women aged 65 years and older, who visited National Osteoporosis Center for diagnostic or treatment procedures. Thereafter a telephone survey was performed using the questionnaire with 28 questions about the number, circumstances, and consequences of falls. The cost of health care due to fall was estimated after calculating the sum of costs for all out-patient visits, procedures or hospitalizations, excluding the cost of medication and medical equipment.

Results
The study population consisted of 878 community-dwelling women (65–90 years old), with mean age of 72.2±4.8 years. Self-reported falls during past 12 months were reported by 310 (35.3%) women, one in seven women had fallen twice or more. Women over 75 years fell more frequently than younger (P=0.021). Of all 407 falls, 90.3% resulted in various injuries, and 77 (18.9%) falls – in bone fractures. There were 41 (53.2%) forearm fractures, 7 (9.1%) vertebral fractures, 6 (7.8%) hip fractures, and 23 (29.9%) other fractures reported. Owing to the fall consequences, 115 women (37.1%) visited an outpatient clinic, 15 (4.8%) were hospitalised. The mean estimated direct health care cost was 194 EUR for the fall with non-fracture injuries, 2571 EUR – with hip fracture, 219 EUR – for fall with forearm fracture.

Conclusions
From all the falls registered in women over 65 years, 90.3% resulted in any injuries, and 18.9% – in bone fracture. The mean cost of direct health care for fall related non-fracture injury was 194 EUR. The costs for fall with hip fracture were the highest and reached 2571 EUR.

Key words: elderly women, falls, health care costs, outcomes.

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Response of mechanically strained tenocytes to different cell culture substrates

David Musson1, Jung-Joo Kim2, Karen Callon1, Dorit Naor1, Vickie Shim3, Iain Anderson1, Ji Hye Kim1, & Ashuka Chhabra1

1Department of Medicine, University of Auckland, Auckland, New Zealand; 2Auckland Bioengineering Institute, University of Auckland, Auckland, New Zealand.

The musculoskeletal system experiences severe mechanical strain, with repetitive or extreme strains causing significant trauma; the result being an increase in mechanobiological studies evaluating mechanical strain on musculoskeletal cells. Currently, most stretching studies utilise fibronectin-coated cultures, as these enhance cell attachment. However, recent studies suggest that fibronectin increases cell turnover and DNA damage and affects cell differentiation. Furthermore, fibronectin fragments cause extracellular matrix degradation. All indicative of diseased states, such as tendinopathy. We employed a novel cell stretching device, where clamp-to-clamp strain is evenly distributed across the culture surface, to determine how coatings affect cell behaviour. We cultured primary rat tenocytes on 0.15 µg/ml collagen type 1 or 10 µg/ml fibronectin-coated, micro-grooved silicone and exposed them to 2 and 4% strain at 0.5 Hz for 12 h. Calcium staining and alamarBlue was used to evaluate cell morphology and viability, while differential gene expression of musculoskeletal cell-specific and inflammatory markers was measured with real-time PCR.

Fibronectin-coated cultures demonstrated greater attachment and growth compared to collagen-coated cultures; while calcium staining suggested cells cultured on fibronectin had a more tenogenic morphology. However, with both coatings, expression of tenocyte markers tenasin-C, tenomodulin, and scleraxis decreased two- to threefold compared to non-coated cultures, with little difference between coatings, non-stretched and 2% stretched cultures. Interestingly, expression of biglycan and fibromodulin, both important in maintaining a tendon stem cell niche, were significantly upregulated in collagen-coated cultures, with biglycan upregulated threefold in non-stretched and 2% stretched cultures, and eightfold in 4% stretched cultures. Neither osteoblast- or chondrocyte-specific markers were altered, while expression of MMP-3 was significantly upregulated in both coated cultures, approximately fivefold in fibronectin-coated and 15–20-fold in collagen-coated cultures.

Overall, we have demonstrated that cells respond differently to different substrates, particularly under higher levels of stretch. Notably, collagen coating may provide a more tenogenic environment for in vitro tendon mechanobiology.

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Sex-specific association of physical activity with bone mass distribution at the femoral neck and trochanter in young adults

PP292

Women suffer more fragility fractures in old age and have a higher incidence of fractures at the femoral neck region compared to men who have higher incidence of anterolateral femoral fractures. The purpose of this cross-sectional study was to analyze associations between physical activity (PA) and bone mass distribution at the femoral neck (FN) and trochanter (TR) in young adults. A left hip DXA scan was used to measure bone mineral density (BMD) at the integral, superolateral (SL) and inferomedial (IM) FN, and TR sub-regions in 38 women (age: 23.5 ± 2.9 years; BMI: 22.3 ± 3.3 kg/m²) and 31 men (age: 28.2 ± 3.6 years; BMI: 23.1 ± 2.3 kg/m²). These sub-regions were used to represent bone mass distribution via two BMD ratios – FN:TR and IMF:SLFN. PA was evaluated with the Actigraph GT1M accelerometers over 7 days. Partial correlation analyses adjusted for body mass revealed in males associations of FN:TR BMD ratio with sedentary (r = 0.449) and active time (r = 0.467) (P < 0.05) and associations of IMF:SLFN BMD ratio with light PA (r = 0.463). In females it was not found significant associations between BMD ratios and PA variables, despite a trend in the association of FN:TR BMD ratio with sedentary time (r = 0.330, P = 0.53) and steps/day (r = 0.304, P = 0.075). In conclusion, PA seems to be related with bone mass distribution in males with a more active lifestyle (independent of PA intensity) to favor the BMD of the TR sub-region and a light PA to favor the SL sub-region of the FN. Potential associations in females appear however to have a contrary relationship with a more active lifestyle to promote the FN compared to the TR sub-region.

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Sarcopenia in patients with spondylarthropathy: is there any relation with radiological damage?

Renata Aguiar1, Tiago Merinhos1, Joana Sequeira2, Catarina Ambrósio1 & Anabela Barcelos1

1Serviço de Reumatologia, Centro Hospitalar do Baixo Vouga, Aveiro, Portugal; 2USF Flôr de Sal, Aveiro, Portugal.

Introduction

The loss of muscle mass (MM) is a serious problem which has been demonstrated in patients with rheumatoid arthritis. There are few studies about the loss of MM in patients with spondylarthropathy (Spa). In a recent case-control study in our department, the risk of sarcopenia in Spa patients was twice than in a healthy control group.

Objective

To assess muscle mass index (MMI) in patients with axial Spa and to search a relation between sarcopenia and radiological damage.

Methods

Observational study, in which modified stoke ankylosing spondylitis spinal score (mSASSS) was assessed in a cohort of patients with axial Spa and muscle mass index (MMI) was determined, from the value of MM, using Lee’s equation. Data were treated using SPSS version 17.0. Values of P < 0.05 were considered significant.
Results
Forty patients were enrolled in this cohort: 19 were males and 21 were females; mean age was 41.1 ± 14.4 years and mean disease duration was 8.8 ± 10.1 years. Mean mSASSS was 8.5 ± 12.1; mean IM was 7.88 ± 1.02 kg/m2 in males and 7.63 ± 0.99 kg/m2 in females; 17 patients had normal IMM, 7 had grade I sacropenia and 16 had grade II sacropenia. No difference with statistical significance was found between the mSASSS value in different sacropenia grades (P = 0.091). There was a moderate negative correlation between IMM and mSASSS in males (P = -0.384), but only weak negative correlation in females (P = -0.016).

Conclusion
In our cohort, a correlation between the presence of sacropenia and a radiological aggressive disease was found in SpA males. However, patients with different grades of sacropenia didn’t present significantly different mSASSS values. This study has some limitations including the sample size, potential confounding factor such the bias of measurement and the use of a non-validated equation to calculate MM. However, this work serves as a stimulus for future studies.

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PP299
Influence of gym high-intensity dynamic exercises and therapeutic exercises on functional status of patients with early rheumatoid arthritis
Evgeniya Orlova1, Dmitry Karateev1, Andrey Kochetkov2 & Tatiana Mozhariak1
1Research Institute of Rheumatology under the Russian Academy of Medical Sciences, Moscow, Russia; 2Central Rehabilitation Hospital of Federal Medical Biological Agency, Moscow, Russia.

Introduction
The patients with rheumatoid arthritis (RA) are less physically active than the general population. The aim of the study is to assess the effect of two exercise programs on the functional status of patients with early RA.

Methods
Fifteen patients with early RA underwent ten high-intensity dynamic exercises using ENSAF-Nonius gym for 45–60 min, including aerobic part (En-Cardio) and 18–20 muscle-strengthening exercises (En-Dynamic Track), 18 patients – 10 therapeutic exercises for 45 min under the supervision of a trainer. The 45-min exercises lasted three times a week for 3 months. 18 patients received only drug therapy (control). HAQ, RAPID3, the average powers of knee extension and ankle flexion by the EN-TreeM movement analysis were evaluated.

Results
Efficacy of the gym exercises was higher than the therapeutic exercises by HAQ and RAPID3 (P<0.05). In the gym group HAQ decreased by 60.7% (0.82 < 0.243, P<0.01), RAPID3 – by 47.5% (4.67 < 0.65, P<0.01). The average extension power of a weaker knee increased by 87.9%, of a stronger – by 70.5% (P<0.01). The average flexion power of a more affected ankle joint elevated by 84.6%, of a less affected – by 68.8% (P<0.01). Adherence to the therapeutic exercises for 3 months was better (83.3%) than the gym exercises (60.0%). Predictors of the regular gym exercises were the young age and the very early stage of RA. The patients of the both groups, who regularly did exercises, had pronounced clinical improvement by HAQ and good response to treatment by RAPID3 more frequently (P<0.01). After 3 months there was statistically significant differences between the exercise groups and the control group in most parameters (P<0.05).

Conclusion
The gym high-intensity dynamic exercises and the therapeutic exercises improve functional status and increase power of motion in patients with early RA.

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PP300
Vitamin D deficiency is associated with nonspecific skeletal pain in Saudi women
Khulood Hussein1,2, Hana Alkadi1, Suzan Lanham-New1,2 & Mohamad Ardawi1
1Department of Physiology, King Abdulaziz University, Jeddah, Saudi Arabia; 2Department of Nutrition and Metabolism, Guildford, UK.

Introduction and aims
Deficiency of vitamin D has been reported in subjects with many types of musculoskeletal pain. The aim of the present study was to determine the association between serum 25-hydroxyvitamin D (25(OH)D) and nonspecific skeletal pain in healthy Saudi women.

Methods
Serum 25(OH)D were measured for 233 healthy women with nonspecific skeletal pain at different regions of the skeletal system including back pain. Serum 25(OH) D was measured by direct competitive chemiluminescence immunoassay using LIASON autoanalyzer (DiaSorin, Inc., Stillwater, MN, USA). Pain information was obtained through a designed questionnaire showing the area and the intensity of pain based on a rating scale from none to severe pain.

Results and discussion
A total of 77% of women had vitamin D deficiency with serum 25(OH)D <50 nmol/L. A significant negative correlation was found between back pain (r=−0.185; P<0.025), bone pain (r=−0.140; P<0.036), daily living activity (r=−0.140; P<0.037), and total pain (back, bone, and muscle) (r=−0.143; P<0.033) and serum 25(OH)D. No differences were seen in age and BMI.

Conclusion
These data indicate a positive association of vitamin D deficiency with a variety of non-specific bone pain. More studies with larger samples are required to confirm these findings. Increasing serum vitamin D to sufficient levels and
Effect of vitamin D status on muscle function and physical performance among Saudi postmenopausal women: a cross-sectional study

Khulood Hussein
King Abdulaziz University, Jeddah, Saudi Arabia.

Introduction

Vitamin D deficiency is common among elderly subjects and is associated with reduced muscle strength. Although vitamin D deficiency is common among Saudi subjects, knowledge about its role in relation to muscle strength and physical performance is lacking. The objectives of the present study are to determine vitamin D status in a sample of randomly selected healthy postmenopausal Saudi women and to investigate the association between their serum 25(OH)D levels and measures of physical performance.

Subjects and method

A total of 223 healthy postmenopausal women (age ≥ 50 years) were randomly recruited from the city of Jeddah, medically examined and provided fasting blood samples for assessment of 25(OH)D and parathyroid hormone (PTH). Physical functions were assessed by the following tests: get up and go (GUG); eight-feet walk (8FW); five-times sit to stand (5-STS). Women were stratified into tertiles of serum 25(OH)D during statistical analysis.

Results

A total of 39, 77, and 95% of women had serum 25(OH)D levels <25, <50, and <75 nmol/l, respectively. A weak correlation approaching significance was found between 25(OH)D and GUG ($r = -0.132; P <0.05$). However, after adjusting for age and BMI, the correlation disappeared. There was no significant difference in any of the measures of physical performance between women in the upper (25(OH)D levels ≥ 38.8 nmol/l) and those in the lower (25(OH)D levels < 22.9 nmol/l) tertiles of 25(OH)D levels. Serum PTH levels was not significantly associated with any of the physical performance measures.

Conclusion

The present study suggests that low vitamin D status (25(OH)D < 50 nmol/l) is not associated with poor physical performance and may be a reflection of muscle adaptation to prolonged duration of vitamin D deficiency.

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Effect of different exercise modes on bone density in middle-aged and older men: a systematic review

Kate A Bolam1, Janinne G Z van Uffelen1,2 & Dennis R Taaffe1,3
1School of Human Movement Studies, The University of Queensland, Brisbane, Queensland, Australia; 2Institute of Sport, Exercise and Active Living, Victoria University, Melbourne, Victoria, Australia; 3School of Environmental and Life Sciences, The University of Newcastle, Ourimbah, New South Wales, Australia.

Although trials have shown that exercise has positive effects on bone mineral density (BMD), not all exercise modalities are osteogenic and the majority of exercise trials have been conducted in older women. The aim of this study was to systematically review trials examining the effect of weight-bearing and resistance-based exercise modalities on the BMD of hip and lumbar spine of middle-aged and older men. Eight electronic databases were searched in August 2012. Only randomised controlled or controlled trials that assessed the effect of weight-bearing exercise interventions on bone density measured by dual-energy X-ray absorptiometry (DXA), and reported effects in middle-aged and older men were included in this review. Eight trials detailed in nine papers were included. The interventions included yoga ($n=1$), walking ($n=2$), resistance training ($n=6$) and impact-loading activities ($n=1$). The methodological quality and reporting of five out of the eight trials was poor. Further, there was heterogeneity in the type, intensity, frequency and duration of the exercise regimens. Effects of exercise varied greatly among studies, with six interventions having a positive effect on BMD, but two interventions having no significant effect. Nevertheless, it seems that resistance training and impact-loading activities are most osteogenic for this population, whereas walking alone had little or no positive effect on bone density. Therefore, regular resistance training and impact-loading activities should be considered as a strategy to prevent osteoporosis in middle-aged and older men. Further high quality randomised controlled trials are needed to establish the optimal exercise prescription for improving BMD for this population.

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higher than those with type I and IV. To be short was experienced to be really difficult by many participants and some thought it was the most difficult part. An important factor was to have a close contact with an orthopedic surgeon specialized in OI. Most participants wanted ‘goal directed activity focused’ physiotherapy, with possibilities to learn and practice to be independent with for example toileting activities and dressing. Moreover, most participants also wanted pool training.

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**PP305**

**Associations of 25-hydroxyvitamin D concentrations with quality of life and self-rated health.** Rachida Rafiq1, Karin Swart2, Natasja van Schoor2, Dorly Deeg2, Paul Lips1-2 & Renate de Jongh1

1 VU University Medical Center, Amsterdam, The Netherlands; 2 VU University Medical Center, EMGO Institute for Health and Care Research, Amsterdam, The Netherlands.

Introduction

Vitamin D deficiency has been associated with impaired physical functioning and several chronic diseases and might thereby affect quality of life and self-rated health. The aim of this study was to assess relationships of serum 25-hydroxyvitamin D (25(OH)D) with quality of life and self-rated health, and to examine whether physical performance and number of chronic diseases mediate these relationships.

Methods

Data were obtained from the LASA, an ongoing cohort study in a representative sample of the older population. Serum 25(OH)D was classified into categories: < 25, 25–50 and ≥ 50 nmol/l. Quality of life was measured with the SF-12 Health Survey in 1998/1999 (n = 1248) and 1998/1999 (n = 1028).

Results

The lowest 25(OH)D category was associated with a lower physical component score of the SF-12 as compared with the highest category (β (95% CI): − 3.9 (− 6.5 to − 1.3)). The lowest 25(OH)D category was associated with a lower cross-sectional score of self-rated health compared to the highest category (odds ratio (95% CI): 0.5 (0.3 to 0.8)). Physical performance and number of chronic diseases were associated with vitamin D status, quality of life and self-rated health. Adding physical performance to the multivariable model decreased the strength of the associations of 25(OH)D category with quality of life and self-rated health. The aim of this study was to assess relationships of serum 25(OH)D category with quality of life and self-rated health. Adding number of chronic diseases decreased the strength of the associations of 25(OH)D category with quality of life with 33%, but did not change the association with self-rated health.

**Conclusion**

Lower 25(OH)D status is associated with lower scores on quality of life and self-rated health. Large part of the associations can be statistically and theoretically explained by physical performance and number of chronic disease.

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**PP306**

**Increased activity associated with exercise does not rescue aged bone’s impaired response to local mechanical loading.**

Lee Meakin1, Chinedu Udoh1, Toshitiro Sugiyama1,2, Gabriel Galea1, Lance Lanyon1 & Joanna Price1

1 University of Bristol, Bristol, UK; 2 Yamaguchi University School of Medicine, Yamaguchi, Japan.

Bones’ fracture resistance is achieved in vivo by adaptation to habitual loading. Aged bone can adapt to exercise but in female rodents ageing impairs the adaptive response to artificial loading1-3. This inconsistency led us to investigate whether treadmill exercise, sufficiently mild to not itself stimulate new bone formation, could rescue aged bone’s diminished response to artificial loading. Young adult 17-week-old (YP) and aged 19-month-old (AF) female C57Bl/6 mice received artificial tibial loading only or loading plus mild levels of treadmill exercise. After treadmill aclimatization, mice were exercised for 30 min every other day at voluntary running speeds (YP 23 cm/s, AF 18 cm/s) for 2 weeks. 3 h later, their right tibiae were subjected to a short period of axial non-invasive loading 40 cycles, peak strain 2250 µε; left limbs were internal controls. Bone was assessed using µCT, serum IGF1 by ELISA, serum corticosterone by RIA. Artificial loading increased cortical bone area and thickness in YF and AF and trabecular BV/TV and thickness in YF. Exercise had no effect on the cortical response to loading but in YF reduced the loading-related increased BV/TV (− 32.1%, P < 0.05). Exercise in YF also increased serum IGF1(15.0%, P < 0.05). In AF exercise decreased serum corticosterone (− 48.1%, P < 0.05) and increased periosteally-enclosed area by 8.9% (P < 0.01) in AF.

This suggests that mild exercise, which would be expected to have beneficial effects in muscle, has no effect on bone’s response to almost concurrent loading in young mice and in aged mice does not rescue their diminished response to loading. The beneficial effects of exercise in the elderly are thus likely to reflect local adaptation to mechanical strain rather than to effects derived from muscle.

**References**


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**PP307**

**Muscle power and force may influence cortical bone strength via distinct mechanisms: findings from a cross sectional study of high bone mass cases and controls.**

Sarah A Hardcastle1, Celia L Gregson1, Jorn Rittweger4, Kate A Ward2 & Jon H Tobias1

1 Musculoskeletal Research Unit, University of Bristol, Bristol, UK; 2 Nutrition and Bone Health, MRC Human Nutrition Research, Cambridge, UK; 3 German Aerospace, Institute of Aerospace Medicine, Center, Cologne, Germany; 4 IRM Research Institute, Manchester Metropolitan University, Manchester, UK.

**Background**

Relationships between muscle function and bone have been examined using a range of techniques, with conflicting results. We aimed to determine these associations within an adult population comprising individuals with high bone mass and family controls.

**Methods**

Recruitment was from four UK sites within the high bone mass (HBM) study; cases and unaffected family controls were pooled. Peak ground reaction force and peak power, during a multiple one-leg jump and single two-leg jump respectively, were recorded using a Leonardo Mechatography Ground Reaction Force Platform, and hip BMD by DXA scanning. A subgroup underwent mid-tibial pQCT (Stratec XCT2000L). Linear regression analysis adjusted for age, gender, height and weight. Force and power were log transformed.

**Results**

189 participants had matching jump plate and hip DXA data (70 males (mean age 58 years), 119 females (mean age 56 years)). Median jump power was 2.25 kW (IQR 1.78, 2.93) and force 1.95 kN (1.68, 2.39). Jump power was positively related to hip BMD (standardised β (95% CI): 0.29 (0.07, 0.51), P = 0.01), but jump force was not (0.03 (− 0.16, 0.22), P = 0.74). In 113 participants with force and pQCT data, power was positively associated with tibial SSI (0.26 (0.09, 0.44), P = 0.01) and with cortical thickness (0.33 (0.06, 0.60), P = 0.02) but not with total bone area (0.10 (− 0.10, 0.30), P = 0.53). Force was also positively associated with SSI (0.24 (0.07, 0.42), P = 0.01), but in contrast to power was associated with total bone area (0.22 (0.03, 0.42), P = 0.02) but not cortical thickness (0.05 (− 0.22, 0.32), P = 0.72).

**Conclusion**

Muscle power and force are both positively associated with cortical bone strength. However, distinct mechanisms appear to be involved, since power was primarily associated with reduced endosteal expansion (reflected by cortical thickness and hip BMD), whereas force was associated with increased periosteal expansion (reflected by total bone area). Based on these findings, interventions targeting both muscle force and power may have the greatest benefit for cortical bone strength.

**References**


**DOI:** 10.1530/boneabs.1.PP307
Fat and lean masses were significantly decreased with age. The maximal

Conclusion

Results

We have found the significantly differences of fat and lean masses in women with age:

- fat mass: 20–24 years – 18 630.12 g; 25–29 years – 18 630.12 g; 30–34 years – 19 210.00 g; 35–39 years – 21 528.15 g; 40–44 years – 24 611.77 g; 45–49 years – 27 505.54 g; 50–54 years – 27 505.54 g; 55–59 years – 29 909.92 g; 60–64 years – 31 600.27 g; 65–69 years – 33 508.25 g; 70–74 years – 33 155.54 g; 75–79 years – 32 284.86 g; 80–84 years – 30 595.53 g; 85–89 years – 30 303.68 g; F = 83.19; P < 0.000001

- lean mass: 20–24 years – 37 271.57 g; 25–29 years – 37 954.09 g; 30–34 years – 39 019.72 g; 35–39 years – 39 928.62 g; 40–44 years – 40 929.67 g; 45–49 years – 41 047.19 g; 50–54 years – 41 936.27 g; 55–59 years – 42 564.79 g; 60–64 years – 42 519.73 g; 65–69 years – 41 758.95 g; 70–74 years – 41 233.77 g; 75–79 years – 41 105.52 g; 80–84 years – 40 308.00 g; 85–89 years – 38 454.61 g; F = 29.15; P < 0.000001

Frequency of sarcopenia in women aged 65 years and older was 7% (women aged 65–69 years (n = 943) – 7.6% (n = 72), 70–74 years (n = 877) – 6.1% (n = 54), 75–79 years (n = 384) – 6.3% (n = 24), 80–84 years (n = 204) – 6.9% (n = 14), 85–89 years (n = 48) – 10.4% (n = 5).

Conclusion

Fat and lean masses were significantly decreased with age. The maximal accumulation of fat and lean masses was in women aged 50–59 years. Frequency of sarcopenia in women aged 65 years and older was 7%.

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PP309

Lean mass, not obesity, is related to total body bone mineral content in boys

Tom Sanchez,1 Jingmei Wang,2 Felix Rajan,3 Terry Schwalenberg4 & Kathy Dudzek5

1Norland—a Cooper Surgical Company, Socorro, New Mexico, USA; 2Norland—a Cooper Surgical Company, Beijing, China; 3Siemens Health-care, Malvern, Pennsylvania, USA; 4Norland—a Cooper Surgical Company, Fort Atkinson, Wisconsin, USA.

The literature has suggested that bone mineral content is modulated by muscle mass and activity. We investigated the relationship between DXA-assessed total body bone mineral content, total body lean mass, appendicular lean mass and obesity (a possible marker of inactivity) in a population of 73 boys between the age of 7 and 19 using a Norland XRX-46 system.

Regression analysis showed that in these growing boys there is a strong positive relationship between total body bone mineral content and either total body lean mass (y = 0.0503x + 3.966; r = 0.9718; P < 0.001) or appendicular lean mass (y = 395.62x – 415.69; r = 0.8737; P < 0.001). Analysis of covariance for total body bone mineral to total body lean mass regressions in groups with normal or low (< 7.26 kg/m²) appendicular lean mass showed that regression slopes did not differ but that subjects with low appendicular lean mass also had lower bone mineral content. When analysis of covariance was carried out on total body bone mineral to total body lean mass regressions for DXA assessed obese and non-obese subjects no difference was seen in regression slopes or in values.

In conclusion, the data show that there is a strong positive relationship between total body lean mass and bone mineral content that is also reflected in calculated appendicular lean mass. The study also shows that, in this population, this relationship is not altered by zero.

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PP310

Osteoporosis: evaluation and imaging

Armine Haroyan1,2 & Liana Ghukasyan2

1Department of Therapeutic Narrow Specialization, Yerevan State University after M. Heratsi, Yerevan, Armenia; 2Department of Rheumatology, Medical Centre ‘Erebouni’, Yerevan, Armenia.

Introduction

The purpose of this study was to determine osteoporosis (OP) and osteopenia in patients with various rheumatic diseases depending on the duration of use glucocorticosteroids (GCS) and daily dose of GCS.

Methods

The study included 125 patients (90 women and 25 men) with various rheumatic diseases receiving GCS therapy. BMD was measured by DXA (Hologic). Patients were divided into two groups. Group I included postmenopausal women and men over 50 years (72 patients), group II- premenopausal women and men younger 50 years (30 patients). The T-score was determined in group I and the Z-score in group II respectively. As it known the loss of bone tissue is more in trabecular bone so we assessed BMD in the spine. In 23 patients (15 women and 8 men) data were normal. 102 patients (22 men and 80 women) had osteoporosis/osteopenia. Average duration of GCS-therapy = 6.8 years (from 2 months to 30 years).

Results

Hundred and two patients had OP or osteopenia (82% of all patients). There was no correlation between the duration of GCS-therapy and OP/osteopenia in both groups. There was positive correlation between the maximal daily dose of GCS and OP and osteopenia in group I (r = 0.13; P < 0.05). No correlation in group II. There was negative correlation between the minimal GCS daily dose and osteopenia (r = -0.2; P < 0.05) in group I.

Conclusion

The majority of the GCS-treated patients regardless of age have high risk of OP/osteopenia of the spine. There was 66% of osteoporosis and 35% of osteopenia in the spine in group I. There was a clear predominance of osteopenia in the spine (60% osteopenia vs 40% OP) in group II. Duration of using GCS had no effect on the risk of the OP.

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PP311

Appropriate osteoporosis treatment by family physicians in response to FRAX vs caroc reporting: a randomized controlled trial

Karen Beattie1, George Joannides1, Joy MacDermid1,3, Ruby Grewal2,3, Joseph’s Healthcare, Hamilton, Ontario, Canada; 2Queen’s University, Kingston, Ontario, Canada; 3Western University, London, Ontario, Canada; 4Hamilton Health Sciences, Hamilton, Ontario, Canada.

Introduction

Current Canadian clinical practice guidelines recommend the FRAX or Canadian Association of Radiologists and Osteoporosis Canada (CAROC) fracture risk assessment tools to report 10-year fracture risk in an individual. CAROC considers sex, age, BMD and previous fracture as risk factors. It is unknown whether one reporting system is more effective in helping general practitioners (GPs) identify individuals who should be recommended for pharmacological treatment. We hypothesized that the FRAX report would result in better identification of patients who should be pharmacologically treated by GPs as compared to the CAROC report.

Methods

Individuals ≥50 years old with a distal radius fracture were included provided they had no previous osteoporosis diagnosis and were not taking any osteoporosis medication. Participants underwent a DXA scan and answered questions about fracture risk factors. Each participant’s GP was randomized to receive either a FRAX report or a CAROC report. Both tools categorize patients as being at low (< 10%), moderate (10–20%) or high (> 20%) fracture risk. The FRAX report, which was pilot tested with six GPs, included a statement recommending treatment for high risk participants. No treatment recommendations were stated on the CAROC report. After 3 months, all participants were called and asked if they were contacted by their GP and if they were recommended for treatment. GP’s treatment decisions were compared to recommendations of a rheumatologist (gold standard).

Results

Sixty non-consecutive participants were enrolled (n = 31 FRAX, 11 low, 16 mod, 4 high risk; n = 30 CAROC; 22 mod, 9 high risk). Of 31 FRAX participants,
remodeling whereas subchondral trabecular bone (STB) parameters were slightly decreased. In OP with severe rate of cartilage damage we found thinning of SCB and significant decrease in BV/TV and trabecular parameters. In OA SCB and STB were thicker. Thinning of SCB in OP was related to the progression of cartilage degeneration what could implicate early-stage OA. Severe cartilage degeneration and intensive activities in SCB in OP and OA suggested that progression of cartilage damage was influenced by altered activities in SCB.

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PP314

Communication of fracture risk and treatment benefit in terms of ‘bone health age’ using FRAX or Qfracture
Bo Abrahamsen1,2, Katrine Hass Rubin1, Carrinna Hansen2,3 & Kim Brixen2,3
1Institute of Clinical Research, University of Southern Denmark, Odense, Denmark; 2Gentofte Hospital, Hellerup, Denmark; 3Department of Endocrinology, OUH, Odense, Denmark.

Introduction
Communication of absolute and relative risks is challenging despite the development of tools to quickly derive absolute fracture risk estimates from risk factors with or without BMD. We speculated that back-transformation of risks to a risk age could make for a clearer message and at the same time increase agreement between risk algorithms.

Results
The algorithms differed less in estimated bone health age than in percent risk. A 60 years old woman with a maternal history of hip fracture has a predicted major osteoporotic fracture risk equivalent to that of a 71 years (FRAX) or 68 years-old woman (Qfracture). Treatment with 40% risk reduction is equivalent to a reduction in risk age by 10 years in both algorithms, reducing risk age to 62 (FRAX) or 60 years (Qfracture; Table 1).

Table 1

<table>
<thead>
<tr>
<th>Assuming no treatment</th>
<th>Assuming treatment with 40% risk reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FRAX Age/10 years risk</td>
</tr>
<tr>
<td>Age 60: maternal hip</td>
<td>71/12%</td>
</tr>
<tr>
<td>fx = own fracture</td>
<td>85/23%</td>
</tr>
<tr>
<td>Age 70: maternal hip</td>
<td>80/18%</td>
</tr>
<tr>
<td>fx = own fracture</td>
<td>90/25%</td>
</tr>
</tbody>
</table>

Conclusions
Conversion of absolute fracture risk to equivalent bone health age is simple and intuitive and can accommodate both baseline BMD and the expected risk reductions on treatment.

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PP315

The stability of intact parathyroid hormone in human blood during different sampling conditions and long-term storage in serum tubes
Camilla Sand Andersen1,2, Hans Christian Hoeck1, Parisa Gazerani1 & Peter Vestergaard1
1Department of Health Science and Technology, Faculty of Medicine, Aalborg University, Aalborg, Denmark; 2Center for Clinical and Basic Research (CCBR), Aalborg, Denmark.

Objective
To investigate i) the analytic stability of PTH in venous blood specimens in serum tubes during 5 years of storage at room temperature, ii) changes in stability caused by storage in different serum tubes (EDTA, K2EDTA, serum), and iii) the stability of PTH with respect to the time from withdrawal of blood to storage.

Methods
Venous blood was collected from ten healthy Caucasian females. One K2EDTA tube and three serum tubes were sampled. The K2EDTA tubes were centrifuged immediately. The serum tubes were processed 30, 60, and 120 min

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after collection. Also, 100 serum samples obtained between September 2007 and February 2008, and stored at −20 °C were analyzed. All samples were analyzed with intact PTH immunoassay (Cobas, Roche Diagnostics).

Results
Using plasma samples processed immediately after collection in the 10 healthy subjects as gold standard, the retrieval in serum samples processed at 30 min was 91.8%. Serum processed at 60 and 120 min showed retrieval of 91.9 and 91.1%, respectively, with no time trend (P > 0.05).

In serum tubes stored for 5 years, PTH values were significantly elevated compared to the reference interval (P < 0.01). No significant difference between PTH from the 5-year-old serum samples and matched K2EDTA tubes measured from March to April 2007 was present (P = 0.58).

Conclusion
It is possible to obtain valid PTH results in serum stored for 5 years. The PTH retrieval in serum compared to plasma was acceptable, and delayed processing for up to 120 min did not affect stability.

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**PP316**

Cortical and trabecular bone parameters from HR-pQCT images at the Tibia: a local comparison with synchrotron radiation micro-computed tomography

Agnès Ostertag1, Françoise Peyrin1,2, Sylvie Fernandez1, Jean-Denis Laredo1, Marie-Christine De Vernejoul1 & Christine Chappard1

1INSERM U606 University Paris Diderot, PRES Sorbonne Paris Cité, PARIS, France; 2CREATIS INSERM U1044; CNRS 5220, University Lyon, Villeurbanne, France; 3ESRF X-Ray Imaging Group, GRENoble, France; 4B2OA UMR CNRS 7052, University Paris Diderot, PRES Sorbonne Paris Cité, Paris, France.

In clinical research protocols, HR-pQCT images (XtremCT Scanco, voxel size: 82 μm) are carried out to evaluate trabecular and cortical bone changes induced by osteoporosis and treatments. Micro-computed tomography (μ-CT) has become a standard tool for examination of trabecular and cortical bone in 3D. The purpose of this study is to evaluate the accuracy of cortical and trabecular measurements derived from HR-pQCT images with morphological measurements from synchrotron radiation (SR) μ-CT. Thirty tibias specimens (mean age: 82.2 ± 9.7 years) were scanned at 3.5 cm from the tibial pilon with the XtremCT using the usual manufacturer acquisition protocol. Samples of cortical and trabecular bone were harvested at the posterior part of the tibia and imaged on ID19 beamline (ESRF, Grenoble) with a voxel size of 7.5 μm. We performed site-matched analyses on the HR-pQCT images with the manufacturer software (HR_local analysis) comparatively to SR micro-CT images. For HR_local analysis, the cortical outcomes were: volumetric bone density (gHA/cm³) (Dcomp), cortical thickness (CTh, mm) and the trabecular bone parameters: bone volume (BV/TV,%), trabecular thickness (Tb.Th, mm), trabecular spacing (Tb.Sp, mm) and trabecular number (Tb.N, per mm). Using the CTAN Skyscan software, the following cortical parameters from SR μCT acquisitions were obtained: Porosity (PoVTV,%), pore diameter (Po.Dm, mm), pore spacing (Po.Sp, mm), pore number (Po.N, per mm). CTh_SR was manually measured. The trabecular parameters were BV/TV_SR(%), Tb.Th_SR, Tb.Sp_SR, Tb.N_SR. Pearson correlation coefficients (r) were used to compare HR-pQCT and SR μCT parameters. The CTh was correlated to CTh_SR (r = 0.61----, Table 1).

<table>
<thead>
<tr>
<th>Cortical</th>
<th>DComp</th>
<th>Trabecular</th>
<th>BV/TV</th>
<th>Tb.Th</th>
<th>Tb.Sp</th>
<th>Tb.N</th>
</tr>
</thead>
<tbody>
<tr>
<td>PoVTV</td>
<td>−0.69 *</td>
<td>BV/TV_SR</td>
<td>0.70</td>
<td>0.62</td>
<td>−0.69</td>
<td>0.62</td>
</tr>
<tr>
<td>Po.Dm</td>
<td>−0.49 †</td>
<td>Tb.Th_SR</td>
<td>0.61</td>
<td>0.72</td>
<td>0.63</td>
<td>0.39</td>
</tr>
<tr>
<td>Po.Sp</td>
<td>0.38</td>
<td>Tb.Sp_SR</td>
<td>0.38</td>
<td>NS</td>
<td>0.70</td>
<td>0.70</td>
</tr>
<tr>
<td>Po.N</td>
<td>NS</td>
<td>Tb.N_SR</td>
<td>0.64</td>
<td>0.47</td>
<td>−0.72</td>
<td>0.68</td>
</tr>
</tbody>
</table>

*P < 0.05, †P < 0.01, ‡P < 10^−4; spearman correlation in italic.

Conclusion
For cortical bone, Dcomp is predominantly related to porosity and for trabecular bone, the most related HR-pQCT parameters are their counterparts derived from the SR μCT images.

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**PP317**

A 3D QCT technique of the thoracic and lumbar spine: integral volume and intervertebral disc space increase and bmd decreases from T6 to L4

Oleg Museyko1, Axel Heinemann2, Mattias Krause3, Reinhard Barkmann3, Michael Ameling2, Claus Gluer2, Klaus Püschel4 & Klaus Engelke3

1Institute of Medical Physics, University of Erlangen-Nuremberg, Erlangen, Germany; 2Institute for Osteology and Biomechanics, University of Hamburg, Hamburg, Germany; 3Molecular Imaging North Competence Center, University of Kiel, Kiel, Germany; 4Institute for Forensic Medicine, University of Hamburg, Hamburg, Germany.

Introduction
QCT of the spine is typically restricted to the BMD analysis of the lumbar vertebrae. However, fractures frequently occur in the thoracolumbar region. Also the load distribution in the spine may depend on the intervertebral disc space (IDS), a good approximation of the intervertebral disc, which itself cannot be reliably assessed by X-ray based methods.

Materials and methods
A QCT 3D acquisition and automated analysis technique (with optional operator interaction) for T6 to L4 was implemented including the segmentation of the IDS defined as the volume between the endplates of two adjacent non-fractured vertebrae and a lateral surface connecting the ridge points of the endplates. QCT data from 12 human cadavers scanned for the purpose of CT acquisition optimization were analyzed. Vertebral fractures with injected cement, or internal metal hardware were excluded.

Results
Percentage of changes in vertebral integral volume, integral and trabecular BMD, and IDS volume, all normalized to values of T12, are shown in the table. The correlation coefficient between IDS volume and volume of the vertebrae underneath was $r = 0.68$ for the thoracic and $r = 0.59$ for the lumbar spine ($P < 0.01$ for both $r$ values; Table 1).

<table>
<thead>
<tr>
<th>% diff. relative to T12</th>
<th>T7</th>
<th>T9</th>
<th>T11</th>
<th>L1</th>
<th>L3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume</td>
<td>−46</td>
<td>−32</td>
<td>−11</td>
<td>+5</td>
<td>+25</td>
</tr>
<tr>
<td>Int BMD</td>
<td>+15</td>
<td>+11</td>
<td>+8</td>
<td>−2</td>
<td>−5</td>
</tr>
<tr>
<td>Trab BMD</td>
<td>+22</td>
<td>+6</td>
<td>+9</td>
<td>−8</td>
<td>−17</td>
</tr>
<tr>
<td>IDS Volume</td>
<td>−62</td>
<td>−36</td>
<td>−8</td>
<td>−17</td>
<td>+16</td>
</tr>
</tbody>
</table>

Conclusion
A largely automatic segmentation of the thoracic and lumbar spine in CT images including the IDS is feasible. Eventually this may improve fracture prediction and amplify finite element models to calculate vertebral strength.

DOI: 10.1530/boneabs.1.PP317

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**PP318**

Binding kinetics of fluorescent bisphosphonates as a tool for monitoring bone dynamics in vivo

Robert Tower1, Graeme Campbell1, Marc Muller1, Olga Will1, Frederieka Grundmann2, Christian Schem2, Claus Gluer2 & Sanjay Tiwari1

1M0IN CC, Kiel, Germany; 2University Hospital Schleswig-Holstein, Kiel, Germany.

Bone resorption and deposition occur in a tightly regulated fashion reflecting the coupled activities of osteoclasts and osteoblasts. Several pathological conditions perturb this balance between bone synthesis and resorption, including osteoporosis and skeletal metastases. The uncoupling of remodeling activities to disseminated tumor cells homing to the bone and to tumor growth in bone. Therefore, a reliable marker of bone remodeling would be useful to provide a strong correlation with the extent of skeletal disease, evaluate the effectiveness of an intervention to suppress resorption associated with metastases or menopause and to predict future bone metastases in cancer patients without malignant spread. The purpose of this study is to determine if the fluorescent bisphosphonate imaging probe osteosense (Perkin Elmer) can predict bone turnover in ovariectomized and parathyroid hormone (PTH)-treated mice. While absolute fluorescence suggests a trend of decreased osteosense binding in ovariectomized mice, no statistical difference was observed. To determine whether bone affinity, rather than total binding capacity, could serve as a more reliable marker of bone mineralization, kinetic analysis of binding was measured. Regressional analysis suggests that decreases in bone mineralization caused by...
PP319
Prevalence of FRAX clinical risk factors: dietary calcium intake habits and osteoporosis screening in Greek women
Sofoclis Bakides, George Sakellariadis, Stavroula Alevizou, Kleaniki Koussi, Anna Rapti, Panayiotis Tsiverdis, Konstantina Kavvadia, Kyriakos Drivas & Charillila-Loukia Ververeli
Molaoi General Hospital, Molaoi, Lakonia, Greece.

Introduction
Osteoporosis-related fractures can cause substantial disability and increase health care costs, and mortality. There are many difficulties to access Greek women residing in remote villages and perform the FRAX tool for osteoporosis evaluation, especially, after the global economy crisis.

Purpose
To estimate the prevalence of FRAX clinical risk factors, calcium intake habits and perform osteoporosis screening in 275 postmenopausal Greek women, aged 40–84 years.

Methods
Clinical risk factors were evaluated with FRAX®. BMD was measured using heel QUS, calcium intake calculation using a food frequency questionnaire.

Results
Mean age was 61.73 years and mean BMI: 27.03 kg/m². In total 51 out of 275 were found eligible for treatment after DEXA (3, 7 and 41 for the age groups: 40–49, 50–65 and over 65, respectively). Secondary osteoporosis was found in 22.54, 14.54% had parental fracture history, 8.36% had a fracture, 14.90% were smokers, 5.81% received steroids, 1.45% had rheumatoid arthritis. Their average calcium intake from dairy products: 605.81, 622.71 and 555.74 mg for the age groups 40–49, 50–64 and over 65, respectively Table 1.

Conclusions
This study revealed that the prevalence of clinical risk factors varies from 1.45 to 22.54%. Further studies will clarify the role of combined use of FRAX and QUS for the best primary care approach, when DEXA is not available.

Table 1

<table>
<thead>
<tr>
<th>Age group/number</th>
<th>Fractured hip</th>
<th>Parent hip fractured</th>
<th>Smoking of use of steroids</th>
<th>Secondary osteoporosis</th>
<th>Osteoporosis R.A.</th>
</tr>
</thead>
<tbody>
<tr>
<td>40–49/48</td>
<td>1</td>
<td>7</td>
<td>13</td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td>50–64/114</td>
<td>6</td>
<td>18</td>
<td>24</td>
<td>6</td>
<td>24</td>
</tr>
<tr>
<td>&gt; 65/113</td>
<td>16</td>
<td>15</td>
<td>4</td>
<td>7</td>
<td>25</td>
</tr>
<tr>
<td>Total 275</td>
<td>23</td>
<td>40</td>
<td>41</td>
<td>16</td>
<td>62</td>
</tr>
</tbody>
</table>

PP321
Use of os calcis quantitative ultrasound for bone mineral density screening in adolescents with menstrual dysfunction
William W K To¹ & Margaret W N Wong²
¹United Christian Hospital, Kwan Tong, Hong Kong; ²The Chinese University of Hong Kong, Shatin, Hong Kong.

Background
Prolonged hypothalamic amenorrhoea with anovulation has been associated with hypo-oestrogenism in adolescents and has been shown to be associated with lower bone mineral density (BMD) values.

Objective
To determine whether differences in BMD between oligo/amenorrhoeic adolescents at risk of low BMD and normal eumenorrhoeic controls can be detectable by quantitative ultrasound (QUS) of the os calcis.

Methods
Adolescents with oligo/amenorrhoea (defined as having amenorrhoea for 3 months or more in past 1 year) and a control group of eumenorrhoeic adolescents were recruited from the Adolescent Gynaecology clinic. All underwent basic anthropometric measurements, body fat composition estimation, hormonal profile assay, DXA of the lumbar spine and hip, QUS of os calcis. BMD, as well as QUS of the os calcis was compared to non-dancers. There were otherwise no significant differences in other basic anthropometric and baseline BMD measurements in the two groups. At the 24-month-assessment, DXA BMD values were consistently higher in both groups, though the increment was significantly greater in the dancers as compared to non-dancers (‡lumbar spine 0.0758 vs 0.0329 kg/cm², P=0.006, ‡neck of femur 0.046 vs 0.019 kg/cm², P=0.004). QUS also showed a larger increment in dance students as compared to non-dancers (¶soundness 18.1 vs 6.99, P=0.033; ¶BMD 0.036 vs 0.01 kg/cm², P=0.048). pQCT showed largely positive increments in both groups, but the magnitude was not significantly different between the two groups.

Conclusion
The findings confirmed that both adolescent dance students and non-dancers showed an increment in BMD values over the 24-month study interval. The differential increments were apparently better detected by conventional DXA as well as by QUS of the os calcis compared to pQCT measurements.

PP330
Quantitative ultrasound of os calcis BMD vs conventional DXA and peripheral QCT in interval assessment of BMD changes in adolescent females
William W K To¹ & Margaret W N Wong²
¹United Christian Hospital, Kwan Tong, Hong Kong; ²The Chinese University of Hong Kong, Shatin, Hong Kong.

Objective
To compare whether interval BMD changes in adolescent females that can be detected using conventional dual energy X-ray absorptiometry (DXA) can also be detected using quantitative peripheral quantitative computed tomography (pQCT) and quantitative ultrasound (QUS) of the os calcis.

Bone Abstracts (2013) Vol 1
**PP322**
Comparative assessment of bone mineral density of the femoral neck between dual-energy X-ray absorptiometry and a new ultrasonic method

Francesco Conversano¹, Ernesto Casciaro¹, Antonio Greco¹, Paola Pisanî¹, Roberto Franchini², Antonella Grimaldi², Eugenio Quarta², Maurizio Muratore¹ & Sergio Casciaro¹
¹National Research Council, Institute of Clinical Physiology, Lecce, Italy; ²O.U. of Rheumatology, Galateo Hospital, San Cesario di Lecce, ASL-LE, Lecce, Italy.

Introduction

Recently reported high incidences of hip fractures emphasize the need of more effective methods for osteoporosis diagnosis, currently performed essentially by dual-energy X-ray absorptiometry (DXA) examinations of the proximal femur. However, high costs and radiation-related issues do not allow DXA employment for population mass screenings. Aim of this study is to carry out a preliminary clinical validation of a new ultrasound (US)-based method to perform femoral bone densitometry at lower costs and without using X-rays.

Methods

A cohort of 90 female patients was recruited according to the following criteria: 60–80 years of age, BMI ≤40 kg/m², no severe deambulation impairments, medical prescription for a femoral DXA, signed informed consent. All the enrolled patients underwent two examinations: a conventional femoral DXA (Hologic Discovery) and an US scan of proximal femur. US data were analyzed by a novel algorithm that processed both echographic images and unfiltered ‘raw’ signals and calculated the same diagnostic parameters provided by DXA (bone mineral density (BMD), T-score, Z-score). Diagnostic accuracy of obtained results was evaluated through a direct comparison with DXA output as a function of patient age and BMI.

Results

For 82.2% of the patients US diagnosis (osteoporotic, osteopenic, and healthy) was the same of the corresponding DXA one. Pearson correlation coefficient (r) between DXA and US measurements was evaluated for each diagnostic parameter, obtaining the following results: r=0.68 (P<0.001) for BMD, r=0.68 (P<0.001) for T-score and r=0.71 (P<0.001) for Z-score, without significant variations as a function of age nor BMI.

Conclusions

The proposed US approach to femoral densitometry showed a very good correlation with DXA measurements performed at the same site, indicating that this innovative non-ionizing method could become extremely useful for early osteoporosis diagnosis through population mass screenings.

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**PP323**
A new ultrasonic method for diagnosis of osteoporosis on hip and spine

Sergio Casciaro¹, Francesco Conversano¹, Ernesto Casciaro², Roberto Franchini², Maria Daniela Renna², Antonio Greco², Eugenio Quarta², Laura Quarta² & Maurizio Muratore²
¹National Research Council, Institute of Clinical Physiology, Lecce, Italy; ²O.U. of Rheumatology, Galateo Hospital, San Cesario di Lecce, ASL-LE, Lecce, Italy.

Introduction

Currently, osteoporosis is mainly diagnosed through dual-energy X-ray absorptiometry (DXA). However, DXA cannot be used for early diagnoses through population mass screenings because of issues related to ionizing radiation employment. Aim of this study is to perform a preliminary clinical validation of a new ultrasound (US)-based method for vertebral densitometry.

Methods

A total of 270 women were included in this study according to the following criteria: 45–80 years of age, BMI ≤40 kg/m², no deambulation impairments, medical prescription for a vertebral DXA, signed informed consent. All the enrolled patients underwent two examinations: a conventional vertebral DXA (Hologic Discovery) and an US scan of lumbar spine. US data were analyzed by a novel algorithm that processed both echographic images and unfiltered ‘raw’ signals and calculated the same diagnostic parameters provided by DXA (bone mineral density (BMD), T-score, Z-score). Diagnostic accuracy of obtained results was assessed through a direct comparison with DXA output as a function of patient age and BMI.

Results

For 87.0% of the patients US diagnosis (osteoporotic, osteopenic, and healthy) was the same of the corresponding DXA one. Specifically, diagnostic accuracy was 87.7% for patients with BMI in the range 25–40 kg/m² (n=114) and 86.5% for those with BMI <25 kg/m² (n=156), with maximum (88.6%) and minimum (78.7%) accuracy in the age range 61–65 and 45–50 years, respectively. All the obtained values of Pearson correlation coefficient (r) between diagnostic parameters provided by DXA and US for patients in the same age and BMI ranges were within the interval 0.72–0.91 (P<0.001).

Conclusions

We proposed an innovative method for US evaluation of BMD directly on the spine which showed a strong and significant agreement with DXA diagnoses. This technique has the potential to revolutionize the approach to osteoporosis diagnosis.

DOI: 10.1530/boneabs.1.PP324

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**PP325**
Relationship between quantitative ultrasound parameters at calcaneous and health-related quality of life domains in postmenopausal Italian women: the FEDRO study

Stefano Gonnelli1, Carla Caffarelli1, Giuseppe Guglielmi2, Stefania Rossi1, Silvano Adami2 & Ranuccio Nuti1
1University of Siena, Siena, Italy; 2University of Verona, Verona, Italy.

Introduction

We aimed to investigate whether QUS parameters at calcaneous may be associated with HRQoL in postmenopausal women without vertebral fracture. To date no data exist in literature about any possible influences of quantitative ultrasonography (QUS) on HRQoL. This study aimed to assess whether QUS parameters at calcaneous may be associated with HRQoL.

Methods

In 1812 ambulatory postmenopausal women aged 60 years or over, referred by their family physicians as outpatients for their specialist visit, we measured HRQoL by the quality of life questionnaire of the european foundation for

Bone Abstracts (2013) Vol 1
osteoporosis (QUALEFFO-41) and stiffness index by using QUS at calcaneus (Achilles Express, Lunar-GE). By grouping the 1812 women on the basis of stiffness index, an highly significant \((P<0.001)\) difference was found for all QUALEFFO-41 domains, but for ‘pain’ and ‘mental status’. Stiffness was inversely correlated \((P<0.01)\) with total QUALEFFO-41 and with all QUALEFFO-41 domains. In stepwise multiple logistic regression analysis Stiffness values were negatively associated with QUALEFFO-41 total score \((\beta=-0.22; \text{95}\% \text{CI}=-0.24 \text{ to } -0.16)\) and all the domains of QUALEFFO-41. The presence of concomitant diseases was associated with a worsening of HRQoL in all domains of QUALEFFO-41 whereas age which was associated with the three domains of physical function, but not with pain and mental function. Finally, the number of pregnancies was significantly associated with a worsening of pain \((\beta=0.50; \text{95}\% \text{CI}=0.24 \text{ to } 0.76)\).

To sum up, in postmenopausal women with no symptoms related to spinal arthritis. Only eight patients were under treatment with antiosteoporotic drugs.

**Conclusions**

To determine bone microarchitecture analysis (BMA) standard curves for wrist and ankle of both sexes: data from EpiReumaPt

**Introduction**

Bone mineral density distribution in early osteoporotic bone

**Table 1**

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**Design**

EpiReumaPt is an ongoing national, population-based, cross-sectional, epidemiologic study developed by the Portuguese Society of Rheumatology to estimate the prevalence of rheumatic diseases in Portugal. Trained interviewers have been randomly applying a standardized questionnaire to 10 000 subjects at their houses. Selected cases are eventually observed by a rheumatologist and ankle and wrist BMA performed. BMA (DIMA systems) is a new imaging technique based on a digital X-ray system that allows bone microarchitecture quantification and osteo-articular imaging at a highest spatial resolution.

**Results**

The study was started on 19 September 2011, and up to now, 5000 interviews were performed and 1700 subjects have been observed by a rheumatologist. Mean age was 53.8 (S.D. 18.4), 61.8% were women and 94% Caucasians. BMA was performed at bone ankle in 747 women and 371 men and at bone wrist in 837 women and 427 men.

Exclusion criteria included: other ethnicities rather than Caucasian and subjects with missing data on birth date. Subjects with left and right sides assessed were considered as ‘duplicates’ and the right side was removed from the analysis. The figures represent the BMA standard curves for women and men ankle and wrist. H parameter (rigidity) was lower in women and decreased with age while measurements in men were very constant along years. A strong and significant correlation was found between measurements at left and right sides. A highly significant but weak correlation \((r=0.30)\) was found between ankle and wrist measurements from the same individuals.

**Conclusions**

These data allow for the first time the development of BMA standard curves for bone ankle in men and for wrist in men and women. Bone quality is a systemic feature, yet differences may occur among sites assessed.

**Table 1**

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**PP328**

Bone mineral density distribution in early osteoporotic bone

Saba Abdulghani1, Luis Santos2, Bruno Vidal1, Rita Cascão1 & João Fonseca

1Instituto de Medicina Molecular, Lisbon, Portugal; 2Instituto Superior Técnico, Lisbon, Portugal.

Osteoporosis (OP) is characterised by low bone mass and microarchitectural deterioration of the bone tissue, leading to enhanced bone fragility and increased fracture risk. OP causes an imbalance in the cellular remodelling process thus inducing changes in the bone’s mineral and organic phases that are responsible for its strength and stiffness. The purpose of this study is to investigate the early effects of OP progression on the arrangement of bone tissue mineral phase by performing quantitative backscattered electron imaging (qBEI) to evaluate the bone mineral density distribution (BMDD) in osteoporotic Balb/c mice (ovariectomized-OVX) and controls (Sham operated). Mice vertebrae (L3) with 0.5; 1; 2 and 3 months of disease duration were evaluated using a well-established method. The following BMDD parameters were measured: \(\text{Ca}_{\text{MEAN}}\) (wt.%Ca); \(\text{Ca}_{\text{WALLTH}}\); \(\text{Ca}_{\text{WALL}}\); \(\text{Ca}_{\text{LOW}}\); \(\text{Ca}_{\text{HIGH}}\); \(\%\) bone area, this parameter also corresponds to the amount of bone area passing primary mineralization and \(\text{Ca}_{\text{HIGH}}\); \(\%\) bone area, also corresponds to the amount of bone area having a fully mineralized bone matrix. Our results revealed a variation in the measured BMDD parameters particularly the \(\text{Ca}_{\text{LOW}}\) and \(\text{Ca}_{\text{HIGH}}\), since the initial phase of the disease where they are shown to be significantly reduced in the OVX mice as a function of disease duration confirming that OP causes a rearrangement in the mineralization density distribution Table 1.

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PP329

Discordance of Z-score in healthy premenopausal women with BMD below the expected range of age and contributing factors: the Korea national health and nutrition examination survey 2008–2009
Sung-Kil Lim, Kyong Hye Park, Kyoung Min Kim, Jung Soo Lim & Yumie Rhee
College of Medicine, Yonsei University, Seoul, Republic of Korea.

Discordance of Z-score is frequently observed and affects to therapeutic strategy in osteoporosis. Z-score discordance in premenopausal women has not been reported yet. In addition, despite of important role of low bone mineral density (BMD) in premenopausal women to predict osteoporotic fracture in postmenopausal age, there are very few reports on the status of low BMD in healthy premenopausal women. To investigate the current status of idiopathic osteoporosis and contributing factors to low BMD in addition to the presence of Z-score discordance in healthy premenopausal women, The Fourth Korea National Health and Nutrition Examination Surveys (KNHANES IV) conducted in 2008–2009. Total 3003 premenopausal women aged 18–50 years without secondary causes of low BMD were included. The prevalence of low BMD in healthy premenopausal women was 2.8%. BMI, total body muscle mass and supplemental amount of calcium and vitamin D were associated with low BMD. By analyzing spine and femur separately, risk factors were different depending on the site; low BMI and vitamin D deficiency were risk factors of low femur neck (FN) BMD, but not of low lumbar spine (LS) BMD. Discordance of Z-score was observed, the prevalence is more than 75%, and major discordance of Z-score was found in 40.9 and 26.2% of the low LS BMD and low FN BMD groups, respectively. Although different definition was applied, major discordance was much higher than expected in Korean. The risk factors were different depending on the site. Long term follow-up design is needed on the subsequent effect of existing Z-score discordance on the fracture risk.

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PP330

Factors that affects on bone density in premenopausal women
Dong Ock Lee1, Hoon Choi2 & Jung Gu Kim3
1Center for Uterine Cancer, Center for Cancer Prevention and Detection, National Cancer Center, Goyang-si, Gyeonggi-do, Republic of Korea; 2Sanggye Paik Hospital Inje University College of Medicine, Seoul, Republic of Korea; 3Seoul National University Hospital, Seoul, Republic of Korea.

Objectives
To find the predicting factors for bone density in premenopausal women.

Methods
Two hundred and forty-five premenopausal women without factors that can cause secondary osteoporosis were analyzed. Age, height, weight, change of height from peak height, history (Hx) of fracture, family Hx of hip or other fracture, Hx of estrogen use, calcium or vitamin D supplementation, smoking and previous Hx of amenorrhea more than 3 months were questioned. Lumbar, femur neck, and hip bone density were measured by dual energy bone absorptiometry. Data were analyzed by analysis of covariance and multiple regression analysis.

Results
Mean age of subjects was 43.6 ± 3.7 years. Smoking women showed significantly lower lumbar spine bone density after adjustment of age and BMI (P = 0.04). Women with Hx of fracture, family fracture, estrogen use, calcium and vitamin D supplementation, and amenorrhea didn’t show significantly different bone density. By multiple regression analysis, bone density of lumbar spine was determined by height, change of height from peak height, weight and smoking (r² = 0.101, P = 0.001). Bone density of femur neck was determined only by weight (r² = 0.050, P = 0.001), and that of hip was by weight and height (r² = 0.088, P = 0.001).

Conclusion
In premenopausal women, smoking affect only on lumbar spine bone density and predicting factors for bone density were height, change of height from peak height, weight and smoking in lumbar spine, weight in femur neck, and weight and height in hip.

Key words
Bone density, premenopausal women.

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PP331

May we screen with FRAX clinical factors?
Carmen Gabriela Barbucci1,2, Catalina Poatina1,2, Dariiana Ionita2, Magda Gascu3, Cristina Stefan3, Aurelia Stefanopol3 & Simona Fica1,2
1Carol Davila University of Medicine and Pharmacy, Bucharest, Romania; 2Elias Hospital- Endocrinology Department, Bucharest, Romania; 3C.I. Parchon Institute, Bucharest, Romania.

Aim
The aim of the study was to evaluate the usefulness of the fracture risk evaluated through the FRAX® model based only on the clinical risk factors as a screening tool for identify the target population for treatment in osteoporosis.

Materials and methods
Two hundred and seventy-six premenopausal women treatment naive referred to two different endocrinology departments for osteoporosis between 2009 and 2011 were evaluated. The FRAX® model for Romanian population was used to calculate the major osteoporotic risk fracture and hip risk fracture either using only clinical risk fracture (without BMD- abbreviated clinical FRAX®) or clinical risk fracture and femoral neck BMD (abbreviated FRAX®). We calculate the negative predictive value of clinical FRAX® outcome comparing to FRAX results to evaluate whether clinical FRAX® evaluation would be a reliable screening tool to select patient for completing DXA evaluation and initiate treatment in osteoporosis.

Results
The mean value of the clinical FRAX® evaluation in the study group was 7% ± 4.7 for the major osteoporotic fracture and 2.4% ± 2.7 for the hip fracture, respective. When femoral neck BMD was included, the mean value was 8.2% ± 3.5 for major osteoporotic fracture and 2.4% ± 2.7 for the hip fracture risk. Using the same cut off value (20% for major osteoporotic fracture and 3% for hip fracture risk) we found a positive predictive value of clinical FRAX® evaluation against complete FRAX® of 33% for major osteoporotic fracture and 77.6% for the hip fracture. The negative predictive value was found to be 95.9% for the major osteoporotic fracture and 90.05% for the hip fracture.

Conclusion
Using FRAX® evaluation based exclusively on clinical risk factors might be appropriate as a screening to identify patients with significant risk for major osteoporotic fracture but not for hip fracture. On the other hand we should highlight that our results are to be applied only in a high risk population like our study group of patients referred to university facilities.

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PP332

Osteoporosis and bone fractures in elder women with rheumatoid arthritis
Kamalya Kasumova, Azamat Satybaldyev & Alexander Smirnov
Research Institute of Rheumatology under the Russian Academy of Medical Sciences, Moscow, Russia.

Introduction
Objective of the study is to assess the frequency of osteoporosis and osteoporotic fractures in rheumatoid arthritis (RA) patients with the onset in the age of 55 and elder.

Methods
Seventy women with RA (mean age of 62.6 years, mean RA duration of 4.6 years) were examined with dual X-ray absorptiometry (DXA) in three locations (vertebral body L1–L4, femoral neck, and distal radius) and X-ray of vertebral with assessment by Felsenberg.

Results
Twenty-one patients (30%) had osteoporosis in three locations, 17 (24.3%) – in two locations. There was the relationship between the risk of osteoporosis and duration of RA BMI, X-ray stage, HAQ, glucocorticoid therapy. Among the patients with RA duration more than 5 years 78% had osteoporosis of femoral neck, 92% – of radius, 60% – of vertebral bodies L1–L4. 89% of patients with body mass index ≤21 had osteoporosis. 69% of patients with fourth X-ray stage had osteoporosis of three positions, 82% – of femoral neck, 100% – of radius. The patients with HAQ more than 1.0 had osteoporosis of femoral neck at 2.5 times more often. 100% of patients, who received glucocorticoids, had osteoporosis of radius, 78.2% – of femoral neck, 69.5% – of vertebral bodies L1–L4. 30 patients had vertebral fractures (9 – crush vertebral fractures), 5 – hip fractures, 1 – humerus fracture, 3 – radius fractures (all of them had body vertebral fractures Th7–L4). The patients with high inflammation activity, system glucocorticoid therapy and long duration of RA had a higher risk of fractures. 82% of patients with RA duration more than 7 years had osteoporosis fractures.

Bone Abstracts (2013) Vol 1
PP334 Bone fragility in patients with alcoholic liver cirrhosis: can hip structure analysis better predict risk of hip fracture

Danijela Djovic1, Djordje Culacic2, Violeta Culacic-Vojinovic3, Svetlana Ignjatovic4, Ivan Soldatovic5, Jelena Vasic6 & Marija Djuric7
1Laboratory for Anthropology, School of Medicine, Institute of Anatomy, University of Belgrade, Belgrade, Serbia; 2Railway Health Care Institute, Belgrade, Serbia; 3School of Medicine, Clinic of Gastroenterology, Clinical Center of Serbia, University of Belgrade, Belgrade, Serbia; 4School of Medicine, Institute of Medical Statistics and Informatics, University of Belgrade, Belgrade, Serbia.

Hepatic osteodystrophy is an important complication of chronic liver disease associated with fractures resulting in pain, deformity and immobility. The aim of the study was to examine association of severity of alcoholic liver cirrhosis with areal bone mineral density (BMD) and to estimate bone geometric strength of the proximal femur in those patients. The study included 27 male patients with alcoholic liver cirrhosis and control group of 36 healthy patients. Laboratory testing included biochemical markers of bone turnover: serum level of osteocalcin and β-cross laps. Areal BMD was measured by dual X-ray absorptiometry of the proximal femora. Structural parameters was obtained using the hip structure analysis software (HSA). Our findings of lower areal BMC and BMD, cross sectional area and section modulus, thinner cortex and higher buckling ratio in neck region of patients with cirrhosis suggest increased risk for fracture. Particular affection of cervical region is in agreement with general epidemiological data indicating more cervical than trochanteric fractures in elderly males.

Decreased osteocalcin values and increased β-cross laps in patients with cirrhosis demonstrated predominantly low bone turnover caused by decreased bone formation with reduced synthesis of collagen matrix. This study confirms that the risk of fracture increases not only due to low bone density but also because of failure of skeletal geometry. This emphasizes the importance of more profound structural analysis of DXA scans in patients with cirrhosis than simple BMD and T scores.

Key words: Alcoholic liver cirrhosis, hepatic osteodystrophy, hip structure analysis, osteocalcin, β-cross laps.

DOI: 10.1530/boneabs.1.PP334

PP336 TBS improves the detection of subjects at risk of fracture irrespectively to the BMD status: a Spanish population-based study

Silvana Di Gregorio1, Renaud Winzenrieth2 & Luis Del Rio3
1CETIR Grup Medic, Barcelona, Cataluña, Spain; 2Med-Imaps, Pessac, Bordeaux, France.

Isn’t uncommon to encounter patients with both a fragility fracture and only a slightly low BMD value or even normal one. Currently the DXA technology can assess information on trabecular microstructural texture supplementing the

Peña et al. (2013) suggested that the hip structure analysis (HSA) improves the estimation of fracture risk in populations with normal BMD. HSA can detect abnormalities in the trabecular microstructure that are not identified by BMD alone. However, up to date there is not enough data about the potential of HSA to detect subjects at risk of fracture irrespectively of the BMD status in a Spanish population.

Objective: To evaluate the potential of the TBS analysis (TBS) to detect subjects at risk of fracture irrespectively of the BMD status in a Spanish population.

Methods: Cross-sectional study including 78 T2DM patients (Hologic QDR 4500). Patients were classified as having or not osteoporosis according to OMS criteria, new criteria from Schwartz et al. and FRAX index. We determined: bone-specific alkaline phosphatase (BSAP) (OCTEIA IDS Ltd); osteocalcin (OC) (DiaSorin, Stillwater, Minnesota USA); TRAP5b (BioTRAP® Assay. IDS Ltd); and CTX (Elecsys β CrossLaps, Roche Diagnostics SL, Barcelona, Spain); results were analysed by SPSS 15.0.

Results: There were no differences in BSAP, OC or TRAP5b according to the three classifications. However, CTX were higher in patients classified as having osteoporosis according the Schwartz criteria, both at femoral neck (0.379 ± 0.173 vs 0.188 ± 0.108 ng/ml, P < 0.001) at lumbar spine (0.306 ± 0.170 vs 0.168 ± 0.087 ng/ml, P < 0.001), and also in patients selected for treatment by FRAX (0.368 ± 0.114 vs 0.199 ± 0.105 ng/ml, P = 0.01). CTX were also higher in patients with osteoporosis by OMS criteria, although only at lumbar spine (0.328 ± 0.182 vs 0.183 ± 0.081 ng/ml, P < 0.001).

Conclusions: Our results suggest that higher CTX concentrations may indicate which T2DM patients are suitable for osteoporosis treatment, although CTX does not constitute a fracture risk factor independently of BMD. However, it may help in the identification of patients in whom DXA must be done.

DOI: 10.1530/boneabs.1.PP336
standard BMD measurement, using a new method: the trabecular bone score (TBS). In order to check TBS, BMD and their combination to discriminate patients with vertebral fracture, we scanned 946 subjects. The cohort was stratified using the WHO diagnostic T-score threshold. TBS was calculated from the same DXA acquisition and region of interests than those used for the LS BMD using TBS Insight. Vertebral fractures were confirmed using lateral vertebral assessment software. The discriminate value of BMD, TBS (L1–L4) or both combined was assessed by ROC curve and using a reclassification index. 6.6% of the cohort, suffered from at least one vertebral fracture. Fracture group have significant lower BMD and TBS than subjects without fracture ($P<0.01)$. Correlation between TBS and BMD is low ($r=0.3)$. The vertebral fractured group had a higher proportion of osteoporosis diagnosis (51%), but surprisingly, 5% of them have normal BMD. In the whole cohort TBS showed a significant higher discrimination power than the BMD (TBS BMD $AUC=0.756 (0.724-0.786)$ vs BMD $AUC=0.638 (0.603-0.672)$, $P=0.013)$. TBS and BMD combination improved the discrimination $AUC=0.767 (0.736-0.797)$. When BMD stratification is used, TBS discrimination was better for normal and osteopenic subjects ($AUC=0.815 (0.747-0.871)$ and 0.795 (0.753–0.832) respectively) than in osteoporotic subjects ($AUC=0.614 (0.541-0.684)$). When reclassification index was used, the combination of 1st TBS tertile threshold and BMD $r=2.5$ T-score threshold improved the overall subject classification by 31 and 29% in comparison with the use of BMD $r=2.5$ T-score and 1st TBS tertile thresholds alone respectively. TBS is a useful tool for patient management in daily clinical routine. One of its main clinical add-value is clearly for normal and osteopenic subjects management.

### PP337

**Dietary calcium and vitamin D intake and serum 25-hydroxyvitamin D and parathyroid hormone levels in healthy elderly Spanish men: the relationship with calcaneal and phalangeal quantitative ultrasound and phalangeal dual energy X-ray absorptiometry**

Alejo Leal, Maria Luz Canal-Macias, Julian Fernando Calderón-García, Raúl Roncoro-Martin, Trinidad Rodríguez-Dominguez & Jose M Moran

**Metabolic Bone Diseases Research Group, Cáceres, Spain.**

**Purpose**

To evaluate whether calcium and vitamin D intake is associated with 25-hydroxyvitamin D (25(OH)D) and parathyroid hormone (PTH) serum concentrations or is associated with either the phalangeal dual energy X-ray absorptiometry (pDXA) or the quantitative bone ultrasound (QUS) at the phalanges and the calcaneus in independent elderly men from southwestern Spain.

**Methods**

Serum PTH and 25(OH)D were measured in 199 healthy elderly men (mean age: 73.31 ± 5.10 year). Food intake was quantified using a dietetic scale on the basis of current 7-day dietary records. Both pDXA and QUS at the phalanges and the calcaneus were assessed.

**Results**

Participants with 25(OH)D levels ≥30 ng/ml and a calcium intake of 800–1200 mg/day exhibited the lowest PTH levels (41.49 ± 16.72 ng/ml). The highest PTH levels (75.60 ng/ml ± 14.16) were observed in the <30 ng/ml group 25(OH)D with a calcium intake >1200 mg/day. No significant differences in the serum PTH levels based on the serum 25(OH)D levels were observed among participants with a calcium intake of 800–1200 mg/day. Serum PTH was inversely correlated with serum 25(OH)D in the entire patient sample ($r=−0.288$, $P=0.019)$. No differences in any of the three densitometry techniques were observed between any of the age groups in the 800–1200 and >1200 mg/day calcium intake groups.

**Conclusions**

PTH levels correlate negatively with serum 25(OH)D levels, and neither calcium nor vitamin D intake exert a strong influence on either of the two parameters.

**DO: 10.1530/boneabs.1.PP337**

### PP338

**Low testosterone levels are associated with poor peripheral bone mineral density and quantitative bone ultrasound at phalanges and calcaneus in healthy elderly men**

Jesus María Lavado-García, Purificación Rey-Sánchez, Carmen Costa-Fernandez, Mariana Martínez, Alejo Leal, Francisco José Rodríguez-Velasco & Juan Diego Pedreira-Zamorano

**Metabolic Bone Diseases Research Group, Cáceres, Spain.**

**Context**

Variations in sex hormones influence bone health in men. Aging in men is associated with a decrease in testosterone (T) levels.

**Aims**

To examine the relationship between T levels and changes in bone health status as measured by quantitative ultrasound (QUS) at the phalanges and the os calcis and by peripheral bone mineral density (pBMD) at the phalanges in healthy elderly Spanish men.

**Methods and material**

We examined 162 men 65–88 years of age, and total serum T concentrations were assessed. Serum total T <300 ng/dl was used as the threshold for biochemical T deficiency. QUS at the phalanges and the os calcis and by pBMD at the phalanges was measured.

**Results**

The sample was divided into low or normal T levels, and both groups were matched for age, weight, height and BMI ($P>0.05$ for all the comparisons). All measured bone parameters were higher in the normal serum T group ($P<0.05)$. Multiple regression analysis revealed that serum T was an independent predictor of both QUS at the calcaneus and pBMD. Serum T exhibited a sensitivity and specificity of 42.1 and 90.2%, respectively, for the detection of lower or higher risks of osteoporosis (based on a T-score < −2 by phalangeal DXA).

**Conclusions**

Our data indicated that T was an independent determinant of QUS at the os calcis and pBMD at the phalanges in elderly Spanish men.

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### PP339

**What is the performance in vertebral fracture discrimination by bone mineral density, micro-architecture estimation, and FRAX in stand-alone, combined or adjusted approaches: the OsteoLaus Study**

Olivier Lamy1,3, Marc-Antoine Krieg1, Delphine Stoll3, Berengère Aubry-Rozière1,2, Marie Metzger1 & Didier Hans1

1Center of Bone Diseases, Lausanne University Hospital, Lausanne, Switzerland; 2Lausanne University Hospital, Rheumatology, Lausanne, Switzerland; 3Lausanne University Hospital, Internal Medicine, Lausanne, Switzerland.

The aim of the study is to compare the performance of FRAX vs TBS adjusted FRAX using Leslie B et al. method to better identify women at high fracture risk. The OsteoLaus cohort (1500 women 50–80 years living in Lausanne, CH) started in 2010. CRF for OP, FRAX, spine and hip BMD, VFA by DXA and TBS were recorded. Sensitivity and specificity in regard to vertebral fracture grade 2 and 3 has been calculated. Net reclassification improvement (NRI) had also been calculated. We included 911 women: mean age 65.2 ± 7.9 year, BMI 25.7 ± 4.4, mean spine BMD 0.93 ± 0.163, TBS 1.289 ± 0.100. As expected, correlation between BMD and site matched TBS is low ($r^2=0.16$). Prevalence of VFx grade 2/3 and MOF are 7.5 and 15.0% respectively.

An incremental improvement in fracture identification was seen by using spine TBS in combination with FRAX. If validated in prospective cohorts, spine TBS may become clinically useful for enhancing fracture prediction from FRAX.

**1. Leslie WD et al. Lumbar Spine TBS is a FRAX independent risk factor for fracture: the Manitoba BMD Cohort. ISCD Annual meeting 2013. Tampa, Florida Table 1.**

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spine BMD</td>
<td>29.4</td>
<td>82.7</td>
</tr>
<tr>
<td>(−2.5 T-score threshold)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spine TBS ($−1,200$ threshold)</td>
<td>51.5</td>
<td>77.1</td>
</tr>
<tr>
<td>FRAX MOF (20% threshold)</td>
<td>38.2</td>
<td>94.8</td>
</tr>
<tr>
<td>Spine TBS or FRAX MOF</td>
<td>63.2</td>
<td>74.4</td>
</tr>
<tr>
<td>(20% thresholds)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBS adjusted FRAX All</td>
<td>50.0</td>
<td>89.9</td>
</tr>
<tr>
<td>fracture (20% threshold)</td>
<td></td>
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</tr>
</tbody>
</table>

NRI for FRAX adjusted by TBS vs FRAX was +7.6% for VFx ($P<0.001$).

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Bone Abstracts (2013) Vol 1
Trabecular bone score and bone mineral density of lumbar spine in healthy women: pros and cons
Vladyslav Povoroznyuk1, O Lamy1,2, Natalia Dzerovych3 & Didier Hans1,2
1Institute of Gerontology NAMS Ukraine, Kyiv, Ukraine; 2Center of Bone Diseases, Lausanne University Hospital, Lausanne, Switzerland.

Areal bone mineral density (aBMD) of the PA spine and proximal femur remained the gold standard for WHO classification of osteoporosis, fracture prediction and patient monitoring. Unfortunately, with age it is not infrequent to observe the presence of degenerative disease such as spinal osteoarthritis which would have a positive artifactual impact on aBMD which could lead to an erroneous interpretation. In a previous study it has been demonstrated that apparently such artifact would have limited impact on the trabecular bone score (TBS). The aim of this study was to evaluate the PA spine TBS and site matched BMD (BMDLS) in healthy women of various ages and verify how the ‘normal’ presence of such artifact would impact the outcome.

All women who had prior exposure to corticosteroids, systemic illness or who were taking medications known to affect bone metabolism were not included. Similarly all fractured subjects were excluded from this analysis. We’ve examined 176 healthy women aged 40–79 years (mean age – 53.4 ± 6.0 years; mean height – 1.64 ± 0.005 m; mean weight – 80.4 ± 1.1 kg). The patients were divided into the following age-dependent groups: 40–49 years (n = 53), 50–59 years (n = 89), 60–69 years (n = 17), 70–79 years (n = 17). BMD of whole body, PA lumbar spine and proximal femur were measured by DXA method (Prodigy, GEHC Lunar, Madison, WI, USA) and PA spine TBS were assessed by TBS iNsight software package installed on our DXA machine (Med-Imaps, Pessac, France).

We observed a significant decrease of TBS (L1–L4) as a function of age (40–49 years – 1.126 ± 0.013 g/cm²; 50–59 years – 1.234 ± 0.013 g/cm²; 60–69 years – 1.343 ± 0.053 g/cm²; 70–79 years – 1.348 ± 0.100 g/cm²; F = 6.56; P = 0.0003) whereas PA spine BMD was significantly increasing with age (BMDLS: 40–49 years – 0.013 g/cm²; 50–59 years – 0.100 g/cm²; 60–69 years – 0.194 ± 0.023 g/cm²; 70–79 years – 1.686 ± 0.205 g/cm²; F = 22.08; P < 0.0001). In this population, BMD of femoral neck didn’t show any significant variations.

TBS decrease with age significantly. BMD of lumbar spine significantly increased in healthy women depending on their age, as it seems to reflect the impact of aggravating spinal osteoarthritis. This contradiction can be traced to the spinal osteoarthritis and degenerative diseases progressing with age in the elderly patients. Thus, TBS is an independent parameter which has a potential diagnostic value of its own, without taking into account the bone mineral density in case of bone degenerative diseases.

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Fracture risk among men, in relation to osteopenia and osteoporosis defined by areal bone mineral density
Julie Pasco1,2, Stephen Lane1,3, Sharon Brennan1,2, Elizabeth Timney1, Gosia Bucki-Smith1, Amelia Dobbins1 & Mark Kotowitz1,2
1Deakin University, Geelong, Victoria, Australia; 2The University of Melbourne, St Albans, Victoria, Australia; 3Barwon Health, Geelong, Victoria, Australia.

Introduction
The purpose of this study was to quantify fracture risk associated with areal bone mineral density (BMD) in older men.

Methods
In this prospective analysis we followed 620 men aged 60–93 years (median 74.3 years) for a median 6.4 years, after baseline BMD assessments (performed 2001–2006) as part of the Geelong Osteoporosis Study. Based on WHO criteria, 33.5% had normal BMD at the femoral neck, 57.6% were osteopenic and 8.9% osteoporotic. Participants were followed until the end of 2010, or until sustaining a fracture, death, or emigration. Post-baseline fractures were ascertained radiologically.

Results
During the study 130 men died, 16 left the region, 63 sustained at least one fracture and 411 remained fracture-free, generating 3592 person years of follow-up. Significant differences in fracture incidence were observed in men with low BMD compared with those with normal BMD (Table 1). Median (IQR) BMDLS was 0.79 (0.75–0.94) g/cm² and median (IQR) TBS was 0.40 (0.35–0.44) in the normal BMD group and 0.81 (0.77–0.87) g/cm² and 0.41 (0.37–0.47) in the low BMD group, respectively (P < 0.0001). In adjusted multivariate models women on statins had higher BMD at femoral neck (P = 0.002) and total hip (P = 0.04) than non-users. No differences were found in men. Simvastatin induced higher increases in BMD than non-statin use at femoral neck (P = 0.002), and total hip (P = 0.009), lovastatin induced lower BMD values at lumbar spine (P = 0.028), and rosvastatin led to higher increases at femoral neck (P = 0.006), in women. In men, solely atorvastatin was associated with higher femoral neck BMD than non-statin use (P = 0.029). Comparing with non-statin users, only lipophilic statins increased BMD at femoral neck in women (P = 0.003) but not in men. According to drug-potency, women on high- or lower-potency agents showed higher BMD values at femoral neck than non-users (P = 0.028 and 0.022 respectively). In men, only high-potency statins were associated with higher femoral neck BMD than non-use (P = 0.021). No differences between dose or length of statin therapy were noted regarding BMD in either sex.

Conclusions
Vitamin D insufficiency and deficieny are very common among Spanish immobilized adults. Mean PTH levels lie around 80 pg/ml, a value that is above the upper limit of normality for this hormone.

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follow-up. Most (86.5%) of the fractures occurred in men without osteoporosis on BMD criteria (17.9% with normal BMD, 68.7% with osteopenia) whereas 3.5% of the fractures occurred in men with osteoporosis. Age-standardised 5-year fracture risk was 2.94% (95% CI 1.20, 6.45) for normal BMD, 6.98% (95% CI 4.27, 11.11) for osteopenia and 17.71% (95% CI 6.62, 68.37) for osteoporosis. Using an age-adjusted Cox proportional hazards model and normal BMD as the referent group, the hazard ratio (HR) for fracture was 2.05 (95% CI 0.34, 4.45) and 4.47 (95% CI 1.54, 13.02) for men of median age with osteopenia and osteoporosis, respectively. The relative risk of those with osteoporosis as compared to those with osteopenia was 2.18 (95% CI 1.92, 5.17). Prior low trauma fracture was significant in the models, with HR 1.85 (95% CI 1.08, 3.15). Conclusion The categories of decreasing BMD defined increasing risk of fracture, with advancing age amplifying this risk. Although men with osteoporotic BMD were at greatest risk, they contributed 3.5% to the total burden of fractures. Two-thirds of the fractures arose from men with osteopenic BMD, who represented approximately half of the population at risk.

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PP344
Bone turnover markers in old old vs postmenopausal women
Charles Inderjeeth1,2, Kien Chan1, Peeti Nair1, Pang Wee Yang1, Anupham Chauhan1 & Ellem Lim1
1North Metropolitan Health Service, Perth, Western Australia, Australia; 2University of Western Australia, Perth, Western Australia, Australia.

Background Osteoporosis is not a homogenous disease. Riggs et al. identified two distinct types of osteoporosis, with different pathophysiology, patterns of bone loss and fracture types. Post-menopausal (PM) osteoporosis is triggered by withdrawal of the effect of oestrogen on bone, which leads to a sharp acceleration of bone turnover with an imbalance towards excessive osteoclastic activity. Senile osteoporosis in the old old (usually after the age of 75) is a disease of reduced formation. However, data on senile osteoporosis is limited.

Aim and hypothesis We aim to compare bone turnover markers in a postmenopausal group to the old old. We hypothesise that the differences in their profiles would reflect the differences in underlying mechanisms of osteoporosis.

Methodology Retrospective audit of all fasting metabolic bone studies (FMBS) performed by the author (CI) in the outpatient clinic during the period 2002–2012. Patients were divided into the postmenopausal (age 50–65) (PM) and old-old (age 75 and above) (OO) groups.

Results Ninety-four and seventy-six FMBS were performed by CI between January 2002 and March 2012. 55 patients met the predefined criteria and were included in the final analysis. PINP and Albumin were significantly lower in the older group (P<0.05). However, there was no significant difference in bone resorption markers between the two groups.

Discussion Lower PINP in the old old group supports the hypothesis by Riggs et al. regarding reduced bone formation in senile osteoporosis. A possible reason for the similar result is the increase in bone remodelling sites in the old old rather than an increased rate of resorption at each individual site. Furthermore, NTx/Creatinine ratios in urine are influenced by reduced muscle bulk vs renal impairment which has the opposite effects.

Conclusion This may have implications for treatment in the old old with predominant cortical osteoporosis. Anabolic treatments may be preferable to anti-resorptive therapies. More research is required in this therapeutic area.

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PP345
Mortality after osteoporosis-related hip fractures in Austria 2008–2010
Wolfgang Brozek1, Berthold Reichardt2, Oliver Kimberger2, Daniela Kritsch1, Klaus Klaushofer1 & Elisabeth Zwettler2
11st Medical Department at Hanusch Hospital, Ludwig Boltzmann Institute of Osteology, Hanusch Hospital of the WGKK and AUVA Trauma Center, Vienna, Austria; 2Sickness Fund Burgenland, Burgenländische Gebietskrankenkasse, Eisenstadt, Austria; 3Clinical Department of General Anesthesia and Intensive Care Medicine, Medical University of Vienna, Vienna, Austria.

Osteoporosis-related hip fractures represent a substantial cause of mortality and morbidity in industrialized countries; nonetheless, past studies in Austria lack mortality figures save during hospitalization (in hospital mortality). We therefore retrospectively retrieved pseudonymized invoice data from Austrian social insurance authorities covering roughly 98% of the entire population including 31,548 subjects over 51 years of age who sustained first hip fractures between July 2008 and December 2010, with follow-up until June 2011. Kaplan–Meier and Cox hazard regression analyses yielded mortalities adjusted for age and gender.

In our cohort, median age of 73.36% female subjects at hospital discharge after first fracture was 83.57 years (IQR: 10.39 and 78.43 years (IQR: 16.26) for men (P<0.0001) (total median age: 82.49 years, IQR 12.27). Total in-hospital mortality in the study interval amounted to 4.03% (women: 3.44%; men: 5.74%; P<0.0001). Amongst survivors of hospitalization after first fracture, total mortality rates within 30 days, half a year, and one year after discharge were 3.13% (95% CI: 2.93–3.33%), 11.94% (95% CI: 11.57–12.31%), and 17.68% (95% CI: 17.23–18.13%), respectively, with shorter survival for male compared with female patients (HR 1.25, 95% CI: 1.19–1.32; P<0.0001). In this group, total one-year mortality rose from 6.33% (95% CI: 4.21–8.46%) amongst patients aged 51–54 years to 40.88% (95% CI: 37.68–44.08%) in patients aged 95 years and above. Total one-year mortality after first fracture, the exact date of which was assessed from hip fracture-related hospital days (median: 16 days, IQR: 17; median per fracture: 15 days, IQR: 9), amounted to 20.18% (95% CI: 19.71–20.65%), male to female HR 1.3 (95% CI 1.24–1.37).

Collectively, next to providing an up-to-date account of osteoporosis-related hip fracture mortality in a cohort comprising the vast majority of first cases aged over 51 treated in Austrian hospitals during a 2.5-year period, mortality rates presented herein are within the lowest compared with recent studies from other countries.

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PP346
Association of osteoprotegerin gene polymorphisms with bone mineral density and bone turnover markers in postmenopausal women and elderly men
Martina Smoljic1, Selma Cvjetic2, Tonislav Kizivat1, Robert Smolic3, Ivana Maric1, Teuta Opacak-Bernardi2, Hrvoje Roguljic1 & Antun Tucak1
1Department of Mineral Metabolism, Faculty of Medicine Osijek, Osijek, Croatia, 2Institute for Medical Research and Occupational Health, Zagreb, Croatia.

Osteoprotegerin gene (OPG) is an important candidate gene of osteoporosis. Association of the OPG polymorphisms and bone mineral density (BMD) have been studied by several research groups, however results are not uniform. The aim of this study was to determine if two polymorphisms in the OPG gene influence bone turnover markers and bone mineral density (BMD) in postmenopausal women and elderly men. A total of 135 patients, aged 41–87 years, were included in this study. Lumbar spine, femoral neck, total-hip and distal radius BMD were measured by dual-energy X-ray absorptiometry (DXA) and bone turnover markers were measured by standard biochemical procedures. OPG gene polymorphisms A163>G and T245>G were detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The frequencies of A163>G and T245>G polymorphisms in the OPG gene were determined by screening 131 DNA samples. The prevalence of genotypes of the A163G polymorphism was 59.4% for GG, 33.3% for AG and 7.2% for AA genotype in group with osteoporosis, whereas in control group the prevalence was 77.8, 16.7 and 5.6%, respectively. The prevalence of genotypes of the T245G polymorphism was 88.4% for genotype TT and 11.6% for genotype TG in group with osteoporosis, whereas in control group the prevalence was 94.4 and 5.6%, respectively. Analysis of BMD in the distal radius of postmenopausal women showed a trend to lower levels in the minor allele homozygote group (GG) vs two other two groups.

Conclusion Our results suggest that OPG polymorphism influence BMD in postmenopausal women, however further biological and/or functional evidence would be needed to confirm the suggestive influence of OPG polymorphisms on BMD.

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Bone Abstracts (2013) Vol I
PP347
Acute effects of glucocorticoids on CRP and bone markers
Jan Stepan1,2, Kristyna Brabnikova Maresova1 & Karel Pavelka1,2
1Institute of Rheumatology, Prague, Czech Republic; 2Charles University Faculty of Medicine 1, Prague, Czech Republic.

Aim
To investigate the acute effects of oral glucocorticoids in doses used in clinical practice on biochemical indices of function of osteoclasts, osteoblasts and osteocytes.

Methods
In 17 adult patients suffering from various medical pathologies requiring systemic steroid therapy that were never before treated with glucocorticoids, the glucocorticoid treatment was initiated (mean prednisolone equivalent dose of 23.1 ± 12.7 mg/day, range 10–50 mg/day). Fasting morning serum concentrations of osteocalcin (OC), amino terminal propeptide of type 1 procollagen (PINP), type 1 collagen cross-linked C-telopeptide (iCTX), soluble receptor activator of nuclear factor kappa B ligand (sRANKL), osteoprotegerin (OPG), sclerostin, Dickkopf-1 (Dkk-1), and the high-sensitivity C-reactive protein (hsCRP) were measured at baseline and on three consecutive days.

Results
Significant reductions in serum OC, PINP, OPG, sclerostin, and hsCRP were observed during 96 h of glucocorticoid administration while serum iCTX showed a significant percentual increase. A significant positive correlation was found between serum concentrations of Dkk-1 and iCTX after 96 h of treatment with glucocorticoids.

Conclusion
Medium-dose, short-term oral GC regimens cause an immediate decrease of the biochemical markers of osteoblast and osteocyte activity (PINP, OC, OPG and sclerostin, respectively), and a moderate increase of the biochemical marker of bone resorption (iCTX) associated with Dkk-1. A significant drop in serum sclerostin, OPG and OC observed in this study may reflect the rapid glucocorticoid induced apoptosis of osteocytes. The results are in good agreement with negative effects of GCs on bone remodelling, despite suppression of systemic inflammation.

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PP348
Variation in osteoporosis patients' service utilization in general practice clinics
Troels Kristensen1,2, Kim Rose Olsen1,2, Charlotte Ejersted3 & Anders Halling2
1Department of Health Economics, Faculty of Health Sciences, Institute of Public Health, University of Southern Denmark, Odense, Denmark/Region of Southern Denmark, Denmark; 2Research Unit of General Practice, Faculty of Health Sciences, Institute of Public Health, University of Southern Denmark, Odense, Denmark/Regions of Southern Denmark, Denmark; 3Department of Endocrinology, Odense University Hospital, Odense, Denmark/Region of Southern Denmark, Denmark.

Background
It is inadequate to use the patient’s age and sex alone to estimate physicians’ workload in the primary setting. The extent to which the morbidity burden of osteoporosis patients would account of the utilization of primary care services has not been examined.

Aim
We analyzed the number of face-to-face visits of osteoporosis patients visiting Danish GP clinics and aimed to assess what proportion of primary care services variation are explained by patient morbidity and GP clinic characteristics.

Methods and data
We use patient morbidity characteristics such as diagnostic markers and multi-morbidity casemix adjustment based on adjusted clinical groups (ACGs) and face-to-face visits for a sample of primary care patients for the year 2010. Our sample included 2057 patients in 59 general practices. We applied a multi-level approach.

Results (preliminary)
The average number of annual face-to-face visits for osteoporosis patients in general practice was about 7.12 visits per patient. Much of the variation in the utilization of primary care services was driven by multi-morbidity characteristics rather than age and gender. The number of face-to-face visits increased progressively with the degree of multi-morbidity. In addition, the number of face-to-face visits was higher for patients who suffered from diagnostic makers based on ICPC-2 (body systems and/or components such as infections and symptoms). Nevertheless, 16–19% of the variation in face-to-face visits was related to the clinic in which the osteoporosis patient was cared for.

Conclusion (preliminary)
Patients’ illness burden and GP clinic characteristics are significant in determining the utilization of primary care service in osteoporosis care. Thus, it may be relevant to introduce differentiated remuneration of GPs according to morbidity status.

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PP349
Women with hormone sensitive breast cancer who have received chemotherapy including prednisolone have reduced bone mass
Sofie Ronn1, Jannie D Hald2, Marianne Thisted1, Louise Andersen1, Louise Grønhøj1, Anders Bonde Jensen1 & Bente L Langdahl2
1Department of Endocrinology and Internal Medicine THG, Aarhus University Hospital, Aarhus C, Denmark; 2Department of Oncology, Aarhus University Hospital, Aarhus C, Denmark.

Introduction
Aromatase inhibitors (AI) used as adjuvant treatment of hormone-sensitive breast cancer reduce the level of circulating estrogen and cause accelerated bone loss. The aim of this study is to investigate the prevalence of osteoporosis in women with breast cancer and the effect of chemotherapy on the risk of osteoporosis.

Methods
Three hundred and sixty women with hormone-sensitive breast cancer who were scheduled to start treatment with AI were included. BMD was measured by DXA, information regarding risk factors for osteoporosis and chemotherapy was obtained by questionnaires and from patient files.

Results
One hundred and five women had been treated with chemotherapy and prednisolone (30%). They were younger than the group not treated with chemotherapy; mean age 57 ± 6 vs 67 ± 7 years (P < 0.001) and had lower BMD Z-scores at the lumbar spine (−0.12 ± 0.24 vs 0.78 ± 1.40), femoral neck (−0.36 ± 1.11 vs 0.14 ± 1.03) and total hip (−0.18 ± 0.97 vs 0.35 ± 1.04) (P < 0.001). The prevalence of osteoporosis was 18 and 14% among women who had or had not received chemotherapy (NS). Regression analyses revealed that BMD was influenced by BMI (P < 0.001), previous fracture (P < 0.05) and chemotherapy (P = 0.08) at the lumbar spine and by BMI (P < 0.001), smoking (P < 0.001) and age (P < 0.001) at the hip sites. If only women with one of the following risk factors; previous fracture, smoking, BMI < 25, age > 70 years were referred for DXA, we would identify 87% of patients with osteoporosis and reduce the need for DXA by 25%. If chemotherapy was added as a risk factor, the corresponding figures would be 93 and 16%.

Conclusion
Women with breast cancer treated with chemotherapy had reduced BMD. This could be due to the chemotherapy and prednisolone treatment but it could also reflect that these women have a more aggressive cancer which could cause bone loss as part of generalized catabolism.

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The relationship between urban–rural migration and bone mineral density in an urban–rural adult population: cross sectional findings from the Hyderabad Indian migration study

Heli Viljakainen1,2, Yoav Ben-Shlomo1, Sanjay Kinra2, Shah Ebrahim2,4, Nicholaos Papachristou1, Elena Kalyvioti1, Irene-Eva Triantaphyllidou1, Eleni Karavia2, Eva Plakoulia1, Harry Blair3, Kyriakos Kypreos2 & Dionysios Papachristou1

1Unit of Bone and Soft Tissue Studies, Department of Histology, School of Medicine, University of Patras, Rion-Patras, Greece; 2Department of Pharmacology, School of Medicine, University of Patras, Rion-Patras, Greece; 3Department of Pathology, School of Medicine, University of Patras, Rion-Patras, Greece; 4Department of Clinical Research, London School of Hygiene and Tropical Medicine, London, UK; 5South Asia Network for Chronic Disease, Non Communicable Disease Epidemiology, London School of Hygiene and Tropical Medicine, London, UK; 6National Institute of Nutrition, Hyderabad, India.

Rural to urban migration is associated with adverse metabolic consequences, but its effect on osteoporosis risk is unclear. We investigated associations between rural to urban migration and bone mineral density (BMD) after accounting for changes in body composition. A cross sectional analysis was performed of rural–urban migrants (RUM) matched with rural non-migrated (RNM) siblings, plus a separate sample of urban-non-migrants (UNM). Participants (n = 764, 54% male, mean age 49 years) were from the Indian Migration Study in Hyderabad. Lumbar spine (LS) and total hip (TH) BMD measured by DXA were the main outcomes. In minimally adjusted models, rural to urban migration was associated with a higher BMD in females; TH BMD: 0.928 (0.014), 0.899 (0.009) and 0.870 (0.012) g/cm² (P = 0.002); LS BMD: 0.923 (0.015), 0.904 (0.010) and 0.855 (0.014) g/cm² (P = 0.056) (mean (s.e.m., UNM, RUM and RNM, respectively). Conversely, no difference was seen in males (P < 0.001 for gender interaction). In regression analyses fat mass, lean mass and insulin were related to BMD, but lean mass was the only independent predictor. In further comparisons of BMD according to migration status, adjusting for lean mass; rural to urban migration was no longer related to BMD in females, whereas a decrease in BMD was seen in males with migration; TH BMD: 0.883 (0.011), 0.904 (0.007) and 0.924 (0.009) g/cm² (P = 0.005); LS BMD: 0.863 (0.015), 0.891 (0.009) and 0.918 (0.012) g/cm² (P = 0.003) (adjusted BMD in males, UNM, RUM and RNM, respectively). In summary rural to urban migration was associated with a higher BMD in females whereas no difference was seen in males. After adjusting for differences in lean mass, no association was evident between urban migration and BMD in females, whereas a negative association was observed in males. Hence, rural to urban migration may represent a risk factor for osteoporotic fracture in males.

DOE: 10.1530/boneabs.1.PP352
Results
i) ApoE/−/− mice fed WTD did not develop obesity, in contrast to the C57BL/6 (control) mice. ii) Osteoclast number was significantly increased, while bone synthesis was significantly reduced in ApoE/−/− mice fed WTD, in contrast to the other groups. iii) Static and dynamic histomorphometry showed that ApoE/−/− mice fed WTD developed osteoporosis.

Conclusions
i) ApoE plays a central role in the regulation of osteoblast and osteoclast function and thus in bone remodeling. ii) The absence of ApoE prevents obesity, but predisposes to the development of osteoporosis after the consumption of high-fat diet.

Acknowledgments
This study is supported by ‘The European Community’s Seventh Framework Programme (FP7-IR-Grant-PG06-GA-256402)’ and The University of Patras ‘Karateedhori’ Research Grant (#D155) (All awarded to DJ Papachristou) and is part of the research network ‘OsteoNet’ of the University of Patras activities.

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PP354
Apolipoprotein A-I deficiency is associated with decreased expression of osteoblast-specific regulators in mice
Elena Kalyvioti1, Nicholas Papachristou1, Irene-Eva Triantaphyllidou1, Eleni Karavia1, Eva Plakoula1, Harry Blair1, Kyriakos Kypreos1 & Dionysis Papachristou1
1Unit of Bone and Soft Tissue Studies, Department of Anatomy-Histology-Embryology, School of Medicine, University of Patras, Rion-Patras, Greece; 2Department of Pharmacology, School of Medicine, University of Patras, Rion-Patras, Greece; 3Department of Pathology, School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania, USA.

Introduction
Recent data suggest that imbalances in lipid metabolism affect the function of both osteoblasts and osteoclasts and thus bone quality. Here we investigated the role of apolipoprotein A-I (ApoA-I), a key element of HDL biogenesis, in the regulation of cardinal genes/proteins that regulate lipoblasts and osteoblasts in mice.

Materials and methods
We used apoA-I deficient (ApoA-I/−/−) and wild-type (C57BL/6) mice (10 animals/group). Following sacrifice, lumbar vertebrae and femora were removed for in vitro experiments. Bone marrow mesenchymal stem cells (BMSC) were isolated from mice femora, and then cultured and differentiated towards osteoblasts. BMSC were assessed for the expression of the lipoblastic and osteoblastic master regulators PPARγ and Runx2, respectively, with the use of western blotting, flow cytometry (FC) and RT-PCR analyses. At day 21, osteoblasts were stained with von Kossa and alkaline phosphatase and examined for the expression of the osteoblast-specific markers osteix, osteopontin and osteocalcin expression using FC.

Results
BMSCs obtained from the ApoA-I/−/− mice displayed significant increase in PPARγ and significant decrease in Runx2 expression at both protein and mRNA levels. Osteix, osteopontin and osteocalcin expression levels were significantly reduced in osteoblasts derived from the ApoA-I/−/− compared to the C57BL/6 group. Von Kossa and alkaline phosphatase stains were reduced in the ApoA-I/−/− compared to the control group.

Conclusions
ApoA-I deficiency reduces the expression of molecules associated with bone formation, while it favors the expression of lipoblastic markers in mice. Our findings highlight the interesting possibility that perturbation in ApoA-I and thus in HDL metabolism may predispose to the development of osteoporosis in mice.

Acknowledgments
This study is supported by ‘The European Community’s Seventh Framework Programme (FP7-IR-Grant-PG06-GA-256402)’ and The University of Patras ‘Karateedhori’ Research Grant (#D155) (All awarded to DJ Papachristou) and is part of the research network ‘OsteoNet’ of the University of Patras activities.

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PP355
Diabetes and obesity as independent risk factors for osteoporosis in postmenopausal women: a population study of 3354 people: first results of the PROF Project (Prevention of Osteoporosis and Fractures)
Cosimo Neglia1,2, Alberto Argentiero1,2, Giovanni Chitano1, Nadia Agnello1, Giuseppe Quarta1, Vincenzo Caiasfa1, Alessandro Distante1 & Prisco Piscitelli1
1ISBEM- Istituto Scientifico Biomedico Euro Mediterraneo, Brindisi, Apulia, Italy; 2University of Pisa, Pisa, Italy; 3University of Salento, Lecce, Italy; 4Department of Orthopedy, Local Health Authority of Taranto, Taranto, Italy.

Objectives
We aimed to analyze bone mineralization in postmenopausal women of Southern Apulia and to evaluate the effect of obesity-related phenotypes as BMI ≥ 30 kg/m2, diabetes, hypertension and cardiovascular diseases.

Methods
The PROF project is a population-based study on 3,356 subjects (40–99 years) analyzed by phalangeal quantitative ultrasound (QUS) to evaluate bone mineral status. A total of 2,756 postmenopausal women were involved and examined by phalangeal QUS based on the transmission of ultrasound through the proximal phalanges (digits II–V). We collected personal, anthropometric and clinical data for each subjects analyzed. The primary outcome of phalangeal QUS was AD-SOS (Amplitude dependent Speed of Sound) T score. Logistic regression analysis was used to evaluated odds ratios (95% CI) of osteoporosis in subjects with obesity, diabetes, cardiovascular disease and hypertension. Results were adjusted by age, physical activity and use of drugs causing osteoporosis. According to the WHO criteria, osteoporosis status was defined as T-Score ≤−2.5 s.d. the average value registered in young healthy women.

Results
Mean age of postmenopausal women was 64±9.5 years and mean BMI was 28.7±3.5.

Pearson correlation analysis revealed a negative association between T score and BMI (r=0.001). Significant odds ratio of osteoporosis status adjusted for age, physical activity and use of drugs causing osteoporosis were observed in women affected by diabetes and obesity, being OR (95% CI) respectively 1.39 (1.05–1.83) and 1.46 (1.20–1.78).

Conclusions
Diabetes and obesity in postmenopausal women with the characteristics of the examined population increase the risk of osteoporosis independently from the effect of the age, physical activity and drugs causing osteoporosis.

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PP356
Microgravity-induced osteoporosis: a challenge for the future of space programs
Prisco Piscitelli1,2, Alberto Argentiero1, Giovanni Chitano1, Cosimo Neglia1, Emiliano Sordi1,2, Giovanni Iolascon1, Alessandro Distante2 & Maria Luisa Brandi1
1Euro Mediterranean Scientific Biomedical Institute, Brinsisi, Apulia, Italy; 2University of Florence, Florence, Italy; 3University of Naples, Naples, Italy.

Objective(s)
We aimed to determine the impact of microgravity-induced osteoporosis on the future of space programs.

Material and methods
We performed a metaanalysis of the available literature, finding out different studies about i) muscle atrophy due to the absence of workload, which can consequently induce bone loss; ii) the effect of long term inactivity on bone mass; iii) the effect of calcium and vitamin D supplementation in women and men in order to prevent bone loss; iv) the effect of bisphosphonates in preventing bone resorption due to long term inactivity (animal models); v) studies concerning osteoporosis carried out during space missions.

Results
Unloading of weight bearing bones as induced by microgravity or immobilization has significant impacts on the calcium and bone metabolism and is the most likely cause for space osteoporosis. During a 4.5–6 month stay in space most of the astronauts develop a reduction in bone mineral density in spine, femoral neck, trochanter, and pelvis of 1–1.6% measured by dual energy X-ray absorption (DEXA). Dependent on the mission length and the individual turnover rates of the astronauts it can even reach individual losses of up to 14% in the femoral neck. Calcaneal mineral density is lost at a 5% rate of its mass each month. Attempts to
prevent disuse osteoporosis with both mechanical and biochemical means, including exercise, skeletal compression, increased hydrostatic pressure to the lower body, supplemental calcium and/or phosphorus, calcitonin, or etidronate were not successful. In Gemini, Apollo, and Skylab astronauts it was shown a negative calcium balance due primarily to hypercalcemia. Altered bone cell activity would probably result in irreversible bone loss with the premature development of senile osteoporosis many years after space flight.

Conclusion(s)
Microgravity-induced osteoporosis represents a challenge for the future of space programs and therefore needs to be further investigated.

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PP357
The prevalence of osteoporosis and risk factors for bone demineralization in Italy: first results from the FIRMO study
Caterina Fossi, Loredana Cavalli, Francesca Giusti, Alessia Metozzi, Simone Patrizi, Andrea Guazzini & Maria Luisa Brandi
University of Florence, Florence, Italy.

Objective(s)
We aimed to determine the prevalence of osteoporosis and risk factors for bone demineralization in the Italian population.

Material and methods
3090 consecutive subjects were screened for osteoporosis by using calcaneal quantitative ultrasounds (QUS) in 16 Italian cities (women: 2635; men: 455) during the extension of the FIRMO study carried out in 2011 on about 7000 people. Anamnestic data were collected to assess the presence of recognized risk factors such as poor sun exposure and calcium intake, physical activity, use of corticosteroids, and conditions associated to osteoporosis.

Results
The mean age was 58 years old. About 19% of examined people was affected by osteoporosis (n = 587), while 31% presented osteopenia (n = 958). About 15% of people had previous fractures due to low energy trauma, and 18.3% (n = 566) disclosed familiar history of osteoporotic and fragility fractures. Forty percent of subjects were smokers (n = 1256), but only 4% declared to assume regularly alcoholic drinks. About 17.5% (n = 537) did not eat dairy products and 9.2% of people (n = 285) was not practicing any kind of physical activity. Sun exposure was extremely scarce (< 10 min/day) in 805 subjects (26.1%). The female gender, age, previous fractures and familiar history of osteoporosus were associated to a higher probability of being osteoporotic (P < 0.05). FRAX value will be computed for each patient, and the results of this 2012 extension of the FIRMO study will be pooled together with those achieved in the previous screening campaign on 7000 subjects.

Conclusion(s)
The study has confirmed that about 20% of Italian women aged 50-59 is osteoporotic, as determined in the ESopo study carried out in year 2000.

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PP358
Osteoporosis and ‘fragility fractures’ in 110 centenarians living at the nursing home of Milan
Ivana Santì1, Monica Gianotto1, Valentina Guercio2, Francesco Cetta2 & Massimo Monti2
1ASP 1MMeS Pio Albergo Trivulzio, Milan, Italy; 2Department of Surgery University, Siena, Italy.

Osteoporosis and fragility fractures correlated, are a major clinical problem in older women and men and a major public health problem worldwide. As the population ages, the incidence of osteoporotic fractures is increasing. These fractures are associated with higher health care costs, physical disability, impaired quality of life, and increased mortality.

Aim
Evaluation of the frequency, type and age of onset of fracture in 110 centenarians (≥ 98 years) living at Nursing Home Pio Albergo Trivulzio of Milan. We studied retrospectively (from 1995 to 2012) demographic and physiopathologic characteristics in 110 subjects (5 males, 105 females; 98–109 years).

Results
92 patients had fractures, while 18 of them never had any fragility fractures. Thirty-one had a single fracture (26 femurs, 2 humerus, 1 pelvis, and 1 knee, 1 vertebra), while 61 multiple. The most frequent fracture sites were the following: femur in 57 cases (52%), both femurs in 7 cases (8%), vertebras in 34 cases (31%), pelvis in 10 cases (12%), humerus in 13 cases (12%), tibia 6 (5%), humerus and femur in 5 cases (4%), ribs 5, knees 4 (4%), wrists 3 (4%), foot 3, clavicle 3, elbow, fingers, radius, 2 (2%) each, other (shoulder, malleolus) 1 (1%) each. 94% cases multiple vertebral fractures were observed. Within the group of patients who had severe osteoporosis with multiple fragility fractures it is to mention the case of a female patient, with a pelvic fracture at 91 years, a right shoulder fracture at 92, a fracture of the left femur at 93 and one of the left elbow at 99 years of age. Another female patient had a left femur fracture at 89 years, a left humerus fracture at 90 and a fracture of the right femur at 91, followed by a vertebral fracture of D12.

Conclusions
Our data show high prevalence of osteoporosis associated with fragility fractures (84%) and of severe osteoporosis associated with multiple fragility fractures (56%). Age is a risk factor of great importance for osteoporosis fractures and it is independent from mineral bone density. The results of our study show the importance of primary and secondary prevention, independently from age. In the ageing population contest, prevention and treatment of osteoporosis is a major public health concern.

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PP359
Ten years of increasing hip fractures incidence in Italy but first good news from the Analysis of National Hospitalizations Records 2000–2009
Prisco Piscitelli1, Maurizio Feola1, Cecilia Rao1, Monica Celi1, Eleonora Piccirilli2, Elena Gasbarra2, Simone Patrizi1, Giovanni Iolascon3, Maria Luisa Brandi1 & Umberto Tarantino1
1Euro Mediterranean Scientiﬁc Bio-Medical Institute, ISBEM Research Centre, Brindisi, Italy; 2Division of Orthopaedics and Traumatology, Tor Vergata Foundation University Hospital, University of Rome Tor Vergata, Rome, Italy; 3Division of Orthopaedics and Rehabilitative Medicine, Second University of Naples, Naples, Italy; 4Department of Surgery and Translational Medicine, University of Florence, Florence, Italy.

Objectives
We aimed to evaluate hospitalization rate of femoral neck fractures in the elderly Italian population over ten years.

Methods
We analyzed national hospitalizations records collected at central level by Ministry of Health from 2000 to 2009. Age- and sex-specific rates of fractures occurred at femoral neck in people ≥ 65 years old. We performed a sub-analysis over a 3-year period (2007–2009), presenting data per five-year age groups, in order to evaluate the incidence of the hip fracture in the oldest population.

Results
We estimated a total of 839 008 hospitalizations due to femoral neck fractures between 2000 and 2009 in people ≥ 65, with an overall increase of 29.8% over 10 years. The incidence per 10000 inhabitants remarkably increased in people ≥ 75, passing from 158.5 to 166.8 (+ 5.2%) and from 72.6 to 77.5 (+ 6.8%) over the ten-year period in women and men, respectively. The oldest age group (people > 85 years old) accounted only for more than 42% of total hospital admissions in 2009 (n = 39 000), despite representing 2.5% of the Italian population. Particularly, women aged ≥ 85 accounted for 30.8% of total fractures, although they represented only 1.8% of the general population. The results of this analysis indicate that femoral neck fractures progressively increased from 2000 to 2009, but a reduction can be observed for the first time in the number of fractures suffered by women ≤ 75 (– 8.5%, between 2004 and 2009).

Conclusion
Hospitalizations for hip fractures in Italy are continuously increasing, although women aged 65–74 years old start showing a decreasing trend.

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PP360
Osteoporosis risk factor correlation with BMD in Latvia
Inese Pavlina1, 2, Inga Tuma1, 2, Inga Adamina1, 2, Ilze Daukste1, 2, Sandra Jaundzeikare1, 6, Dains Kaneps1, 6, Ingrida Kaze1, 6, Agita Medne1, 6 & Signe Zelca1, 6
1Riga East Clinical University Hospital, Riga, Latvia; 2Pauls Stradiņa Clinical University Hospital, Riga, Latvia; 3Riga 2nd Hospital, Riga, Latvia; 4Health Centre 4, Riga, Latvia; 5Latvian Maritime Medicine Center, Riga, Latvia; 6Latvian Osteoporosis and Bone Metabolism Diseases Association, Riga, Latvia.

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Objective(s)
To assess osteoporosis risk factors for postmenopausal women and correlation with bone mineral density (BMD) in Latvia.

Material and methods
A national cross-sectional study conducted in Latvia about osteoporosis risk factors in postmenopausal women and BMD determination with DXA. 1,598 women who had a DXA scan visit, took part in a study (May–October 2012). The women filled out a questionnaire with 25 multiple choice questions on osteodensitometry, calcium and vitamin D usage, smoking, physical exercises, glucocorticosteroids use, and anti-osteoporosis medications. On the basis of DXA examination, the physicians then filled out BMD results.

Results
The average age of the patients was 65.6 ± 9.0 years and the body weight was 71.9 ± 13.7 kg; the height was 159 ± 6.3 cm; the menopause recorded from 49.3 ± 4.6 years, 63% had previously done DXA. Osteoporosis previously diagnosed in 41.9%. Previous fractures due to bone fragility recorded in 38.6%, 26.8% had a family history of fractures. Calcium used 60.7% of all the patients and 36.2% used vitamin D as dietary supplements. 8.2% used alcohol less than once a month. 13.7% exercised daily. 59.5% had no physical activities. 7.4% of the patients took glucocorticosteroids of which 69.6% had been taking the medication for more than three months. We obtained a statistically correlation that as the age increased, BMD decreased in the lumbar spine as well as in left and right hip (P < 0.001 for all sites). We also found that if the body weight decreases, BMD in the lumbar spine and left and right hip also decreases (P < 0.001 for all sites). Another correlation concerns the menopause and BMD. The earliest menopause and the lower BMD was in the lumbar spine (P < 0.001 for all sites).

Conclusion(s)
This study suggests that osteoporosis not sufficiently diagnosed and undertreated in Latvia. Insufficient attention paid to osteoporosis risk factors.

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PP361
Referral rate of Lebanese males for osteodensitometry
Yasser Yaghi1,2, Ghassan Maaloul1, Ahmad Ghazzawi1 & Kinda Yaghi3
1Hamoud Hospital UMC, Saida, Lebanon; 2Bellevue Medical Center UMC, Beirut, Lebanon; 3Lebanese welfare Association for the Handicapped, Sarafand, Lebanon; 4Beirut Arab University, Beirut, Lebanon.

Introduction and objectives
Male osteoporosis in the Arab World and the Middle East should be considered a major public health issue, and a problem in clinical medicine which deserves adequate attention similar to post-menopausal osteoporosis. There is a great lack of awareness among men about osteoporosis, and treatment is not as well codified as in women.

The aim of this study was to evaluate referrals of male patients to Osteodensitometry Unit in a University Hospital in Southern Lebanon.

Materials and methods
All records of patients (7002) referred for dual X-Ray absorptiometry over a period of 14 years (1997–2010) were reviewed and assessed.

Age, height, weight, risk factors, reason for submission to DXA study and BMD results of spine (L1–L4), Femur (neck and total) and forearm (33%) were documented on paper forms/questionnaires as well as on SPSS-17 Software Program.

Results
The total male referral made up 4.6% (321/7002) for the period 1997–2010. Referral rate did not increase over years.

Mean age of patients was 66.4 years with a mean BMI 27.03.

The most common reason for performing the DXA study was check up (48.6%) and bone pain and myalgia in 43.9%.

Mean bone densities of lumbar spine (L1–L4), femur (total), femur (neck), and forearm (33%) were indicative of osteopenia.

Conclusion
Our study showed a low referral rate of males for DXA study and this suggests that osteoporosis is still viewed as a disease of females.

Mean low bone densities in males are to be seriously considered.

We call for greater attention to be paid to the risk factors in males before the admission for a fracture. So male osteoporosis would be timely diagnosed and timely treated.

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PP362
Assessment of osteoporosis knowledge among Lebanese physicians
Yasser Yaghi1,2, Fatima El Hobb3, Youssef Mousse3, Kinda Yaghi3 & Nancy Maan
1Bone and Joint Decade, Saida, Lebanon; 2Lebanese Welfare Association for the Handicapped, Sarafand, Lebanon; 3Beirut Arab University, Beirut, Lebanon.

Aims and background
Osteoporosis is a growing public health problem in developing countries. Awareness can lead to reduction in the incidence of the disease and consequently the fragility fractures.

Osteoporosis knowledge is an important contributor to improving management and treatment of patients. The aim of this study was to measure osteoporosis knowledge among Lebanese physicians.

Subjects and methods
Representative random samples of Lebanese physicians in two referral health centers in Lebanon were asked to answer 30-item standardized questionnaire addressing their knowledge about osteoporosis. The response time was assumed to take 10 min. The questionnaire distributed covered 15 specialties working in both centers. Cut off points were applied and assessment based on questionnaires ranged from poor to very good.

Results
Answers were received from 102 physicians, 83 males and 19 females, mean age was 34.91 years, and mean time elapsed since graduation was 10.59 years.

Lebanese physicians appeared well informed about general knowledge, risk factors and prevention strategies of osteoporosis. 80% of them considered that veiled ladies are more prone to have vitamin D insufficiency and 65% of them knew about VDR and its importance. Lebanese doctors considered vitamin D (95%), physical activity and sun exposure (92%) as crucial issues in preventing osteoporosis. Most difficult questions appeared to be those concerning different kinds of diets and its impact on bone health. Whereas 44% of responders had limited knowledge about PTH treatment regimen and considered nasal calcitonin as first line of treatment. Thirty percent never hear about strontium ranelate but 80% of them had considerable knowledge of bisphosphonates.

Conclusion
Lebanese physicians have considerable awareness of the importance of preventing osteoporosis. They were active in identifying groups at risk but our findings stress the need to extend the knowledge of physicians regarding different treatment regimens and clinical methods. Proper dissemination of osteoporosis treatment knowledge may further enhance evidence based treatment for the disease.

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PP363
Adolescents’ lifestyle and bone health: what about the young bones in Norway?
Anne Winther1, Elaine Dennison2,3, Luai Awad Ahmed1, Anne-Sofie Furberg4, Guri Grimnes1,4, Rolf Jorde1, Clara Gram Gjesdal5 & Nina Emuns1
1University of Tromsø, Tromsø, Norway; 2MRC LifeCourse Epidemiology Unit, Southampton, UK; 3Victoria University, Wellington, New Zealand; 4University Hospital of North Norway, Tromsø, Norway; 5Haukeland University Hospital, Bergen, Norway.

Introduction
Norway has one of the highest reported incidences of osteoporotic fracture. Since, bone mineral density (BMD) is a strong predictor of future fracture risk, high peak bone mass achievement is essential. This study is the first to examine BMD in a population-based study including Norwegian adolescents. Here we compare the measured BMD with international reference ranges and explore predictors of BMD in this population.

Methods
In 2010–2011 all first year comprehensive school students (1100 participants, aged 16–17 years) in the Tromsø region were invited to participate in the Fit Futures study, an expansion of the Tromsø study. Altogether 508 girls and 530 boys attended the survey providing an attendance rate >90%. BMD at the total hip and femoral neck was measured as g/cm² by DEXA (GE Lunar prodigy, Lunar Corporation, Madison, WI, USA). Lifestyle variables were collected by self-administered questionnaires and interviews.

Results
The mean BMD was 1.060 (s.d. 0.124) g/cm² at the total hip and 1.066 (s.d. 0.123) g/cm² at the femoral neck in girls, in boys 1.116 (s.d. 0.147) and 1.103 (s.d. 0.123) g/cm². Differences were significant between boys and girls.
Osteoporosis an independent predictor of mortality in hip fracture patients

Andreas P Diamantopoulos1,2, Mari Hoff2, Marc Hochberg3 & Glenn Haugeberg1,2
1Hospital of Southern Norway Trust, Kristiansand, Norway; 2NTNU University, Trondheim, Norway; 3University of Maryland, Baltimore, Maryland, USA.

Introduction
Mortality after hip fracture is increased. However, only a few studies have explored for predictors beyond gender and age. Thus our aim was to study risk factors associated with increased mortality in hip fracture patients.

Methods
Hip fracture patients (> 50 years) admitted to a county hospital in 2004–2005 were consecutively invited for assessment at the hospital osteoporosis centre. A broad spectre of data was collected. Standardized bone density measurements at lumbar spine L2-4 and hip (femoral neck and total hip) were performed using DXA (Lunar Prodigy). DXA osteoporosis was defined as T-score ≤ -2.5 at lumbar spine and/or hip.

Results
Hip fracture patients (129 men and 303 women) were identified and 296 (85 men and 211 women); (mean age 80.7 (S.D. 9.1) were assessed at the Osteoporosis center. DXA osteoporosis was found in 218 (74.1%) patients (53 males, 165 females). In bivariate analysis, variables significantly associated (P<0.05) with increased mortality included no snow, indoor activity, osteoporosis, restricted mobility, stroke, dementia, inability to walk outdoors, visual impairment, older age > 80 years and male gender. In the table variables independently associated with increased mortality is displayed. Table 1

<table>
<thead>
<tr>
<th>Gender (ref group female)</th>
<th>OR</th>
<th>95% CI for OR</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5.8</td>
<td>2.8–11.9</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Age (ref group &lt;80 years)</td>
<td>3.0</td>
<td>1.7–5.4</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Dementia (ref group no.)</td>
<td>5.0</td>
<td>1.6–16.2</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Osteoporosis (ref group no.)</td>
<td>2.2</td>
<td>1.1–4.2</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Conclusion
Osteoporosis was found to be an independent predictor of mortality. This is in particular interesting as treatment with bisphosphonates has been shown not only to reduce fractures but mortality too.

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PP365
Effect of sitagliptin on bone growth and remodeling in non-ovariectomized and ovariectomized rats

Maria Pytlík1, Joanna Folwarczna1, Justyna Françzec-Sokół1, Adam Smyla2, Patyczka Węsioł1 & Maria Zych2
1Department of Pharmacology, Medical University of Silesia, Katowice, Sosnowiec, Poland; 2Department of Pharmacognosy and Phytochemistry, Medical University of Silesia, Katowice, Sosnowiec, Poland.

Type 2 diabetes and osteoporosis often occur together in postmenopausal women. Sitagliptin is a new drug used in the therapy of type 2 diabetes; it affects incretin system as a result of inhibition of dipeptidyl peptide-4. So, far, the impact of the drug on bone remodeling processes is unknown. The aim of this study was to investigate the effect of sitagliptin on bone growth and remodeling in non-ovariectomized and ovariectomized rats.

The experiments were carried out on 3-month-old female Wistar rats divided into 4 groups (n=9–10 per group): I – non-ovariectomized control rats, II – ovariectomized control rats, III – non-ovariectomized rats receiving sitagliptin (15 mg/kg p.o.), IV – ovariectomized rats receiving sitagliptin (15 mg/kg p.o.). Bilateral ovariectomy was performed 7 days before the start of the experiment, under ketamine-xylazine anesthesia. Sitagliptin was administered once daily for 28 days. Bone growth and remodeling after the use of sitagliptin was assessed based on macrometric parameters (the length and diameter in the mid-length of the tibia and femur), and histomorphometric parameters including measurements of the tibial and femoral diaphysis (endosteal and periosteal transverse growth, transverse cross-section area of the cortical bone and marrow cavity) and femoral epiphysis and metaphysis (width of trabeculae and epiphyseal cartilage). Bone mass, mineral mass, calcium and phosphorus content, as well as serum estradiol, ostecalcin and RatLaps levels were also studied.

In ovariectomized rats, estrogen deficiency caused increased bone remodeling with intensification of bone resorption and impairment of mineralization. Sitagliptin favorably affected the skeletal system of ovariectomized rats, inducing intensification of bone formation and mineralization, and inhibition of bone resorption. In non-ovariectomized rats, sitagliptin only slightly intensified bone formation.

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PP366
Caffeine at a moderate dose favorably affected bone mechanical properties in ovariectomized rats

Joanna Folwarczna1, Mari Pytlík1, Maria Zych2, Urszula Cegiela1, Ilona Kaczmarczyk-Sedlak2, Barbara Nowińska1 & Leszek Sliwiński1
1Department of Pharmacology, Medical University of Silesia, Katowice, Sosnowiec, Poland; 2Department of Pharmacognosy and Phytochemistry, Medical University of Silesia, Katowice, Sosnowiec, Poland.

Caffeine, a methylxanthine present in coffee, tea, cocoa-cola and other beverages, is considered to be responsible for an increased risk of osteoporosis in coffee drinkers, however the data from human and experimental studies are inconsistent. The aim of this study was to investigate the effects of a moderate dose of caffeine on the skeletal system of rats with normal and decreased estrogen level (developing osteoporosis due to estrogen deficiency).

Caffeine (20 mg/kg p.o. daily) was administered for 4 weeks to non-ovariectomized and ovariectomized rats, inducing intensification of bone formation and mineralization, and inhibition of bone resorption. In non-ovariectomized rats, sitagliptin only slightly intensified bone formation.

Caffeine favorably affected the skeletal system of the ovariectomized rats, slightly inhibiting development of bone changes induced by estrogen deficiency (increasing bone mineralization, and improving the strength and structure of cancellous bone). Moreover, caffeine favorably affected mechanical properties of compact bone. There were no significant effects of caffeine in rats with normal estrogen level, however two-way ANOVA revealed significant main effects of caffeine, indicating increased bone strength regardless of the estrogen status. It may be speculated that the favorable caffeine effects were mediated via blockade of A1 adenosine receptors, known to be involved in regulation of bone resorption.

In conclusion, results of this study indicate that moderate caffeine intake may be safe and even exert some beneficial effects on the skeletal system.

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PP367
Estimated glomerular filtration rate is associated with bone fragility in the elderly
Maria João Gonçalves1, Ana Rodrigues1, Joana Caetano-Lopes1, Emília Raquel1, Ana Lopes1, Bruno Vidal1, Ana Cataria Val1, Marco Sarmento1, Maria Fátima Vaz1, Jacinto Monteiro1, João Eurico Fonseca1 & Helena Canhão1
1Rheumatology Research Unit, Instituto de Medicina Molecular, Lisbon, Portugal; 2Orthopaedics Department, Santa Maria Hospital, Lisbon, Portugal; 3Mechanics Engineering Department, Instituto Superior Técnico, Lisbon, Portugal.

Introduction
Osteoporosis is frequently associated with renal disease, namely the bone metabolism disturbances caused by secondary hyperparathyroidism of chronic kidney disease (CKD). The increased risk of fragility fractures is well demonstrated in patients with end-stage renal disease (ESRD). There is recent evidence that bone pathological changes start early in the course of CKD. Our aim is to evaluate whether chronic renal disease, before ESRD, is associated with bone fragility. Bone fragility was assessed considering history of fragility fracture events, 10-year risk for major osteoporotic fractures and hip fractures (FRAX), biochemical bone turnover markers (PINP and CTX) and mechanical testing to determine bone stiffness.

Design
We studied patients admitted for total hip replacement surgery. They were asked for clinical data and blood samples. Blood biomechanical studies were performed and a bone cylinder was drilled from their femoral epiphyses. Glomerular filtration rate (GFR) was estimated using Cockcroft-Gault formula; we excluded patients with obvious limitations to the application of the formula. We also excluded from the analysis patients with terminal renal impairment, with eGFR ≤15 ml/min or history of renal replacement therapy.

Results
We included 111 patients. Mean age 74.31±10.03, 70% of subjects were female, 98% Caucasian. Fracture history had an inverted relation with eGFR (P=0.023). The clinical score FRAX was inverted related with eGFR (for both risks P=0.000). The biomarker CTX also showed an inverted relation with eGFR (P=0.056) and bone stiffness (coefficient 2.42, P=0.073) showed a trend for association with eGFR. All analysis were adjusted for age and gender.

Conclusion
Renal impairment in early stages, measured by eGFR, was associated with increased bone fragility assessed by fracture events, FRAX and bone turnover and biomechanics biomarkers, after adjustment to age and sex.

DOI: 10.1530/boneabs.1.PP367

A comparative analysis of risk fracture evaluation by Portuguese and Spanish FRAX tool was made on 1444 subjects observed by a rheumatologist. Mean age was 57.98 years old (s.d. 15.34), 67.3% were women and 95% were Caucasians. In the total sample the difference between the mean MOFR assessed by the Portuguese algorithm (4.74 (s.d. 5.9)) and the Spanish (4.4 (s.d. 5.25)) was statistically significant (P=0.000). The same was observed to mean HFR difference between Portuguese algorithm (1.8 (s.d. 3.96)) and the Spanish one (1.6 (s.d. 3.77)) (P=0.000).

The results from Portuguese and Spanish FRAX® tool were statistically significant in women for MOFR and for both sexes for HFR (Table 1).

| Major Osteoporotic risk | | Hip Fracture risk | |
|-------------------------|-----------------|-----------------|
|                        | Portuguese tool | Spanish tool | P value | Portuguese tool | Spanish tool | P value |
| Female                  | 5.56            | 5.09           | 0.0000   | 2.09           | 1.84           | 0.0000   |
| Male                    | 3.07            | 2.99           | 0.252    | 1.17           | 1.08           | 0.0063   |

Conclusions:
Significant statistically differences were observed in 10 year major and hip fracture risk when the Portuguese or the Spanish FRAX algorithms were applied. Yet the clinical impact of these differences is unknown, it suggests that the FRAX tool should be validated and selected for the specific population.

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PP369
Changes in bone mineral density of the both proximal femur after total knee arthroplasty
Kwang Kyoun Kim
Konyoung University Hospital, Daejeon, Republic of Korea.

Introduction
The authors experienced a ipsilateral femoral stress fracture after total knee arthroplasty (TKR). Decreased bone mineral density (BMD) after TKR have been reported as cause. However, reports regarding changes of BMD in the proximal hip after TKR have been rare. Therefore, first, we studied the question of whether TKR can effect change of proximal hip BMD? Second, if so, does TKR have different effects on BMD of the operative and non-operative sides?

Materials and methods
Forty-eight patients scheduled to undergo unilateral TKA because of primary knee OA were included in this study, conducted at a medical center, between October 2006 and October 2009. In these 48 patients, 96 hips were evaluated. Measurement of BMD was performed preoperatively and 1 month, 3 months, 6 months, and 1 year after unilateral TKA.

Results
BMD of both femoral neck areas was significantly lower than preoperative BMD at 1 month and 3 months after TKA. BMD of both trochanter areas was significantly lower than preoperative BMD at 1 month and 3 months after TKA. However, no statistical differences of changes in BMD of femur neck and trochanter were observed between the operative and non-operative sides at each measurement time.

Conclusion
Total knee arthroplasty was found to affect both proximal femurs during the early period after TKA. However, it does not affect the ipsilateral side and contralateral side differently. Therefore, we thought that a temporary decrease in BMD after TKA was not the direct cause of ipsilateral femoral stress fracture.

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**PP370**

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

Heung Yeol Kim1 & Hoon Choi2

1Kosin University, Busan, Republic of Korea; 2Inje University, Seoul, Republic of Korea.

**Aim**

Alendronate enhances bone morphogenetic proteins (BMP)-mediated osteoblast differentiation. A balanced regulation of inhibitors of DNA binding/differentiation (Id) plays an important role in BMP-mediated osteoblast differentiation. However, there are no studies on the possible roles of Id genes in alendronate-induced osteoblast differentiation. This study investigated the effect of alendronate on the expression of Id genes in osteoblast differentiation.

**Methods**

C2C12 cells were treated with alendronate for various concentrations and time periods. For evaluation of alendronate-induced osteoblast differentiation in C2C12 cells, alkaline phosphatase (ALP) activity was measured. The expression of osteoblast differentiation markers such as ALP, type 1 collagen (Col 1), and osteocalcin (OCN), and the expression of Id-1 and Id-2 were measured by RT-PCR. In order to understand the mechanism underlying the regulation of Id genes, the promoter region of the Id-1 gene was identified. Database analysis of the promoter region for Id-1 using known consensus sequences identified several putative response elements, including CCAAT/enhancer-binding protein β (C/EBPβ).

**Results**

Alendronate treatment significantly increased not only ALP activity but also expression of ALP, Col 1, and OCN, Id-1 and Id-2. C/EBPβ and alendronate cooperatively increased the promoter activity and expression of Id-1.

**Conclusion**

C/EBPβ cooperatively increased the promoter activity and expression of Id-1.

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**PP371**

Comparative analysis of vitamin D level with or without osteoporotic spinal compression fracture in Korean elderly patients

Ye-Soo Park1, Hong-Sik Kim1, Dong-Yi Kong1, Ye-Yeon Won2 & Byung-Moon Kang3

1Department of Orthopaedic Surgery, Hanyang University College of Medicine, Guri Hospital, Guri City, Gyeonggi-do, Republic of Korea; 2Department of Orthopaedic Surgery, Ajou University Hospital College of Medicine, Suwon City, Gyeonggi-do, Republic of Korea; 3Department of Obstetrics and Gynecology, Asan Medical Center, Ulsan University College of Medicine, Seoul City, Republic of Korea.

**Introduction**

To compare serum vitamin D levels in elderly patients with or without osteoporotic spinal compression fractures (OSCF) and to evaluate a correlation between serum vitamin D level and several variables such as age, and bone mineral density (BMD), bone turnover markers.

**Methods**

The medical records of 78 patients with OSCF (fracture group) and 84 age-matched control patients who were diagnosed osteoporosis without OSCF (control group) were reviewed. Serum vitamin D levels were compared between the two groups with consideration of age and seasonal variations and compared according to sex and living environment (living and nursing home) in each group. BMD and bone turnover markers (osteocalcin and c-telopeptide) were compared between the two groups. In all subjects (162 patients), the correlation between vitamin D level and age, BMD, and bone turnover markers was evaluated.

**Results**

In the serum 25(OH) vitamin D3 level, 62 patients (78.6%) in fracture group and 46 patients (59%) in control group were insufficient. Both the mean 25(OH) vitamin D3 levels and BMD were significantly lower in the fracture group compared to the control group (P < 0.0001 and < 0.0001 respectively). In particular, there were significant differences of 25(OH) vitamin D3 levels between the two groups in patients in their 60s with consideration of age and in spring and autumn with consideration of seasonal variations. But, 25(OH) vitamin D3 levels were not significantly different in each group according to sex and living environment. 25(OH) vitamin D3 level was negatively correlated with age (r = −0.183, P = 0.02) and positively correlated with BMD (r = 0.251, P = 0.001).

**Conclusions**

Vitamin D level was insufficient in most patients with OSCF and patients with OSCF were found to have significantly lower vitamin D levels than patients without fracture. So, it is important to maintain the optimal range of serum vitamin D level in elderly patients with osteoporosis to prevent OSCF.

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**PP372**

Decreased bone mineral density is associated with coronary atherosclerosis in healthy postmenopausal women

Mihyun Jo1,2, SiHyun Cho1,2, Young Sik Choi1,2, Byung Seok Lee1,2 & Seok Kyo Seo1,2

1Yonsei University College of Medicine, Seoul, Republic of Korea; 2Institute of Women’s Life Medical Science, Seoul, Republic of Korea.

**Objective**

The aim of this study was to assess the association between bone mineral density (BMD) and coronary atherosclerosis in healthy postmenopausal women.

**Methods**

We performed a retrospective review of 252 postmenopausal women who visited the health promotion center for a routine checkup, after excluding participants who had factors affecting BMD and coronary artery disease. BMD of the lumbar spine and proximal femur was evaluated by dual-energy X-ray absorptiometry. Coronary atherosclerosis was assessed by 64-row multidetector computed tomography. Participants were divided into normal bone and osteopenia–osteoporosis groups according to the T-scores of their lumbar spine or femur neck.

**Results**

Participants with osteopenia-osteoporosis had a significantly higher proportion of coronary atherosclerosis than those with normal BMD at the lumbar spine (P = 0.003) and femur neck (P = 0.004). Osteopenia-osteoporosis at the lumbar spine (OR, 2.86; 95% CI, 1.12–7.27) and femur neck (OR, 3.35; 95% CI, 1.07–10.57) was associated with coronary atherosclerosis after controlling age and cardiovascular risk factors.

**Conclusion**

Decreased BMD is associated with coronary atherosclerosis in healthy postmenopausal women, independent of age and cardiovascular risk factors.

**PP373**

Prevalence of vitamin D deficiency and low bone mineral density in healthy Saudi women

Khulood Hussein1,2, Hanan Alkadi1,2, Susan Lanham-New1 & Mohamad Ardawi1,2

1Surrey University, Guildford, UK; 2King Abdulaziz University, Jeddah, Saudi Arabia.

**Introduction and aims**

Vitamin D deficiency is a prevalent disorder in developing countries. Clinical manifestations of the deficiency include musculoskeletal disorders, such as nonspecific muscle pain, poor muscle strength and low bone mineral density (BMD). The aim of this study was to determine the prevalence of vitamin D deficiency and low BMD in healthy Saudi women.

**Methods**

The subjects of this cross-sectional study comprised 449 healthy Saudi women who were randomly recruited from the city of Jeddah through Primary Health Care Centers. Fasting blood samples were collected for assessment of 25(OH)D and bone turnover markers. Lumbar spine and femoral neck BMD were measured using DXA. Vitamin D deficiency was defined as serum 25(OH)D < 50 nmol/l.

**Results and discussion**

The mean age was 43.9 ± 15.9 years and the mean serum 25(OH)D was 28.8 ± 21.8 nmol/l. A total of 80.5% of women studied were vitamin D deficient and 55% exhibited severe vitamin D deficiency (25(OH)D < 25 nmol/l). The mean BMD for lumbar spine and femoral neck was 1.062 ± 0.161 and 0.889 ± 0.137 respectively. Osteopenia was evident in more than one quarter of the women at both sites and 6.5% were osteoporotic. Circulating C-terminal telopeptide of type I collagen (CTX) level correlated significantly with lumbar spine (r = −0.09, P = 0.04) while a trend was found with femoral neck BMD (r = −0.30, P = 0.09).

**Conclusion**

These data suggest that low vitamin D status is associated with low bone mass in this healthy population. Further investigations are currently underway to explore concomitant effects of other lifestyle factors on bone health in these women.

DOI: 10.1530/boneabs.1.PP373
**PP374**

**Effect of two types of bariatric surgery (gastrojejunal bypass and sleeve gastropasty) on gene expression of bone remodeling markers in Goto-Kakizaki rats**

José-Luis Pérez-Castillón, José-Antonio Riancho, Daniel DeLuís, Manuel González-Sagrado, Marta Ruiz-Mambrilla, María Domingo-Anfres, Rosa Conde, David Primo, Antonio Dueñas-Laita.

Hospital Rio Horta, Valladolid, Spain; Hospital Marques de Valdecilla, Retiefe, Santander, Spain.

**Background**

Surgical treatment of type 2 diabetes, specially in obese patients, has provided good results in the control of blood glucose and Hb1Ac although its effect on bone health is not clear. The aim of this study was to evaluate gene expression of bone remodelling markers in type 2 diabetic Goto-Kakizaki (GK) non-obese rats after gastrojejunal bypass and sleeve gastropasty, and their relationship with hormonal parameters.

**Materials and methods**

We designed an experimental study in GK rats non operated, rats with gastrojejunal bypass and sleeve gastropasty. Gene expression of markers of bone remodeling was measured. Levels of insulin, leptin, and glucagon-like peptide-1 (GLP-1) were determined.

**Results**

GK rats had decreased levels of osteocalcine expression, a marker of bone formation, compared with Wistar rats. Gene expression of markers of bone remodeling in GK rats was similar in the three groups studied (control, gastrojejunal bypass, and gastropasty) although there was a trend to decreased RANKL in gastropasty group. Significant differences in the osteocalcin: RANKL ratio were observed between controls and gastrojejunal bypass rats compared with gastropasty rats. The behaviour of gastrointestinal hormones was antagonistic between both techniques as expected (GLP-1 gastrojejunal bypass 1.54 ± 0.24 ng/ml vs GLP-1 gastropasty 0.67 ± 0.09, \( P = 0.0001 \); leptin gastropasty 1178 ± 0.474 pg/ml vs leptin gastropasty 7391 ± 4054 pg/ml, \( P = 0.002 \)).

There was an induction in the latency of the secretory process associated with an increase in gastrectomized rats. In gastrectomized rats there was an inverse relationship between leptin and RANKL (\( r = -0.771 \), \( P = 0.072 \)).

**Conclusion**

Our results show a more favourable profile of sleeve gastropasty bone on remodeling. There was a trend to an increase in the expression of the osteocalcin gene, which is probably mediated by increased expression of leptin that inhibits bone remodeling. Levels of insulin, leptin, and glucagon-like peptide-1 (GLP-1) were determined.

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**PP375**

**25-Hydroxyvitamin D values in liver transplant candidates**

Ana Monreal, Pilar Peris, Andrea Cuervo, Africa Muxí, Laia Gifré & Nuria Guaitabens.

Hospital Clinic, Rheumatology Service, Barcelona, Spain; Hospital Clinic, Nuclear Medicine Service, Barcelona, Spain; CIBERh, Barcelona, Spain.

**Introduction**

Liver transplant candidates have bone and mineral metabolism disorders that may influence the development of fractures after liver transplantation (LT).

**Objective**

To analyze the levels of 25-hydroxyvitamin D (25-OH-D) in patient candidates for LT and the factors associated with vitamin D deficiency.

**Methods**

Between January 2010 and May 2012, 116 liver transplant candidates (85 male and 31 female patients) were included in the study. In all patients, we analyzed the clinical and laboratory characteristics (including serum 25-OH-D and PTH values), densitometry of the lumbar spine and femur (DXA) and spinal X-rays in lateral projection.

**Results**

In liver transplant candidates 25-OH-D mean levels were 14.1 ± 10.3 ng/ml. 25-OH-D levels were < 10 ng/ml in 48%, < 20 ng/ml in 81.9% and < 30 in 93% of patients. Nevertheless only 9% of patients were supplemented with calcium and/or vitamin D. 25-OH-D values were related with the severity of the liver disease. Thus, child A patients showed higher values of 25-OH-D than child B and C patients (A: 18.5 ± 12; B: 12.65 ± 8.9; C: 9.36 ± 5.2 ng/ml; \( P < 0.001 \)).

In control patients 25-OH-D levels were inferior to 10 ng/ml in 25% of child A, 59% of child B and 68% of child C patients (\( P < 0.001 \)). Moreover, patients with vitamin D deficiency (25-OH-D < 10 ng/ml) showed lower values of lumbar Z-score (\(< 10 \text{ ng/ml: } -1.35 \pm 1.5 \text{ vs } > 10 \text{ ng/ml: } -0.6 \pm 1.5; \ P < 0.05 \) ) femoral neck Z-score (<10 ng/ml: −0.5 + 1.2 vs > 10 ng/ml: −0.1 + 1; \( P < 0.05 \) ) and total femur Z-score (< 10 ng/ml: −0.7 + 1.2 vs > 10 ng/ml: −0.2 + 1; \( P < 0.05 \) ).

**Conclusions**

Vitamin D deficiency is frequent among liver transplant candidates. However, calcium and/or vitamin D supplementation is uncommon. Vitamin D deficiency was most frequent in the most severe liver disease patients. Moreover, patients with vitamin D deficiency had low bone mass.

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**PP376**

**Ursodeoxycholic acid protects osteoblastic cells from bilirubin and lithocholic acid induced apoptosis**

Silvia Ruiz-Gaspa, Marta Dubreuil, Andrés Combalia, Pilar Peris, Ana Monreal, Albert Parés & Nuria Guaitabens.

1Metabolic Bone Diseases, CIBERh, Hospital Clinic, Barcelona, Spain; CIBERh, Hospital Clinic, Barcelona, Spain; 2Liver Unit, CIBERh, Hospital Clinic, Barcelona, Spain.

**Introduction**

Osteoporosis is a common complication in patients with chronic cholestasis, usually characterized by reduced bone formation. Ursodeoxycholic acid (UDCA) improves differentiation and mineralization and counters the damaging effects of bilirubin and lithocholic acid (LCA) in osteoblastic cells. Moreover, UDCA decreases apoptosis in a number of cell lines, but this antiapoptotic effect has not been investigated in bone cells.

**Aims**

To assess the antiapoptotic effects of UDCA on osteoblastic cells.

**Material and methods**

Primary human osteoblasts (hOB) and Saos-2 cell cultures were incubated with UDCA (100 µM), with and without bilirubin (50 µM) and LCA (10 µM) – the highest concentrations with no major effects on osteoblast viability –, and camptothecin (0.5 µM) as a proapoptotic agent. Apoptosis was assessed by DNA fragmentation, flow cytometry analysis (annexin V-FITC labeling), and gene expression of Bcl-2-associated X protein (BAX) and BCL2-like 1 protein (BCL2L1) as antiapoptotic and proapoptotic genes respectively.

**Results**

LCA and bilirubin resulted in a significant (\( P < 0.01 \) ) and 5.7-fold induction of DNA fragmentation, respectively, with parallel effects in the flow cytometry analysis in Saos-2 cells. Similar results were found in hOB. UDCA alone had no consequences on apoptosis, but UDCA significantly (\( P < 0.01 \) ) decreased the apoptotic effects of LCA and bilirubin by 71 and 75%, respectively, as observed by DNA fragmentation in Saos-2 cells, and with lower effects in hOB. These results were found with flow cytometry as well. Moreover, UDCA neutralized the effects of LCA and bilirubin on the up-regulated BAX, and on the down-regulated BCL2L1 gene expression.

**Conclusions**

Bilirubin and lithocholic acid stimulate apoptosis in osteoblastic cells. Ursodeoxycholic acid has clear antiapoptotic effects countering the consequences of these two substances increased in cholestasis. These results suggest that ursodeoxycholic acid may have further beneficial effects on bone formation in patients with cholestasis.

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**PP377**

**Effect of glucocorticoid treatment on Wnt signalling antagonists (sclerostin and Dkk-1) and their relationship to bone turnover and bone mass**

Laia Gifré, Pilar Peris, Silvia Ruiz-Gaspa, Ana Monreal, Benet Nomdedeu & Núria Guaitabens.

1Metabolic Bone Diseases Unit, Rheumatology Department, Hospital Clinic, Barcelona, Spain; CIBERh, Hospital Clinic, Barcelona, Spain; 2Hematology Department, Hospital Clinic, Barcelona, Spain.

**Introduction**

Wnt-β-catenin signalling and its antagonists (sclerostin and Dkk-1) play an important role in the regulation of bone mass and osteoblastogenesis. Glucocorticoid therapy (GCCT) is a well known factor related to decreased bone formation and osteoporosis development. Therefore, we analyzed the effect of GCCT on Wnt signalling antagonists (sclerostin and Dkk-1) and their relationship to bone mass and bone turnover.

**Methods**

22 patients (11M/11F, aged 48 ± 20 years) recently initiating GCCT were prospectively included (≥ 7.5 mg/day, ≤ 6 months), excluding patients with...
associated metabolic bone diseases or on antiosteoporotic treatment. Bone turnover markers (bone formation: P1NP, bone AP; bone resorption: sCTx), Wnt antagonists (serum sclerostin and Dkk-1, determined by ELISA, Biomedica Gruppe, Austria) were assessed in all patients (at baseline and 12 months). Bone mineral density (BMD) was performed to assess osteoporosis. The results were compared with 20 healthy controls.

Results

The mean daily GCCT dose was 66±16 mg/day. Idiopathic thrombocytopenic purpura (73%) and hemolytic anemia (14%) were the most frequently associated conditions. Patients on GCCT showed a significant decrease in bone formation markers vs controls (P1NP: 19.6±9.4 vs 44.1±8.9 ng/ml, P=0.001) and increased bone resorption (sCTx: 0.59±0.23 vs 0.4±0.17 ng/ml, P=0.049). Patients on GCCT had decreased Dkk-1 compared to controls (31.8±28.1 vs 46.8±15.3 pmol/l, P=0.028) with similar sclerostin values (39.7±21.3 vs 32.9±19.3 pmol/l, P=0.399). 20% had demineralistic osteoporosis. Sclerostin correlated positively with GCCT doses (r=0.505, P=0.016) and lumbar BMD (r=0.554, P=0.008), and negatively with bone AP (r=-0.510, P=0.015). At 12 months, Dkk-1 significantly decreased compared to baseline (16.6±13.8, P=0.02), and sclerostin tended to increase (49.2±12.0, P=0.496).

Conclusion

The effect of GCCT on the serum levels of the Wnt signalling parameters differs depending on the antagonist evaluated. Dkk-1 levels decreased after the initiation of GCCT whereas sclerostin values tended to increase and showed a relationship to the dose of GCC and bone formation parameters.

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PP379

Role of Wnt antagonists (sclerostin and Dkk-1) on bone turnover markers and bone mass, in patients with complete spinal cord injury: preliminary results

Laia Gifre1, Joan Vidal2, Silvia Ruiz-Gaspa2, Enric Portell2, Ana Monegal1, Africa Muxu2, Nuria Gutabens1 & Pilar Peris1,3

1Groupe Metabolic Diseases Unit, Rheumatology Department, Hospital Clinic, Barcelona, Spain; 2Surgical Cord Unit, Neurorehabilitation Institute Guttmann, Badalona, Spain; 3CIBEREHd, Hospital Clinic, Barcelona, Spain; 4Nuclear Medicine Department, Hospital Clinic, Barcelona, Spain.

Spinal cord injury (SCI) has been associated with a marked increase in bone loss. This study analysed the effect of Wnt signalling antagonists (sclerostin and Dkk-1) and their relationship with bone turnover markers and BMD evolution in patients with a recent SCI.

Methods

Patients with a recent complete motor SCI (AIS A or B); (<6 months) were prospectively included. Bone turnover markers (bone formation: P1NP, bone AP; bone resorption: sCTx), Wnt antagonists (serum sclerostin and Dkk-1, determined by ELISA, Biomedica Gruppe, Austria) and bone mineral density (BMD) were assessed in all patients at baseline and at 6 months. The results were compared with 23 healthy individuals of similar age and sex.

Results

25 men with a mean age of 37±15 years were included at 101±33 days of SCI onset (AIS 24A, 1B). 56% had paraplegia. Thirteen patients were assessed at 6 months of follow-up. Patients with SCI showed a significant increase in bone turnover markers compared to controls (P1NP 194±87 vs 49±15 ng/ml, P<0.001; sCTx 1.39±0.47 vs 0.48±0.21 ng/ml, P<0.001) and decreased levels of Dkk-1 163.5±32.8 vs 39.9±15.7 pmol/l, P=0.003). No differences in sclerostin levels were observed vs controls (39.7±15.4 vs 35.9±20.5 pmol/l, P=ns). 60% had a low BMD. At 6 months, sclerostin levels increased significantly (40%, P=0.013), bone turnover markers decreased (P1NP ~37%, P=0.003 and sCTx ~32%, P=0.007) and BMD decreased about 11% at total femur (P=0.002) compared to baseline. Dkk-1 levels also significantly decreased (~35%, P=0.041). Changes in Dkk-1 levels were positively correlated with changes in total femur BMD (r=0.6, P=0.05), while changes in sclerostin were negatively correlated with bone AP change (r=-0.668, P=0.025).

Conclusions

Patients with complete SCI have a marked increase in bone turnover markers and early bone loss over 10% at femur. Wnt signalling antagonists seem to be related to bone loss in acute SCI.

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PP380

Hip fracture trends in Denmark 1980–2010 with age-period-cohort-effects

Bjorn Rosengren1,2, Jonas Bjork4, Cyrus Cooper5 & Bo Abrahamsen1,2

1Gentofte Hospital, Hellerup, Denmark; 2University of Southern Denmark, Odense, Denmark; 3Clinical and Molecular Osteoporosis Research Unit, Department of Orthopedics and Clinical Sciences, Skåne University Hospital, Lund University, Malmö, Sweden; 4Unit for Medical Statistics and Epidemiology at R and D Centre Skåne, Skåne University Hospital, Lund, Sweden; 5MRC Lifecourse Epidemiology Unit, University of Southampton, Southampton, UK.

The origin of the recent levelling-off in hip fracture incidence in several settings is unknown.

Methods

Using Danish national inpatient data for individuals aged ≥50 years during 1980–2010, we examined annual number and incidence of hip fractures and age, period, and cohort effects by log-likelihood estimates in Poisson regression models. Age adjustment was done by direct standardization, time-trend analysis by linear regression, and identification of breakpoints in linear trends by join-point analyses.

Results

There were 240 121 hip fractures, 74% in women. Before 1993, the annual age-standardized hip fracture incidence increased (2.8% per year (95% CI 2.3 to 3.5%)), and the annual number of hip fractures increased (4.4% per year (3.8 to 5.0%)). After 1993, the age-standardized hip fracture incidence decreased (~1.2% per year (~1.5 ~0.9%)) and the number of hip fractures was stable (~0.3% per year (~0.7 ~0.6%)).

The combined period+cohort effects were more marked in men, with an incidence rate ratio (IRR) ranging from 0.4 to 1.2 depending on 6-year birth cohort and 0.7 to 1.1 depending on 3-year period. In women the corresponding results were IRR 0.8 to 1.4 and 0.9 to 1.2.

Analyses of specific cohort effects (estimated by deviations from underlying linear trends in cohort) in the full APC-model showed higher risk in men born
PP381
Impact of hip fracture on mortality and life expectancy
Karl Michaelsson1, Peter Nordström2, Anna Nordström3, Hans Garmo4, Lisa Byberg5, Nancy Pedersen1, & Håkan Melhus6
1Section of Orthopaedics, Department of Surgical Sciences, Uppsala University, Uppsala, Sweden; 2Section of Geriatric Medicine, Department of Community Medicine and Rehabilitation, Umeå University, Umeå, Sweden; 3Section of Rehabilitation Medicine, Department of Community Medicine and Rehabilitation, Umeå University, Umeå, Sweden; 4Division of Cancer Studies, School of Medicine, King’s College, London, UK; 5Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden; 6Section of Clinical Pharmacology, Department of Medical Sciences, Uppsala University, Uppsala, Sweden.

Several studies have shown a higher mortality after hip fracture but the reasons and the duration of the excess risk is not well understood. We aimed to determine whether there exists a higher mortality after hip fracture when controlling for genetic constitution, environmental and lifestyle risk factors and comorbidity. All 286 identical Swedish twin pairs discordant for hip fracture (1972–2010) were identified by use of the National patient register and the Swedish twin registry, the largest in the world. Comorbidity and lifestyle information was retrieved by registers and questionnaire information by surveys within the twin registry done before the hip fracture event. We used intrapair Cox’s regression to compute multivariable adjusted hazard ratios (HRs) for death. During 3877 person-years of follow-up, 244 of 572 twins died. Through the first year after hip fracture, the rate of death increased fourfold in women (HR 4.46; 95% CI 1.47–13.56) and sixfold in men (HR 6.51; 95% CI 1.37–30.97). The higher rate in women only persisted during the first year after hip fracture (HR after 1 year 0.97; 95% CI 0.64–1.48), whereas the higher risk in men lost 5 years with a successive decline in risk during this 5-year period. On average, the hip fracture contributed to 0.9 years of life lost in women (95% CI 0.1–1.7) and 2.7 years in men (95% CI 1.7–3.7). The potential years of life lost due to the hip fracture was especially pronounced in older men (>75 years), with an average loss of 47% (95% CI 31–61) of the expected remaining lifetime. In conclusion, the impact on mortality by a hip fracture event per se lasts 1 year in women and 5 years in men, halving the expected remaining lifetime. In conclusion, the impact on mortality by a hip fracture event per se lasts 1 year in women and 5 years in men, halving the expected remaining lifetime.

PP382
The relationship between cardiovascular risk and bone mineral density: an important role for anthropometry
Renate de Jongh1, Karen Jameson1, Holly Syddall1, Avan Sayer1, Martin den Heijer2, Cyrus Cooper1, & Elaine Dennison1
1MRC Epidemiology Resource Centre, Southampton, UK; 2U University Medical Centre, Amsterdam, The Netherlands.

Introduction
Cardiovascular disease and osteoporosis have often been reported to coexist in older people. However, the literature is conflicting regarding size and indeed direction of the association. The aim of the present study was therefore to assess associations between the Framingham general cardiovascular risk score and bone characteristics in a cohort of older adults.

Methods
We studied 374 men and 379 women, born 1931–1939, who participated in the Hertfordshire Cohort Study and were without cardiovascular disease at baseline (1998–2004). Data on demographic and lifestyle factors, anthropometry, blood pressure and blood lipid concentrations were collected and the Framingham general cardiovascular risk score (FRS) was calculated. DXA scans were conducted. After an average of 4.5 years (± s.d. 0.9) DEXA (n = 447) was repeated and peripheral quantitative computed tomography (pQCT) of the tibia and radius (n = 499) was performed. All analyses were adjusted for gender and age.

Results
FRS (mean range, 14.4 (5–26) points) was positively associated with BMD at the lumbar spine (β (95% CI), 0.058 (0.022 to 0.094) g/cm² per 10 points change, P < 0.01) and proximal femur (0.056 (0.028 to 0.083), P = 0.01). These associations were unaltered by adjustment for additional cardiovascular risk factors except for anthropometry. This effect was strongest for weight (lumbar spine 0.014 (–0.020 to 0.049, P = 0.42); proximal femur 0.016 (–0.010 to 0.042), P = 0.23). No relationships were identified between FRS and bone loss rate over follow-up. Analysis of pQCT data demonstrated relationships between FRS and volumetric trabecular BMD (tibia 1.29 (0.15 to 2.43), P = 0.03; radius 1.15 (–0.02 to 2.31, P = 0.05), but not with volumetric cortical BMD, areal measurements or measurements of bone strength. Adjustment for anthropometric measurements attenuated the relationship between FRS and pQCT data.

Conclusion
General cardiovascular risk is positively associated with bone mineral density. Anthropometry, in particular weight, is an important contributor to this association.

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PP383
Detection of autoantibodies to osteoprotegerin in patients with rheumatoid arthritis and their association with disease activity
Barbara Hauser1, Philip Riches1, Tamara Gilchrist2, Jim F Wilson5, William D Fraser3 & Stuart H Ralston1
1Rheumatic Disease Unit, Institute of Genetics and Molecular Medicine, Edinburgh, UK; 2Centre for Population Health Sciences, The University of Edinburgh Medical School, Edinburgh, UK; 3Faculty of Medicine and Health Sciences, University of East Anglia, Norwich, UK.

Introduction
Osteoporosis and fragility fractures are recognized complications of rheumatoid arthritis (RA). Previously Riches et al. described a patient with celiac disease and severe osteoporosis in whom neutralizing antibodies to osteoprotegerin (OPG) were present. The aim of this study was to determine if OPG autoantibodies were present in patients with RA and other rheumatic diseases and to relate these to clinical features.

Methods
We developed a novel ELISA to detect OPG autoantibodies, using recombinant human OPG as the capture antigen with detection of antigen-antibody complex through HRP conjugated anti-human antibodies. We screened for the presence of OPG autoantibodies in 75 patients with RA, 47 with SLE, 31 with rheumatoid arthritis and their association with disease activity described a patient with celiac disease and severe osteoporosis in whom neutralizing antibodies to osteoprotegerin (OPG) were present. The aim of this study was to determine if OPG autoantibodies were present in patients with RA and other rheumatic diseases and to relate these to clinical features.

Results
Two patients in the control group (1%) had detectable OPG antibodies when compared with 7/75 patients with RA (9.3%, P = 0.01 compared with controls), SLE n = 5/31 (16.1%, P = 0.001 from controls) and SLE n = 3/49 (6%, P = 0.05 from controls). In the RA group the presence of OPG antibodies was associated with disease duration and DAS28 score, but not with BMD (Table 1). No association was found between antibody levels and BMD and disease activity (BASDAI) in AS, or BMD in the other disease groups (not shown).

Table 1 Characteristics of RA patients with OPG autoantibodies.

<table>
<thead>
<tr>
<th>Characteristics of RA pts</th>
<th>Positive OPG Ab</th>
<th>Negative OPG Ab</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n = 7)</td>
<td>(n = 68)</td>
<td>(n = 68)</td>
</tr>
<tr>
<td>Age</td>
<td>63.3 ± 11.8</td>
<td>61.5 ± 13.2</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>16.0 ± 12.3</td>
<td>6.4 ± 7.8</td>
</tr>
<tr>
<td>DAS 28</td>
<td>6.1 ± 0.6</td>
<td>5.4 ± 1.4</td>
</tr>
<tr>
<td>Hip BMD (g/cm²)</td>
<td>0.86 ± 0.26</td>
<td>0.84 ± 0.16</td>
</tr>
</tbody>
</table>

Conclusions
We conclude that OPG antibodies can be detected in a variety of autoimmune diseases. They are particularly common in SLE but also found in RA where they are associated with duration of disease and disease activity. Further research is in progress to evaluate the functional activity of OPG antibodies identified in patients with rheumatic diseases, and correlate this with clinical outcomes.

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Does vitamin D status impact on hip fracture incidence?: evidence of fracture variation with latitude and season in Sweden
Eugene McCloskey, Helena Johansson, Anders Odén & John Kanis University of Sheffield, Sheffield, UK.

Although the optimal requirement of vitamin D for skeletal health in the general community remains uncertain, vitamin D deficiency impacts bone mineralisation, increases bone turnover, accelerates bone loss and increases fracture risk. Seasonal variation in the hip fracture incidence, reported in several studies, supports a role for vitamin D deficiency in the epidemiology of hip fracture. We hypothesised that if the association is causal, then the amplitude of the seasonal variation and the hip fracture risk should vary by latitude. We have examined the incidence of hip fracture in men and women aged 50 years or more from Sweden (latitudes 55° to 69°) between 1987 and 2009. In order to avoid double counting, only one fracture in a period of a year was counted per individual. The effects of season and latitude were examined by Poisson regression.

As expected hip fracture rates were higher in women than in men. Men contributed 104 822 hip fractures in 33 313 065 person-years of observation and women contributed 263 993 hip fractures in 38 387 660 person-years. After adjustment for age and seasonality, hip fracture incidence increased by 2.6% (95% CI: 2.3–2.8%) per degree increase in latitude for men and by 1.7% (95% CI: 1.5–1.9%) for women. The increases were even more marked when additionally adjusting for population density (as a surrogate of urban vs rural lifestyle). There was a marked seasonal variation of hip fracture. The highest risk was observed in February and the incidence was 37.6 and 23.5% lower in men and women respectively during the summer. Importantly, there were significant interactions of amplitude of the seasonal variation with latitude (P < 0.001 for both men and women), indicating that seasonal variation during the year was more pronounced in the north of Sweden than in the south.

These associations strengthen the hypothesis that vitamin D status has an important impact in the causation of hip fracture.

Conclusion

Patients at higher fracture risk are being treated, with Maj.FR10 three times and Hip.FR10 five times greater in those on treatment. However, even using these conservative estimates from GP data, patients with Hip.FR10 up to 47% remain untreated. Variations may be due to differences in demography, current case finding strategies or quality of routine data collection. However, using FRAX and RAIDR, we can identify GP practices with poorer data or higher untreated fracture risk (e.g. 6.5% with Hip.FR10 > 10%) in order to target treatment.

DOI: 10.1530/boneabs.1.PP384

Fracture risk assessment in a primary care population: case finding using routine GP data, FRAX® and RAIDR® in the United Kingdom
Terry Aspray1,2, Erica Whalley3, Mike Scott1, Steve Summers1, Steve Turley4, Rachel Weight5, Valerie Maddison1, Sharon Aedy1 & Lesley Kay1
1Musculoskeletal Unit, Freeman Hospital, Newcastle upon Tyne Hospitals Trust, Newcastle upon Tyne, UK; 2Institute for Ageing and Health, Newcastle University, Newcastle upon Tyne, UK; 3NHS North of Tyne, Newcastle upon Tyne, UK; 4Newcastle West Clinical Commissioning Group, Newcastle upon Tyne, UK; 5Newcastle North and East Clinical Commissioning Group, Newcastle upon Tyne, UK.

Introduction

Fracture risk assessment using FRAX® estimates 10-year fracture risk (FR10) at major sites (Maj.FR10) and hip (Hip.FR10). In 2012, in Newcastle, UK, a strategy was agreed to share data between General Practice (GP), Clinical Commissioning Groups and local hospitals to identify patients at high FR10.

Methods/design
FRAX® and RAIDR® (a health reporting, analysis and intelligence delivery tool) were used to assess routine GP data for 120 478 patients (50.2% female), aged 40–90 years, from 37 GP practices, patients with Hip.FR10, up to 47% remain untreated. Variations may be due to differences in demography, current case finding strategies or quality of routine data collection. However, using FRAX and RAIDR, we can identify GP practices with poorer data or higher untreated fracture risk (e.g. 6.5% with Hip.FR10 > 10%) in order to target treatment.

DOI: 10.1530/boneabs.1.PP386

Sclerostin associated with vertebral bone marrow fat in older men but not women
Vivian Ma1, Xiaojian Li1, Sigurdur Sigurdsson2, Gudny Eriksdottir2, Alda Hauksdottir2, Lisa Palermo1, Trisha Hue1, Thomas Lang1, Tamara Harris3, Clifford Rosen4, Eric Vittinghoff5, Kristin Siggeirsdottir3, Gunnar Sigurdsson3,6, Diana Oskarsdottir2, Vilhjulmund Guðnason2,5 & Ann Schwartz1
1University of California, San Francisco, California, USA; 2Icelandic Heart Association, Kopavogur, Iceland; 3National Institute on Aging, Bethesda, Maryland, USA; 4Maine Medical Center Research Institute, Scarborough, Maine, USA; 5University of Iceland, Reykjavik, Iceland; 6Landspítali University Hospital, Reykjavik, Iceland.

Previous studies found a negative correlation between vertebral bone marrow fat (MF) and bone density (BMD). Proposed mechanisms for this include i) a shift in stem cell lineage allocation from osteoblasts towards adipocytes, and ii) an increase in osteoclast-promoting cytokines with greater MF. However, little is known about the relationship between MF and bone markers. To assess these relationships in older adults, we used data from the AGES-Reykjavik cohort. MF was measured in 301 participants with magnetic resonance spectroscopy (MRS; 1.5 Tesla) at L1-L4 and expressed as ratio of fat to water plus fat (%). After excluding subjects with diabetes (n = 17), inadequate serum (n = 2), or bone-active medication use (n = 44), analyses included 111 men and 127 women (mean age = 79 years, mean BMI = 27.6 kg/m²). Hip and spine scans were obtained using quantitative computed tomography (QCT). Blood was drawn fasting. Serum CTX, PINP, and sclerostin were batch assayed and compared to MF and BMD using Spearman rank correlations. There was a trend towards a negative conclusion.

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Table 1 Correlations with serum sclerostin in older men and women.

<table>
<thead>
<tr>
<th>Vertebral MF</th>
<th>PINP</th>
<th>CTX</th>
<th>Trabecular spine vBMD</th>
<th>Trabecular hip vBMD</th>
<th>Cortical hip vBMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men (n=111)</td>
<td>0.27r</td>
<td>-0.04r</td>
<td>0.18r</td>
<td>0.27r</td>
<td>0.42r</td>
</tr>
<tr>
<td>Woman (n=127)</td>
<td>-0.06</td>
<td>-0.20r</td>
<td>-0.32r</td>
<td>0.54r</td>
<td>0.27r</td>
</tr>
</tbody>
</table>

Participants with diabetes excluded. Spearman rank correlations. *P<0.01.

correlation between MF and CTX in men (r = -0.18, P=0.063), but none of the correlations between MF and PINP or CTX were statistically significant. MF was correlated with sclerostin in men only (Table). Sclerostin correlated negatively with bone turnover markers and positively with BMD. The results for MF and sclerostin suggest a gender-dependent relationship between bone formation and marrow fat.

The association of leptin: adiponectin ratio with bone in overweight and obese postmenopausal women

Yi-Chih Chi1, Pei-Yang Liu2, Maria Spicer1 & Jasminka Ilich1

1Florida State University, Tallahassee, Florida, USA; 2University of Akron, Akron, Ohio, USA.

Leptin and adiponectin have an opposing relationship in circulation; leptin is higher and adiponectin lower in overweight/obese individuals, and vice versa. Studies showed that both leptin and adiponectin can be either beneficial or harmful to bone depending on the mode of action. The objective was to investigate the association of serum leptin:adiponectin ratio (L:A) with BMD of various skeletal sites and markers of bone turnover in overweight and obese postmenopausal women. Participants included (n=184) healthy Caucasian women (BMI range 25.0–40.0 kg/m², age 55.7±4.4 years, mean±s.d., at baseline), instructed to reduce energy intake. BMD and body composition were assessed by iDXA. Serum leptin, adiponectin and bone markers (osteocalcin, serum NTx and urine CTx) were analyzed with immunoassay kits. Pearson’s and partial correlation, and repeated measures ANOVA were calculated using SPSS. After 6-months, participants lost ~5 and ~ 2% of body weight and fat, respectively, as well as some of the bone mass in several skeletal sites (although NS). As expected, serum leptin significantly decreased while adiponectin increased with weight loss. Yet, leptin was still significantly positively correlated with total femur BMD (the same noticed at baseline) before and after controlling for age, years since menopause, physical activity, and dietary calcium and vitamin D intake. Adiponectin was significantly negatively correlated only with serum NTx before and after controlling for the above confounders. In conclusion, 6-month weight loss resulted in slight bone loss and decreased leptin and increased adiponectin levels. The positive effect of leptin on femoral BMD remained even after its decreased levels caused by weight loss.

Serum leptin and adiponectin in overweight and obese postmenopausal women after the 6-month weight loss program and their relationship with BMD

Yi-Chih Chi1, Pei-Yang Liu2, Maria Spicer1 & Jasminka Ilich1

1Florida State University, Tallahassee, Florida, USA; 2University of Akron, Akron, Ohio, USA.

The connection between osteoporosis and obesity is becoming a topic of increasing research. The adipocyte-secreted hormones, leptin and adiponectin, may be the mediators between adipose tissue and bone. The aim was to examine the changes in leptin and adiponectin with the weight and body composition (fat and lean mass) change during the 6-months weight loss program. Additionally, the relationship between two adipokines and BMD of various skeletal sites was also examined. Participants were healthy Caucasian women, n = 100 (BMI range 25.0–40.0 kg/m², age 55.7±4.4 years, mean±s.d., at baseline), instructed to reduce energy intake. BMD and body composition were assessed by iDXA. Serum leptin, adiponectin and bone markers (osteocalcin, serum NTx and urine CTx) were analyzed with immunoassay kits. Pearson’s and partial correlation, and repeated measures ANOVA were calculated using SPSS. After 6-months, participants lost ~5 and ~ 2% of body weight and fat, respectively, as well as some of the bone mass in several skeletal sites (although NS). As expected, serum leptin significantly decreased while adiponectin increased with weight and fat loss. Yet, leptin was still significantly positively correlated with total femur BMD (the same noticed at baseline) before and after controlling for age, years since menopause, physical activity, and dietary calcium and vitamin D intake. Adiponectin was significantly negatively correlated only with serum NTx before and after controlling for the above confounders. In conclusion, 6-month weight loss resulted in slight bone loss and decreased leptin and increased adiponectin levels. The positive effect of leptin on femoral BMD remained even after its decreased levels caused by weight loss.

Osteoporosis: treatment

Abstract withdrawn.
Electronic clinical decision support for the management of osteoporosis in primary care

Yvonne Selecki, Jaqui Center, Tuan Nguyen & John Eismann
Garvan Institute of Medical Research, Sydney, New South Wales, Australia.

The gap between osteoporosis clinical guidelines and their implementation exists in all countries. The increasing use of computerised patient records offers new opportunities to aide clinical decision making. We have developed a fracture and osteoporosis investigation and treatment clinical decision tool to aide primary care management of osteoporosis. Uniquely, this tool is designed to be integrated with existing patient data software.

Electronic clinical decision support systems are one of the most promising interventions to improve uptake of guideline-based recommendations in clinical practice. There is solid scientific evidence for their use. A recent systematic review of RCTs found two thirds demonstrating improvement in clinical decision making. Studies have also found several features essential for the optimum use of the tools: automatic provision of the clinical decision tool as part of clinical workflow; recommendation rather than just assessments are provided; decision support is supplied at the time and location of decision making and computer based, rather than paper based, decision support. All these features are incorporated into our tool as well as an audit function to data mine for patients who may require screening for osteoporosis and a fracture risk calculator with specific functions for communicating risk to patients. Uniquely this tool is designed to be incorporated into existing patient data software.

Design of such electronic tools require unique collaborations between clinicians and software development professionals with clinicians providing the detail of clinical decision thought pathways and IT professionals translating this into computer screens. They then need to be tested for acceptability, and feasibility in primary care and, above all, effectiveness, that is, their ability to improve patient health outcomes. We will describe the process required for the development of electronic decision support as well as the unique features of the osteoporosis tool.

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Remarkable bone mineral density increases on teriparatide in patients with glucocorticoid-induced osteoporosis and Crohn’s disease

Danny Ko-Wu Kuo1, Kenny To2 & David Kendler2
1Porthealth Clinical Research, Vancouver, British Columbia, Canada; 2University of British Columbia, Vancouver, British Columbia, Canada; 3El Elisabeth Canada Inc., Toronto, Ontario, Canada.

Crohn’s disease often results in abnormalities in bone strength, and ultimately increases the risk of fragility fracture. Up to 55% of patients with Crohn’s disease have bone mineral density in the osteopenia range up to 50% of osteoporosis. Glucocorticoid is frequently used in the treatment of Crohn’s disease and is associated with osteoporosis and increased fracture risk. It has been reported that osteoporotic fractures in patients with Crohn’s disease are 40% more likely than in patients with ulcerative colitis. Malabsorption, vitamin D insufficiency, amenorrhea/hypogonadism, glucocorticoid, and chronic inflammation have all been linked to bone loss in Crohn’s disease. Indicated therapies include bisphosphonates and teriparatide. We report on the novel initial use of teriparatide specifically in two cases of Crohn’s disease. Both reviewed patients had severe osteoporosis with spine fractures. Both experienced remission from Crohn’s disease at the same time as initiating teriparatide therapy and calcium and vitamin D supplementation. Both had the introduction of zoledronic acid intravenous annual infusion antiresorptive therapy subsequent to teriparatide. Increases in spine bone density over the course of this therapy were 48- and 72% in each of our patients, observed over five years and three years, respectively. Similar but lesser magnitude increases in BMD were seen at hip sites over the same timeframe. We attribute these remarkable improvements in bone mineralization to the young age of the patients, the stabilization of their underlying Crohn’s disease, discontinuation of glucocorticoid therapy, improved nutrition, the initial use of bone anabolic therapy followed by antiresorptive therapy, as well as calcium and vitamin D supplementation. A possible role for initial bone anabolic therapy in such patients should be investigated further.

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Effects of a new conjugate drug in a rat model of postmenopausal osteoporosis

Careesa Liu1, Robert Young2 & Marc Gryppas1,3
1University of Toronto, Toronto, Canada; 2Simon Fraser University, Burnaby, Canada; 3Mount Sinai Hospital, Toronto, Canada.

Introduction

Standard clinical treatments for postmenopausal osteoporosis utilize resorption-inhibiting drugs such as bisphosphonates, which selectively bind to bone mineral but also suppress bone formation over time. Prostaglandin E2 (PGs) has bone-anabolic effects in vivo, but its clinical utility is hindered by side effects upon systemic administration. Since PGs acts on bone via the EP receptor, our approach utilizes a specific EP receptor agonist (EP4a) to promote bone formation. The EP4a is reversibly linked with the bisphosphonate alendronate (ALN) to create an ALN-EP4a conjugate drug. When administered systemically, the bone-targeting property of ALN directly delivers EP4a to bone sites, where hydrolytic enzymes in the bone environment slowly cleave the chemical link. This liberates EP4a to promote bone formation while leaving ALN bound to bone.

Methods

We used the ovariectomized (OVX) rat model to investigate the in vivo effects of ALN-EP4a in a curative experiment. Three-month-old female Sprague–Dawley rats were OVX, allowed to lose bone for 6 weeks, then treated for 6 weeks before sacrifice (n = 9–12/group). Treatments consisted of conjugate in low (5 mg/kg i.v. weekly) and high (25 mg/kg i.v. week 1, 15 mg/kg weeks 2, 4, 6) doses, vehicle for OVX (i.v. weekly) and sham-operated (s.c. daily) rats, co-dosed unconjugated EP4a and ALN (2.5 mg/kg each i.v. weekly), and PG(3) (4 mg/kg s.c. daily).

Results

Uncalculated histomorphometry of the proximal tibial metaphysis shows that conjugate low dose significantly increases MAR by 69% and BFR/BS by 131% compared to OVX. Micro-computed tomography indicates that, compared to

PP392

Anti-osteoporosis treatment amongst austrian hip fracture patients: status quo, and effects on mortality and subsequent fracture risk

Wolfgang Brozek1, Berthold Reichardt2, Oliver Kimberger1, Daniela Kritsch1, Klaus Klausschofer1 & Elisabeth Zettl1
1Ludwig Boltzmann Institute of Osteology, Hanusch Hospital of the WGK and AUVA Trauma Center, 1st Medical Department at Hanusch Hospital, Vienna, Austria; 2Sickness Fund Burgenland, Burgenlandische Gebietskrankenkasse, Eisenstadt, Austria; 3Clinical Department of General Anesthesia and Intensive Care Medicine, Medical University of Vienna, Vienna, Austria.

Osteoporosis is commonly known as the prime risk factor for hip fracture in the elderly. We thus evaluated status and effect of osteoporosis treatment amongst hip fracture patients in a large Austrian cohort.

Retrospectively retrieved pseudonymized invoice data from Austrian social insurance authorities covering roughly 98% of the entire population included 31 548 subjects over 50 years with first hip fractures between July 2008 and December 2010, with follow-up until June 2011. Information on anti-osteoporosis treatment before and after first fracture was available between July 2007 and June 2010 for various drugs including bisphosphonates. χ2-testing and Cox regression analysis were used to identify differences between treatment groups.

Alendronic acid was administered to 10.8% of patients (13.27% women, 10.39% men) before and to 13.34% of patients (15.74% women, 13.43% men) after first fracture. Corresponding figures for the other drugs (overall, women, men, before/after first fracture): ibandronic acid: overall 2.70/4.06%, women 3.39/5.01%, men 0.81/1.46%; risedronic acid: 5.27/3.58%, 6.57/4.42%, 1.78/1.26%; zoledronic acid: 0.34/0.93%, 0.40/1.10%, 0.18/0.48%; calcitonin: 1.98/1.47%, 2.42/1.67%, 0.77/0.90%; treatment frequencies of strontium, raloxifen, teriparatide, PTH, and denosumab were below 1% in all groups. As many as 72.76% of patients (66.97% women, 86.83% men) were untreated. Amongst survivors, we observed a significantly decreased proportion of subsequent fractures when receiving bisphosphonates only before or before and after first fracture, compared with bisphosphonate treatment only after first fracture (χ2 = 21.841, P < 0.0001), the same being true for non-bisphosphonate drugs (χ2 = 6.269, P < 0.05). Moreover, whereas individuals receiving bisphosphonates at least before first fracture showed prolonged survival after hospital discharge relative to untreated patients (HR 0.69, 95% CI: 0.64–0.74; P < 0.0001), there was no such difference for other drugs.

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PP394

Ectopic calcification in bone: potential role in the pathogenesis of osteoporosis and coronary artery disease

Joanne Quigley1,2,3,4,5,6,7
1University of Melbourne, Melbourne, Australia; 2Royal Children’s Hospital, Melbourne, Australia; 3University of Queensland, Brisbane, Australia; 4Children’s Health Research Institute, Queensland Children’s Health, Brisbane, Australia; 5Women’s and Children’s Hospital, Melbourne, Australia; 6University of Edinburgh, Edinburgh, United Kingdom; 7Institute for Molecular Biosciences, The University of Queensland, Brisbane, Australia.
PP395
Renal function and safety result after 1 year treatment of zoledronic acid in Chinese women with postmenopausal osteoporosis
Huiyong Shen1, Yue Sun2 & Xin Liu2
1Department of Orthopaedics, the Second Affiliated Hospital of Sun Yat-Sen University, Guangzhou, China; 2Beijing Novartis Pharma Co., Ltd., Beijing, China.

Objective
Zoledronic acid (Zol) has been demonstrated to be an effective therapy to treat postmenopausal osteoporosis (PMO) in Chinese women in a 12-month post-marketing observational study (ZOOM study). As an I.V. bisphosphonate, Zol are exclusively excreted via the kidneys. We present a report of the renal function and safety data of once-yearly Zol 5 mg treatment.

Subjects and methods
A total of 373 PMO patients from 30 different centers in China with baseline Ccr > 35 ml/min received a single 15-μg infusion of Zol. Renal function (serum creatinine, creatinine clearance rate (CCR), and BUN) was assessed at baseline, 6 and 12 month after therapy. Safety was assessed from adverse events and serious adverse events recorded by the investigators.

Results
Renal function assessment for all the patients showed that mean serum creatinine and BUN maintained 12 month after treatment (creatinine 62.64 ± 14.59 vs 63.21 ± 15.04 μmol/l and BUN 5.66 ± 3.46 vs 5.44 ± 3.18 μmol/l, respectively). Creatinine increase of more than 0.5 mg/dl compared to baseline was found in only one patient (0.38%) at 12 month. The baseline average CCR was 70.57 ± 23.67 ml/min. During 1 year follow-up, CCR declined under 35 ml/min in nine patients (9/257, 3.50%) with moderate renal impairment at baseline (CCR 35.52 ± 47.63 ml/min). A total of 42 patients (11.26%) experienced adverse events (AEs). The most common AEs were fever (6.17%) and musculoskeletal pain (1.61%), while other AEs occurred below the ratio of 1%. Serious adverse events were reported in four cases, including three deaths due to pneumonia, lung cancer and gastric perforation, which were not considered by the Investigator to be drug related.

Conclusion
Once yearly zoledronic acid administration was associated with a good safety profile and generally well tolerated in Chinese PMO patients.

PP396
Characterization and risk factors of acute-phase response following a first-dose administration of zoledronic acid for treatment of osteoporosis
Decai Chen
West China Hospital, Sichuan University, Chengdu, China.

Objective
To explore the characterization and risk factors of acute-phase response (APR) following a first-dose administration of 5 mg zoledronic acid for treatment of osteoporosis.

Method
We conducted clinical data of the zoledronic acid users for treatment of osteoporosis in Department of Endocrinology, West China Hospital, Sichuan University from January 2009 to November 2012.

Results
A total of 178 patients were eligible for inclusion in the study, of which 108 patients has experienced the APR. 80 (45%) patients developed fever, 14 (9.6%) chills, 48 (27%) musculoskeletal pain, 19 (10.7%) gastrointestinal symptoms, 10 (5.6%) headache and dizziness, 7 (3.9%) palpitation, and 3 (1.7%) rash. APR was more common in patients with higher baseline tartrate-resistant acid phosphatase 5b (TRACP-5b) and new-onset vertebral compression fractures (new-onset VCF). Stepwise logistic regression showed that the odds ratio (OR) to have APR in higher baseline TRACP-5b and new VCF was 3.3 and 2.5, respectively.

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PP397
Intact heparin inhibits BMP6 osteogenic activity
Jelena Brkljacic, Martina Pauk, Igor Erjavec & Slobodan Vukicevic Laboratory for Mineralized Tissues, School of Medicine, Center for Translational and Clinical Research, University of Zagreb, Zagreb, Croatia.

Introduction
One third of patients in the long-term heparin therapy show reduction in bone density. We have shown that heparin binds to bone morphogenetic protein 6 (BMP6) and inhibits its osteogenic activity in vitro. Here we explored whether heparin effects BMP6 mediated bone efficacy in vivo.

Methods
We have used a mouse model of postmenopausal osteoporosis and tested the effect of heparin on BMP6 therapy and its osteogenic activity using DEXA and μCT of femur and tibia.

Results
We showed that BMP6 restored the quality and microarchitecture of osteoporotic bone. In combination with heparin, femur bone volume over tissue volume was reduced for 24% (tibia 30%) and the trabecular number by 29% (tibia 35%), while trabecular separation was increased by 25% (tibia 17%), as compared to BMP6 therapy alone. These results were supported by BMD values measured by DEXA. In heparin-induced osteoporosis model, BMP6 prevented heparin-induced osteoporosis when used simultaneously, but not after heparin therapy, as shown by BMD of femur and tibia. The results were confirmed by μCT, where BMP6 used simultaneously with heparin improved all measured parameters of trabecular bone.

Conclusion
We confirmed that heparin binds to BMP6 in vivo and prevents dose- and time-dependently its osteogenic activity, which might be the mechanism of heparin-induced bone loss.

PP398
Health economic consequences of fractures in patients with osteoporosis: a national register based study of total and incremental health costs following fracture
Kim Rose Olsen1, Carrinna Hansen1,2 & Bo Abrahamsen2,3 1eMpirisk Aps, Frederiksberg, Denmark; 2Gentofte Hospital, Hellerup, Denmark; 3University of Southern Denmark, Odense, Denmark.

Introduction
Osteoporotic fractures are known to be costly to society but estimates tend to be based on small scale prospective studies. In the following we report national data for healthcare costs due to fractures in patients with osteoporosis.

Study population and methods
All Danish residents aged 35+, mean age 70.5 years, 13% men, 27.3% prior major osteoporotic fracture, who began bisphosphonates for osteoporosis between 1/1997 and 12/2002 (n = 39,058) were followed for incident fractures using national health registers (primary care, medications, hospital visits). Cost of residential or home care was not included.

Results
Change in healthcare costs (2010 prices) in the year following fracture compared with the year before irrespective of vital status: (Table 1)

<table>
<thead>
<tr>
<th></th>
<th>Hip</th>
<th>Spine</th>
<th>Humerus</th>
<th>Forearm</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Costs</td>
<td>n=1,538</td>
<td>n=369</td>
<td>n=733</td>
<td>n=1,038</td>
<td>n=1,884</td>
</tr>
<tr>
<td>Baseline</td>
<td>8,583</td>
<td>9,221</td>
<td>7,623</td>
<td>6,027</td>
<td>7,830</td>
</tr>
<tr>
<td>After fracture &amp; Change</td>
<td>17,145</td>
<td>15,914</td>
<td>13,064</td>
<td>10,996</td>
<td>13,888</td>
</tr>
</tbody>
</table>

Healthcare costs were substantial in the year following hip fracture with a total healthcare cost of (mean) USD 19,745. However there were considerable baseline costs prior to fracture (mean USD 8,583), chiefly for inpatient treatment.
Conclusions
Substantial healthcare costs were observed in patients who had been diagnosed with osteoporosis and subsequently sustained fractures. These costs are compatible with those reported in a smaller longitudinal study from Sweden (Acta Orthop. 2008 79 269–280). However, the present study also shows that patients had considerable healthcare costs in the year before fracture, highlighting the importance of using the change in healthcare costs in models that estimate cost savings due to fractures avoided.

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**PP399**
Assessment of serum 25-hydroxyvitamin D concentrations in postmenopausal osteoporotic women: a retrospective study to evaluate long-term treatment with vitamin D3

Camilla Sand Andersen1, Peter Vestergaard1, Parisa Gazerani1 & Hans Christian Hoeck2
1Department of Health Science and Technology, Faculty of Medicine, Aalborg University, Aalborg, Denmark; 2Center for Clinical and Basic Research (CCBR), Aalborg, Denmark.

Introduction
The purpose of this study was to evaluate whether daily treatment with 400 IU vitamin D3 was sufficient to maintain 25-hydroxyvitamin D (25(OH)D) concentrations above 60 nmol/l over a 3-year period. In addition, the study aimed to clarify if any differences existed in serum 25(OH)D between pre-supplemented women and women who already had serum 25(OH)D above 60 nmol/l at screening.

Methods
Serum samples drawn from 251 postmenopausal osteoporotic women over a 3-year period were analyzed for 25(OH)D, parathyroid hormone (PTH), and phosphate. Furthermore, a comprehensive patient file review was performed to collect patient characteristics and laboratory results. RM-ANOVA was applied to evaluate treatment effect over time. Approval was obtained from The Danish Scientific Ethical Committee, Region North Jutland, Denmark (N-20120060).

Results
Serum 25(OH)D increased significantly over the 3-year period ($P<0.001$). The mean increase was 24 nmol/l. Pre-supplemented women had a significantly lower mean 25(OH)D concentration compared to non-pre-supplemented women ($P<0.001$) but remained above 60 nmol/l. Season significantly interacted with 25(OH)D concentrations measured during winter were significantly elevated compared to other seasons ($P<0.05$).

Conclusions
It appears that daily treatment with 400 IU vitamin D3 over a 3-year period is sufficient to maintain serum 25(OH)D above 60 nmol/l.

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**PP400**
Osteonecrosis of the jaw and non-malignant disease

Amélie Coudert1, Géraldine Lescaille2, Vanessa Baaroun1,2, Jean Azerad2, Martine Cohen-Solal1, Ariane Bordan1 & Vianney Descroix3
1Laboratory of Oral Molecular Physiopathology, INSERM, UMR 872, Cordeliers Research Center, Team 5, Universités Paris-Diderot, Paris 7, Pierre et Marie Curie and Paris-Descartes, Paris, France; 2Oral Surgery Department, Pitié-Salpêtrière University Hospital, Paris Diderot University, Paris, France; 3INSERM UMR-S 606, Hôpital Lariboisière, Ambroise Paré Street, Paris, France.

Bisphosphonates (BP) are powerful bone resorption inhibitors. They are used for the symptomatic treatment of malignant osteolytic bone disease (e.g. multiple myeloma and bone metastasis), as well as bone diseases associated with high bone resorption (e.g. postmenopausal osteoporosis, cortisone-induced osteoporosis). However, recent data showed that a rare, but serious, adverse effect of BP therapy is osteonecrosis of the jaw (BRONJ). Given the increasing number of persons on bisphosphonate treatment for non-malignant disease, it is important to accurately identify pathogenesis, risk factors and management strategies for BRONJ in patients with non-malignant disease. The objective of this study was to review cases of BRONJ occurring in association with benign disease and to describe and compare the clinical course and outcome for patients with BRONJ and rheumatoid arthritis or osteoporosis. We retrospectively reviewed observations of all patients referred for treatment and follow-up for BRONJ from January 2007 to December 2011. Demographic data, medical history, maxilofacial findings, BRONJ treatment and follow-up were reviewed for each case. Over a 5-year period, we diagnosed 112 patients with BRONJ. Among these patients, 15 received bisphosphonate treatment for non-malignant disease. Patients received bisphosphonates for a variety of reasons: eight (53%) to prevent osteoporosis in association with underlying rheumatoid arthritis; six (40%) to prevent idiopathic osteoporosis, and one (7%) to treat ankle algodystrophy. The mean oral bisphosphonate exposure period was 48.4 months. In 13 cases (86.6%), BRONJ was diagnosed following dental extraction. Major surgery, sequestrectomy or alveolectomy was performed in nine patients (60%), all of whom healed within 3–36 months. Comparative analysis of all the variables showed no statistically significant differences between patients with rheumatoid arthritis and others in conclusion, within the limits of our study, we were unable to demonstrate a difference in BRONJ disease spectrum, clinical course or outcome between patients with or without rheumatoid arthritis.

DOI: 10.1530/boneabs.1.PP400

**PP401**
Effectiveness of Strontium Renalate therapy for osteoporosis in men

Lali Kilasonia, Medea Kopaliani, Nana Kirvalidze, Luba Lagvilava & Neriman Tsintsadze
LTD ‘Medulla’ clinic, Tbilisi, Georgia.

Introduction
Since the identification of the fact that osteoporosis represents not quite rare disease in men, its preconditions-diseases increasing the likelihood of osteoporosis in men has been intensively studied. As a result, a new direction ‘male osteoporosis’ has been established in medicine, although, there is not sufficient information on how the basic medications are selected for men: whether certain drugs have priority effect for the treatment of osteoporosis in this category of patients.

Materials and methods
Our research studied 125 male patients (aged 40–70) with osteoporosis who have been on Bivalos (Strontium Renalate) treatment for 2 years, with the standard scheme: daily 2.0 mg Ca and D3 with combined drugs (Ca D3 Nicomed Forte). Osteoporosis was diagnosed through X-ray densitometry method (Hologic®-100). On selecting Bivalos as a basic medication, its pathogenesis, its anabolic effect on osteoblasts and an antiresorptive effect caused by influencing on RANKL in OPG conditions; BMD basic index was also considered in the studied category. Despite the clinical form of the disease, average index of T-criteria in spinal ribs and hip fluctuated between 2.5S.D and 2.7S.D, which is more likely to be related to the empirically higher peak bone mass index in males. In 36 cases out of 125 patients, hypogonadotropic hypogonadism was verified; 30 patients were with thyroid gland diseases; 15 patients with diabetes mellitus; 24 patients with rheumatoid arthritis.

Results
As a result of densitometric study after 2 year treatment, it was established that: i) Bone mineral density in the treated patients increased, in 58% of cases reaching 7.2% in hip proximal part, while it was 4.8% in 48% of patients. Bone increase was identified in spinal ribs. ii) Our data proves that besides antiosteoporotic effect, Bivalos has analgesic effect too which was identified in 70% of patients. 7.2% in hip proximal part, while it was 4.8% in 48% of patients. Bone increase was identified in spinal ribs. iii) All patients underwent the treatment well, without possible undesirable complications.

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**PP402**
Do Bisphosphonates remain basic drugs for the treatment of osteoporosis?

Lali Kilasonia, Luba Lagvilava, Nana Kirvalidze, Medea Kopaliani & Neriman Tsintsadze
LTD ‘Medulla’ clinic, Tbilisi, Georgia.

Introduction
If we pay more attention, we will notice that the frequency of drugs against osteoporosis is increasing on world markets every year. At the same time, there is ongoing compromises on medicines, targeted activity mechanisms of new drugs are multifull studies. To be short, recent knowledge gives use impression that only Bisphosphonates maintain their strong positions if the medicines and most importantly, the length of treatment is adequately selected.
Materials and methods
We would like to share our experience on the results and effectiveness of treatment of 500 patients. 300 out of these 500 patients were receiving Peroral Bonviva injections for 3 years, while 150 patients were receiving Bonviva i.v. also for 3 years. In parallel to Bonviva, each patient in these two groups was receiving combined preparation of Ca and D3 vitamins. Patients were aged between 35–85; 3 age categories were identified: Group I – 175 patients aged 35–50; Group II – 200 patients aged 51–75; Group III – 125 patients aged 75–85. Gender: 110 male and 390 female patients. Patients were divided into the following categories by their clinical forms of osteoporosis: 135 women with post menopausal diagnosis; 75 patients with rheumatoid arthritis; 65 patients with male osteoporosis; 116 patients with thyroidal osteoporosis; 18 patients with osteoporosis during osteoarthrosis; 31 patients with senile osteoporosis.

Results
Treatment effect of Bonviva after 3 year treatment reaches 60–70% notwithstanding the age of the patients and clinical form of the disease. Fracture risks in spinal ribs have decreased by 34% and 39% in peripheral bones after 3 year treatment with peroral Bonviva. Maximal index of decreasing fracture risks was identified in lumbar vertebrae, reaching 44% as a result of 3 year Bonviva intra-vein treatment. This one again proves that clinical effects of Bisphosphonates are likely to be related to its cumulative level in the bone rather than the frequency of its administration. Clinical effects of Bonviva in older ages (Group II) is less, which, in parallel to antiresorptive effect, should be related the lack of formation in the age, causing difficulties to Bisphosphonates. (Table 1)

Table 1 Increase of BMD as a result of Bonviva treatment during different clinical forms of osteoporosis 3 year experience.

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>Post menopausal osteoporosis</th>
<th>Osteoarthritis</th>
<th>Thyroidal osteoporosis</th>
<th>Male</th>
<th>Rheumatoid</th>
<th>Senile</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=750</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intra-vein Bonviva (3 mg·once every 3 month)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥5.4%</td>
<td>≥5.8%</td>
<td>≥6.1%</td>
<td>≥5.1%</td>
<td>≥6.8%</td>
<td>≥5.2%</td>
<td></td>
</tr>
<tr>
<td>Number of patients</td>
<td>Post menopausal osteoporosis</td>
<td>Osteoarthritis</td>
<td>Thyroidal osteoporosis</td>
<td>Male</td>
<td>Rheumatoid</td>
<td>Senile</td>
</tr>
<tr>
<td>N=150</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥5.8%</td>
<td>≥6.3%</td>
<td>≥6.3%</td>
<td>≥6.4%</td>
<td>≥7.1%</td>
<td>≥5.5%</td>
</tr>
</tbody>
</table>

DOI: 10.1530/boneabs.1.PP402

PP403
Parathyroid hormone changes following denosumab treatment in postmenopausal osteoporosis
Polyzoi Makras1, Stergios Polyzos2, Athanasios Papadodemou3, Panagiotis Kokkoris1,1, Daniel Chatziotiadis4, & Athanasios Anastasilakis1
1Department of Endocrinology and Diabetes, 251 Hellenic Air Force and VA General Hospital, Athens, Greece; 2Ippokration Hospital, Second Medical Clinic, Aristotle University of Thessaloniki, Thessaloniki, Greece; 3Department of Medical Research, 251 Hellenic Air Force and VA General Hospital, Athens, Greece; 4Division of Nuclear Medicine, 251 Hellenic Air Force and VA General Hospital, Athens, Greece; 5Department of Endocrinology, 424 Military Hospital, Thessaloniki, Greece.

Purpose
Denosumab is a new potent antiresorptive treatment of osteoporosis that can potentially induce a compensatory increase of parathyroid hormone (PTH) levels. We aimed to evaluate the alteration of PTH 1 and 6 months after denosumab’s administration (60 mg) with different regimens of calcium and vitamin D (Ca/D) supplementation, as well as the association of PTH with serum Ca and bone markers.

Methods
This was a prospective, multicenter, study among 47 postmenopausal women requiring onset or continuation of osteoporosis treatment who were followed for 6 months. The intervention included administration of 1 g calcium carbonate and 800 IU cholecalciferol daily for 6 months (Group A) or the double dose (2 g/1600 IU) for the first month followed by the 1 g/800 IU Ca/D regimen for the next 5 months (Group B).

Results
PTH levels were significantly higher at month 1 and 6 only in Group A; Ca levels were significantly decreased at month 1 and returned to baseline values at month 6 within the same Group. The mean percent change between month 1 and baseline for PTH ([ΔPTH1,0]/P1) was significantly higher in Group A than B (63.5 ± 28.2 vs. −3.0 ± 4.7%, P = 0.029). [ΔPTH1,0] was correlated with the reciprocal Δ-changes of Ca (r = −0.610, P = 0.002), and collagen type I C-terminal telopeptide (r = −0.697, P = 0.003) only in Group A.

Conclusion
An increase of PTH should be expected, at least following the first administration of denosumab in common clinical practice. The effect of this compensatory consequence in bone metabolism warrants further investigation.

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PP404
Sequential therapy after PTH 1–84 treatment: comparison among bisphosphonates and strontium ranelate
Renato Pastore1, Daniela Mentuccia1, Patrizio Pasqualetti1,2, & Gaetano Frazese2
1UOC Endocrinologia, Ospedale S. Giovanni Calibita, Fatebenefratelli, Isola Tiberina, Rome, Italy; 2SeSMT AfaR, Fatebenefratelli, Isola Tiberina, Rome, Italy.

Introduction
Evidence in literature shows how is useful to use antiresorptive drugs such as bisphosphonates, in severe osteoporosis severe after PTH (1–34 or 1–84) treatment.

Methods
This study was divided into two parts: the first one analyzed BMD changes by DXA at the lumbar and femoral and serum osteocalcin and β-CTX, monitoring their performance after 6, 12 and 18 months in 71 women with severe postmenopausal osteoporosis, treated for 18 months with PTH 1–84. In the second phase of the study, 66.66% (50 of 71 treated patients) was divided into five groups (each with ten patients), who received Calcium (1 g/day) and vitamin D (5600 IU/week). Patients, respectively, taking alendronate, risedronate weekly, ibandronate monthly and strontium ranelate daily and in the last group only vitamin D and Calcium. After 18 months was evaluated again BMD at the spine and femur.

Results
We observed a slight increase in femoral T-score at the end of treatment, (P = 0.07, Wilcoxon test), more significant at the lumbar spine (baseline = −3.3 ± 0.9 and −2.7 ± 1.2 at the end of treatment (P < 0.001, Wilcoxon test). Osteocalcin was increased (ANOVA, P < 0.001), 4.4 times from baseline at 6th month, 5.4 and 3.1, respectively, at 12th and 18th months (Bonferroni, P < 0.001). β-CTX levels showed an increase of 2.5 times from baseline at month 6th, 2.6 and 1.8 respectively at 12th and 18th months (P < 0.001). After 18 months of therapy with other bisphosphonates, strontium ranelate and calcium and vitamin D further significant increases were evidenced in T-scores after ibandronate (+0.9, 95% CI: +0.2, +1.5, P < 0.05), ranelate (+ 0.8, 95% CI: +0.4, +1.3, P < 0.05), ranelate (+ 1.6, 95% CI: +1.0, +2.3, P < 0.05).

Conclusions
These results suggest that in severe osteoporosis the treatment of choice would include a first cycle of 18 months with PTH 1–84, followed by subsequent therapy with antiresorptive drugs or ranelate strontium.

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PP405
Adherence to therapy: outcomes after seven years of treatment with bisphosphonates
Renato Pastore & Daniela Mentuccia
UOC Endocrinologia, Ospedale S. Giovanni Calibita, Fatebenefratelli, Isola Tiberina, Rome, Italy.

Introduction
Bisphosphonates are the first-choice treatment for osteoporosis. However, the efficacy observed in clinical trials may not be realized in a real-life setting, partly due to poor adherence to therapy, with a significant worsening of clinical outcomes. The aim of this study conducted on an outpatient cohort is to quantify their performance after 6, 12 and 18 months in 71 women with severe postmenopausal osteoporosis, treated for 18 months with PTH 1–84.

Materials and methods
Two hundred and thirty-six women suffered from osteoporosis (mean age 66.4 years; s.d. 9.3; range 44–88) were studied with bisphosphonates (BP) between January 2004 and December 2011 were examined. We assessed the association
between adherence to oral BP and incidence of osteoporotic fractures. Adherence was quantified using the medication possession ratio (MPR) per year for each patient. Adherence to treatment was defined as having MPR ≥ 80%.

Results
Adherence rates decreased from 53% for treatment lasting 0–2 years to 43% for treatment lasting 2–4 years, returning to 49% for treatment lasting more than 4 years. In the whole sample mean MPR was 60.6%. Among the motivations of therapy drop-out co-morbidities, self-made decision, GI intolerance and death were the most frequent. Non-adherent patients had higher risk of fracture (adjusted odds ratio = 3.4, 95% CI 1.1 to 10.5, P = 0.032). Problems incompliance were reported in 85 visits (37.8%) on 51 patients (21.6%). The mean MPR per year adherence was associated with age (<65 years (P = 0.040), absence of co-morbidities (P = 0.023), positive history of fracture (P = 0.044); having the same physician in follow-up (P = 0.025).

Conclusions
From our results it emerges the importance of the relationship between physician and patient in improving the adherence. Adherence to BP in osteoporosis management is suboptimal in a real-life setting. A significant positive association exists between poor adherence and increased risk of osteoporotic fractures which becomes augmented with longer treatment duration.

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**PP406**

In real clinical practice osteoporosis drugs are taken for a very short period: analysis of persistence in the campania region

Giovanni Iolascon1, Annarita Capaldo1, Valentina Orlando2, Enrica Menditto2 & Francesca Gimaglio1
1Orthopaedics and Rehabilitation Medicine, Second University of Naples, Naples, Italy; 2CIRFF/Centre of Pharmacoeconomics Faculty of Pharmacy University of Naples, Federicoll, Naples, Italy.

Introduction
Persistence is defined as the period between the start and the interruption of a pharmacological treatment. In osteoporotic patients, persistence to therapy is poor, resulting in reduced benefits and increased risk of fracture. The aim of this study is to analyze persistence with drug therapy in osteoporotic patients in the Campania region.

Material and methods
We conducted a retrospective population-based cohort study to examine prescription data of 30 348 subjects, males and females, aged ≥ 40 years, in Campania Region (Southern Italy). They received at least one prescription for osteoporosis medication in the period between January 1, 2009 and December 31, 2009. Subjects had not received osteoporosis medication in the year prior to the start of the study. They were followed for 1 year from the first prescription of an antosteoporotic drug and persistence was assessed with the method of medication gaps. In addition, a survival analysis was performed by the Kaplan–Meier method and univariate sensitivity analysis.

Results
The mean age of our samples was 69.1 years. 54.8% of subjects were persistent at 3 months, 32.8% at 6 months, 21.9% at 9 months and 15.9% at 12 months. The results of analysis of persistence for each drug are shown in Table 1.

Conclusion(s)
Taken together, these results suggest that the study mixture has an overall beneficial effect on bone metabolism, by improving the anabolism and in parallel reducing bone resorption. Given the relatively low amount of calcium and vitamin D3 present in the mixture, the addition of inulin and soy isoflavones had likely contributed to the observed effects.

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**PP407**

Effect of a mixture of calcium, vitamin D, inulin and soy isoflavones on bone metabolism in post-menopausal women: a retrospective analysis

Maurizio Bevilacqua1, Vellela Righini1, Diana Certan2, Matteo Alemanni2 & Giorgio Gandolfini3
1Endocrinology and Diabetes Unit, Department of Medicine, Luigi Sacco Hospital (Vialba) – University of Milan, Milano, Italy; 2Medical Affairs, Medical Department, Bayer S.P.A. – Pharmacuticals, Milano, Italy; 3IRCSS S, Maria Nascente, Rheumatology and Bone Metabolism Unit, Don Guocchi Foundation ONLUS, Milano, Italy.

Introduction
A retrospective analysis we previously performed on post-menopausal women showed that the addition of inulin (3 g) and soy isoflavones (40 mg) to daily calcium (500 mg) and vitamin D3 (300 UI) supplementation was able to increase calcium absorption by 60%, while reducing circulating parathormone and leaving vitamin D3 levels unchanged. Therefore, we tested whether such a mixture could affect also bone metabolism.

Methods
Otherwise healthy post-menopausal women presenting to our ambulatory and that received the study mixture for at least 3 months were retrospectively analysed for the following markers of bone metabolism: IGF1, collagen-teleopeptide (CTX) and osteocalcin.

Results
The retrospective analysis included 28 women. 3 months of supplementation induced an increase of IGF1 levels, from a mean value of 107.59 (s.d. 51.13) ng/ml at baseline to 124.86 (61.77) ng/ml, P = 0.01, suggesting an increase in bone anabolism. On the other hand, CTX levels were significantly reduced, from 315.57 (211.11) pg/ml at baseline to 263.43 (154.60) pg/ml; P = 0.04, pointing out a positive effect on bone resorption, too. A modest reduction of osteocalcin levels was observed, from 22.91 (10.25) ng/ml at baseline to 20.82 (8.34) ng/ml, although it did not reach statistical significance (P = 0.07).

Conclusions
Taken together, these results suggest that the study mixture has an overall beneficial effect on bone metabolism, by improving the anabolism and in parallel reducing bone resorption. Given the relatively low amount of calcium and vitamin D3 present in the mixture, the addition of inulin and soy isoflavones had likely contributed to the observed effects.

**Table 1**

<table>
<thead>
<tr>
<th>% Persistent</th>
<th>Raloxifene</th>
<th>Alendro-nate</th>
<th>Ibandr-onate</th>
<th>Risedro-nate</th>
<th>Alendro-nate + cholecalciferol</th>
<th>Stronium renodate</th>
</tr>
</thead>
<tbody>
<tr>
<td>99 (%)</td>
<td>49.1</td>
<td>51.4</td>
<td>70.2</td>
<td>61.3</td>
<td>59.7</td>
<td>43.3</td>
</tr>
<tr>
<td>180 (%)</td>
<td>33.0</td>
<td>29.2</td>
<td>49.2</td>
<td>39.7</td>
<td>38.4</td>
<td>20.3</td>
</tr>
<tr>
<td>270 (%)</td>
<td>24.5</td>
<td>20.1</td>
<td>36.1</td>
<td>26.7</td>
<td>27.4</td>
<td>10.9</td>
</tr>
<tr>
<td>365 (%)</td>
<td>18.9</td>
<td>14.6</td>
<td>26.5</td>
<td>19.6</td>
<td>20.3</td>
<td>6.8</td>
</tr>
</tbody>
</table>

Our study showed that in Southern Italy <30% of patients treated with antosteoporotic drugs is persistent with therapy at one year and <40% at six months. Therefore most people don’t make any therapeutic benefit in order to reduce risk fracture.

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**PP408**

Vertebroplasty vs kyphoplasty in osteoporotic vertebral fractures: a finite element comparative analysis

Luca Pietrogrande1, Claudia Ottardi2, Luigi La Barbera2, Emanuela Raimondo1 & Tomaso Villa3
1Dipartimento di Scienze della Salute, Università degli Studi, Milano, Italy; 2Laboratory of Biological Structure Mechanics, Department of Structural Engineering, Politecnico di Milano, Milano, Italy; 3IRCSS Istituto Ortopedico Galeazzi, Milano, Italy; 4UO Ortopedia, AO San Paolo, Milano, Italy.

Introduction
Vertebroplasty (VP) and ballon kyphoplasty (BKP) are used in the treatment of the vertebral compression fractures (VCF), that usually result in a typical wedged deformation. It is still under debate which technique is the best, in terms of efficacy, costs, and safety, mainly about the risk of a adjacent new fractures. The aim of this study is to evaluate the biomechanical outcome of vertebroplasty and kyphoplasty by a computational comparative analysis with finite element models.

Material and methods
A finite element model of intact T9–T11 spinal segment has been realized and then modified in order to simulate a wedge shaped VCF with a reduction of 25% (angle 13°) and 50% (angle 26°) of the original anterior height of T10 vertebral body. The following conditions have been considered for each model: osteoporotic bone (OP), vertebroplasty on T10 (VP), and kyphoplasty on T10 (KP).

Results
Vertebroplasty causes only a negligible variations in the intradiscal pressure (IDP) and on the stress values on the end-plates (EPs). Kyphoplasty with a total restoration produces a reduction of 5% in the IDP below the fracture, while in the EPs a significant reduction of the stress is noticed (20–50%). The presence of the cement core (effect of material) has a negligible, while the wedged shape of the fractured vertebra (effect of geometry) has a significant effect.

Conclusions
In conclusion it can be stated that the effect of cement injection in the fractured vertebra causes slight variations in stress distribution, as already found in previous
studies, that the effect of the geometry of the fractured vertebral body on stress
distribution on the EPs is significant, and, consequently, that kyphoplasty offers
some advantages respect to vertebroplasty in reducing the stress distribution, in
particular on the EPs, if the height of the vertebral body is restored.
DOI: 10.1530/boneabs.1.PP408

PP409
25-OH vitamin D and γ-3 TCR lymphocyte interplay in the pathogenesis of acute phase reaction after zoledronic acid infusion for osteoporosis treatment
Chiara Crotti, Francesca Cavaciocchi, Maria De Santis, Angela Ceribelli, Gianluigi Fabbriciani & Carlo Selmi
Humanitas Clinical and Research Center, Rozzano (Milan), Italy.

Background
Zoledronic acid (ZA) for the treatment of osteoporosis (OP) is associated with a transient post-infusional acute phase reaction (APR) due to ZA-mediated activation of γ-3 TCR lymphocytes (γ-3TCR) and production of cytokines.

Primary objective
To investigate if OP patients developing APR (APR+) after ZA infusion have lower 25-OH vitamin D (25-OHvD) levels and a higher percentage of γ-3TCR compared to patients without APR (APR−). Secondary objectives: to identify 25-OHvD level associated with a lower risk of APR; to investigate if there is an inverse correlation between 25-OHvD levels and γ-3TCR.

Methods
We enrolled 38 OP patients treated with 5 mg i.v. ZA. Before the first drug infusion, serum 25-OHvD levels were recorded and peripheral blood were drawn for T lymphocyte subpopulations FACS analysis (FACS Cytometer, La Fortessa, BD). APR occurrence was recorded by phone call 1 week after the infusion.

Results
19/38 (50%) patients developed APR. APR+ patients had significantly lower 25-OHvD levels compared to APR− patients (mean 22.1±2.9 ± 8.2 ng/ml vs mean 35.4±5.1 ± 17 ng/ml, P=0.0028). γ-3TCR were higher in APR+ patients compared to APR− patients (0.6±0.3 vs 0.38±0.3%, P=0.13). Patients with 25-OHvD levels >30 ng/ml had a significantly lower frequency of APR (2/19, 11% vs 11/19, 63%, P=0.0008; OR=14.57, CI 95% 2.57–82.73), and significantly higher γ-3TCR percentage (1.60±0.4 vs 0.56±0.6, P=0.024). 25-OHvD levels did not correlate with γ-3TCR percentage (r=−0.24, P=0.14). The lower APR frequency in patients previously treated with oral alendronates vs naïve patients (21 vs 63.2%) was dependent on 25-OHvD levels on logistic regression.

Conclusion
APR+ after ZA infusion have lower serum 25-OHvD levels, those with levels <30 ng/ml had a 25-fold higher risk for APR, suggesting that this concentration should be obtained before ZA infusion by supplementation. The possibility of a higher 25-OH vitamin D (25-OHvD) level associated with a lower risk of APR; to investigate if there is an inverse correlation between 25-OHvD levels and γ-3TCR.

DOI: 10.1530/boneabs.1.PP409

PP410
Bone turnover markers and radiographic progression of vertebral compression fractures during anti-osteoporotic therapy
Costantino Corradini1, Vittorio Macchi1, Stefano Pasqualotto1, Francesca Boisio1, Daniele Tradati1, Calogero Crapanzano2 & Cesare Verdúla1
1Orthopaedic and Traumatologic Clinic, State University of Milan c/o AO Orthopaedic Institute G.Pini, Milan, Italy; 2Unit of Clinical Pathology, AO Orthopaedic Institute G.Pini, Milan, Italy.

Introduction
In postmenopausal women with vertebral compression fractures (VCF) the mechanisms regulating healing processes and an anti-osteoporotic treatment are not completely clarified. The aim of this prospective study was the evaluation of bone turnover markers, bone mineral density and radiographic progression of one or more VCF during assumption of risedronate, strontium ranelate or teriparatide.

Materials and methods
Women with recent osteoporotic VCF verified through magnetic resonance were assigned to receive either risedronate (RIS group, n=19) or strontium ranelate (SR group n=16) or teriparatide (TPTD group, n=24) following guidelines of Italian regulatory agency. Serum and urinary bone turnover markers and lateral thoraco-lumbar spine X-rays were obtained at 0, 1, 3 and 6 months of therapy.

Bone Abstracts (2013) Vol 1 Lumbar BMD was measured by DEXA before and 6 months after treatment initiation.

Results
At time 0 serum markers of bone formation alkaline phosphatase (ALP), osteocalcin (OC) and of bone resorption desoxipiridoline (DIPD) but also osteoprotegerin (OPG) were around higher level of normality, while sclerostin (SOST) was substantially unchanged. Between 1st and 3rd month within the consolidation process OC peaked in TPTD group while those in RIS group and SR group remained significantly lower. In the same period ALP levels decreased in RIS group, unchanged in SR group and increased in TPTD group. DPD remained high in TPTD group; while in all groups were significantly and constantly reduced in 6 months. Serum OPG levels remained unchanged in RIS group and SR group while reduce in TPTD group. SOST was significantly increased 6 months in RIS group, whereas remained statistically unaffected in the TPTD group. Lumbar BMD increased significantly at 6 months in all groups and in particular in TPTD group. An inconstant progression in VCF on radiograms were detected in RIS and SR groups.

Conclusions
In recent osteoporotic VCF a divergence between the formation and resorption markers has been revealed between anti-osteoporotic therapies with a different radiographic progression.

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PP411
Changes in low back pain and upper gastrointestinal symptoms in Japanese osteoporotic patients after switching to once-monthly oral minodronate from daily or weekly bisphosphonates
Nobukazu Okimoto1,2, Akimori Sakai3, Satoshi Ikeda4, Toru Yoshioka4, Kita Teshima5, Hidehiro Matsumoto6, Hiroshi Tsukuraki7, Yuichi Okazaki8, Masato Nagashima9,10, Fumio Fukuda11 & Shinobu Arita12
1Okimoto Clinic, Hiroshima, Japan; 2Okimoto Orthopaedics and Sports Clinic, Hiroshima, Japan; 3University of Occupational and Environmental Health, Fukuoka, Japan; 4Ren-Ai Memorial Hospital, Fukuoka, Japan; 5Saka Midorior Hospital, Hiroshima, Japan; 6Teshima Orthopaedic Clinic, Fukuoka, Japan; 7Sanzai Hospital, Miyazaki, Japan; 8Tsukuraki Orthopaedic and Rheumatoid Clinic, Kumamoto, Japan; 9Makujama Central Hospital, Fukuoka, Japan; 10Katsuki Neurosurgery and Orthopaedic Clinic, Fukuoka, Japan; 11Kitakyushu General Hospital, Fukuoka, Japan; 12Osaka Hospital, Fukuoka, Japan.

Introduction
Minodronate, a new-generation bisphosphonate (BP), is the first BP available as a once-monthly oral minodronate (MIN50 mg) on low back pain (LBP) associated with osteoporosis. We also evaluated the changes in upper gastrointestinal (GI) symptoms (common adverse effects with the use of BPs) after switching from daily or weekly BPs to MIN50 mg.

Methods
We conducted a prospective multicenter study involving 11 institutions in Japan. A total of 389 patients (367 females) using BPs for the treatment of osteoporosis were enrolled. Participants completed a self-administered questionnaire to investigate patient preference for monthly dosing regimens, and were assigned to either the MIN50 mg (n = 258) or their current BP (n = 131) according to their preference. Upper GI symptom scores of heartburn, epigastralgia and epigastric fullness in the MIN50 mg-switched group were all significantly improved early at one month after switching, and the improvement was significantly superior compared with the previous BP-continued group (P < 0.05). Conclusion
MIN50 mg significantly improved LBP in patients previously treated with other BPs, and upper GI symptoms were significantly reduced after switching to MIN50 mg. These QOL-related benefits of MIN50 mg, together with the dosing convenience, may improve treatment adherence, thereby optimizing outcomes.

DOI: 10.1530/boneabs.1.PP411
Preoperative bisphosphonate treatment in patients with neuromuscular scoliosis improves bone strength of vertebral body
Masatumi Kashii, Yukitaka Nagamoto, Takahito Fujimori, Hirotugu Honda, Takashi Kaito, Hideki Yoshikawa & Motoki Iwasaki
1Osaka University Graduate School of Medicine, Suita, Osaka, Japan; 2Osaka National Hospital, Osaka, Japan.

Background
Boys with muscular dystrophy as presented by Duchenne muscular dystrophy (DMD) lose muscle strength and are usually confined to a wheelchair prior to 13 years. Furthermore they often have development of myogenic scoliosis, and scoliosis surgery is necessary to acquire sitting balance. Osteoporosis is one of the major concerns to perform surgical treatment. Patients with DMD or congenital muscular dystrophy (CMD) have fragile bones due to loss of ambulation, glucocorticoid therapy and DMD itself.

Object
To investigate BMD and bone metabolism in patients with muscular dystrophy and to verify efficacy and safety of preoperative bisphosphonate (BP) administration for osteoporosis associated with myogenic scoliosis.

Materials and methods
BMD and bone turnover markers were examined in 11 boys with muscular dystrophy who had undergone spinal surgery. A mean age was 14.5 years at surgery and all were non-ambulatory. BMD measurement was performed at lumbar spine (L2-4) and on total body. Patients were administered oral BP (Aldronate 35 mg) once a week, and BMD and bone turnover markers were measured before BP administration and after surgery.

Results
Mean lumbar BMD (L2-4) was 0.49 g/cm² (Z-score: -4.5) and mean thoracic, lumbar and pelvic BMD measured by total body scan were 0.50, 0.57 and 0.45 g/cm², respectively. All patients had severe osteoporosis with extremely high bone turnover (mean bone alkaline phosphatase: 60.6 μg/ml, TRACP5b: 928 μM/L). Mean duration of BP administration was 5.3 months. All patients could continue the drug without any side effects. Preoperative BP administration revealed a significant increase of L2-4 BMD (6.4%) and a significant decrease of bone turnover markers.

Conclusion
Patients with muscular dystrophy had severe osteoporosis with high bone turnover and preoperative BP administration improves bone fragility, myogenic scoliosis.

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Early response to once-monthly oral minodronate after switching from daily or weekly bisphosphonates in Japanese osteoporotic patients
Akinori Sakai, Satoshi Ikeda, Nobukazu Okimoto, Kitau Teshima, Shinobu Arita, Hidetoshi Matsumoto, Hiroshi Tsunukami, Yuichi Okazaki, Masato Nagashima, Fumio Fukuda & Toru Yoshio

University of Occupational and Environmental Health, Kitakyushu, Japan;
Ken-Ai Memorial Hospital, Onga, Japan;
Okimoto Clinic, Kure, Hiroshima, Japan;
Okamoto Orthopaedics and Sports Clinic, Hiroshima, Japan;
Okamoto Orthopaedic Clinic, Kitakyushu, Japan;
Obase Hospital, Kanda, Miyako, Japan;
Sanai Hospital, Saito, Japan;
Tsunukami Orthopaedic and Rheumatoid Clinic, Tamana, Japan;
Makizama Central Hospital, Kitakyushu, Japan;
Katsuki Neurosurgery and Orthopaedic Clinic, Nogata, Japan;
Kitakyushu General Hospital, Kitakyushu, Japan;
Saka Midorii Hospital, Hiroshima, Japan.

Introduction
Minodronate, a highly potent, new-generation bisphosphonate (BP), is the first BP available as a once-monthly oral regimen in Japan. The aim of the present study was to investigate the effects of once-monthly oral minodronate on bone turnover markers (BTM) and bone mineral density (BMD) in osteoporotic patients previously using daily or weekly BPs in real clinical practice.

Methods
We conducted a prospective multicenter study involving 11 institutions in Japan. A total of 389 patients (367 females) using BPs for the treatment of osteoporosis were enrolled. Participants were divided into two groups depending on their preference for dosing regimens as follows: MIN50 mg group (n=258) was switched to once-monthly minodronate (50 mg), and daily BP group (n=131) continued their current daily or weekly BP. Serum TRACP-5b was measured at baseline and 1, 2 and 6 months post-treatment. Serum P1NP was measured at baseline and 2 and 6 months post-treatment. BMD of lumbar spine, total hip, and/or 1/3 distal radius were measured at baseline and 6 months post-treatment.

Results
In MIN50 mg group, significant reductions were seen in TRACP-5b at 1 month post-treatment (~10.3%, P<0.01) and onward, and in P1NP at 2 months post-treatment (~8.0%, P<0.01) and onward, while remaining within the reference range for a healthy young adult. BMD in the MIN50 mg group was significantly increased at lumbar (+1.4%, P<0.01) and radius (+1.1%, P<0.01) at 6 months after therapy; however no significant changes were seen in the daily BP group.

Conclusion
Once-monthly minodronate after switched from daily or weekly BPs demonstrated prompt BTM suppression within the normal reference range and superior BMD gains compared with continuing previous BPs. Thus, once-monthly minodronate provides an effective and convenient alternative to current BP therapies.

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Treatment with eldecalcitol (ED-71) and raloxifene combined increases cancellous and cortical bone strength in ovariectomized rats
Sadaoki Sakai, Satoshi Takeda, Ayako Shiraishi, Nobuo Koike, Masahiko Mihara & Koichi Endo
Chugai Pharmaceutical Co., Ltd., Gotemba, Japan.

Eldecalcitol (ED-71; ELD), a 25-hydroxyproloxy derivative of 1α,25(OH)2D3, was approved to treat osteoporosis in Japan in 2011. Raloxifene (RAL), a selective estrogen receptor modulator, is available to treat or prevent postmenopausal osteoporosis. In this study, we compared the effects of combining ELD and RAL after each monotherapy in osteoporotic rats. Eight-month-old female Wistar–Imamichi rats were ovariectomized (OVX) and administered either ELD (7.5 mg/kg), RAL (0.3 mg/kg) or ELD plus RAL daily by oral gavage for 12 weeks. Urinary deoxypyridinoline (DPD), a marker of bone resorption, was reduced significantly in the combination therapy group compared to either the ELD or RAL groups after 4 weeks of treatment. DPD in combination therapy group remained a lower level than in the RAL monotherapy group until the end of experiment. Both lumbar spine and distal femur bone mineral density (BMD) were higher in combination group than either monotherapy group. Bone strength of lumbar vertebra in compression and the femoral midshaft in three-point bending were significantly higher in combination group than vehicle treatment group. Bone histomorphometric analysis revealed that osteoblast surface (Ob./SBS) and osteoclast surface (Oc./SBS) decreased in all the agent-treated groups. Ob./SBS in the combination group was significantly lower than in both monotherapy groups, but not less than sham control group. Mineral apposition rate (MAR) and bone formation rate (BFR) were significantly reduced in the combination group to sham control level. ELD (10⁻⁷ M) and RAL (10⁻¹⁰ M) inhibited in vitro osteoclastogenesis of mouse bone marrow cells, and ELD combined with RAL more potently inhibited osteoclast differentiation than RAL alone.

In summary, the simultaneous administration of ELD and RAL enhanced cancellous and cortical bone strength in ovariectomized rats. It reduced bone turnover in vivo and inhibited bone marrow osteoclastogenesis in vitro, without excess suppression of bone formation.

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Patient preference and adherence to once-monthly oral minodronate in Japanese osteoporotic patients previously using daily or weekly bisphosphonates
Satoshi Ikeda, Akinori Sakai, Nobukazu Okimoto, Kitau Teshima, Shinobu Arita, Hidetoshi Matsumoto, Hiroshi Tsunukami, Yuichi Okazaki, Masato Nagashima, Fumio Fukuda & Toru Yoshio
Ken-Ai Memorial Hospital, Onga, Japan;
University of Occupational and Environmental Health, Kitakyushu, Japan;
Okimoto Clinic, Kure, Hiroshima, Japan;
Okamoto Orthopaedics and Sports Clinic, Hiroshima, Japan;
Obase Hospital, Kanda, Miyako, Japan;
Sanai Hospital, Saito, Japan;
Tsunukami Orthopaedic and Rheumatoid Clinic, Tamana, Japan;
Makizama Central Hospital, Kitakyushu, Japan;
Katsuki Neurosurgery and Orthopaedic Clinic, Nogata, Japan;
Kitakyushu General Hospital, Kitakyushu, Japan;
Saka Midorii Hospital, Hiroshima, Japan.

Introduction
Minodronate, a highly potent, new-generation bisphosphonate (BP), is the first BP available as a once-monthly oral regimen in Japan. The aim of the present study was to investigate the effects of once-monthly oral minodronate on bone turnover markers (BTM) and bone mineral density (BMD) in osteoporotic patients previously using daily or weekly BPs in real clinical practice.

Methods
We conducted a prospective multicenter study involving 11 institutions in Japan. A total of 389 patients (367 females) using BPs for the treatment of osteoporosis were enrolled. Participants were divided into two groups depending on their preference for dosing regimens as follows: MIN50 mg group (n=258) was switched to once-monthly minodronate (50 mg), and daily BP group (n=131) continued their current daily or weekly BP. Serum TRACP-5b was measured at baseline and 1, 2 and 6 months post-treatment. Serum P1NP was measured at baseline and 2 and 6 months post-treatment. BMD of lumbar spine, total hip, and/or 1/3 distal radius were measured at baseline and 6 months post-treatment.

Results
In MIN50 mg group, significant reductions were seen in TRACP-5b at 1 month post-treatment (~10.3%, P<0.01) and onward, and in P1NP at 2 months post-treatment (~8.0%, P<0.01) and onward, while remaining within the reference range for a healthy young adult. BMD in the MIN50 mg group was significantly increased at lumbar (+1.4%, P<0.01) and radius (+1.1%, P<0.01) at 6 months after therapy; however no significant changes were seen in the daily BP group.

Conclusion
Once-monthly minodronate after switched from daily or weekly BPs demonstrated prompt BTM suppression within the normal reference range and superior BMD gains compared with continuing previous BPs. Thus, once-monthly minodronate provides an effective and convenient alternative to current BP therapies.

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PP416 Meta-analysis of the effects of vitamin D supplements on bone mineral density in adults
Ian R Reid, Mark Bolland & Andrew Grey
Department of Medicine, University of Auckland, Auckland, New Zealand.

Recent meta-analyses of vitamin D without co-administration of calcium have not demonstrated fracture prevention, possibly through lack of power, inappropriate choice of doses, or failure to target the intervention to deficient populations. Bone mineral density (BMD) is able to detect biologically significant effects in much smaller cohorts, so is a relevant surrogate measure with which to re-assess the skeletal efficacy of these supplements. We searched Web of Science, Embase and the Cochrane Database for randomized trials comparing interventions that differed only in vitamin D content (D3 or D2, but not a vitamin D metabolite), and presented BMD results. Studies in groups with other metabolic conditions were excluded, so was a relevant surrogate measure with which to re-assess the skeletal efficacy of these supplements. We therefore analyzed bone mineral properties in a bone biopsy obtained at the fracture site from an 88-year-old female with AFF, who had been treated with alendronate for 8 years. The whole body quantitative computed tomography (qCT) and dynamic quantitative computed tomography (dQCT) showed a decrease in lean mass and bone density, and an increase in bone trabecular density, except at the femoral neck where there were small increases of uncertain clinical significance. The widespread use of vitamin D supplements in the management of osteoporosis should, therefore, be re-examined.

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PP417 A case of atypical femoral fracture with abnormal cortical bone characterized by impaired mineralization and pyrophosphate accumulation
Maziar Shabestari1, Erik Fink Eriksen2, Paul Rosagher3, Eleftherios Paschalis4 & Adolfo Diez-Perez2
1University of Oslo, Oslo, Norway; 2Oslo University Hospital, Oslo, Norway; 3Hanusch Hospital of WGiK, Vienna, Austria; 4Department of Internal Medicine, Barcelona, Spain.

Impaired bone material properties have been invoked as being responsible for the development of atypical femoral fractures (AFF) after long term bisphosphonate use. We therefore analyzed bone material properties in a bone biopsy obtained at the fracture site from an 88-year-old female with AFF, who had been treated with alendronate for 8 years. We used conventional histology, quantitative back-scattered electron imaging (qBEI), and Raman spectroscopy (RS). Histology revealed numerous eroded surfaces, widened osteoid seams, and osteocytic osteolysis. qBEI exhibited a scaffold of highly mineralized, porous bone matrix with numerous enlarged, osteocyte lacunae. Bone mineralization density distribution (BMDd) was shifted towards lower and more heterogenous mineralization compared to a normal reference database: mean calcium content (CaMean = 4.1%, CaPeak = 1.8%), mineralization heterogeneity (CaWidth = 29.3%), bone with reduced mineralization (CaLow = 11%), bone with increased mineralization (CaHigh = 2%). RS data obtained at open osteons were compared with iliac crest biopsies from 35 healthy premenopausal, 16 treatment-naive osteoporotic women (PMGc) and osteoporotic females (OP) treated with different bisphosphonates. The mineral/matrix ratio of AFF bone was similar to two alendronate and two risedronate groups, lower than PMC, and higher than either OP or OP-zoledronate groups. The proteoglycan content was higher in the AFF biopsy compared to all other groups. The mineral crystallinity of AFF bone was similar to both ALN groups, but higher compared to all other groups. Most significantly, however, we detected increased levels of pyrophosphate at osteoid/mineralized bone interfaces in AFF bone, a feature absent in other biopsies obtained from subjects after long term bisphosphonate treatment.

In conclusion, bone from this case of AFF showed several abnormalities: i) altered arrangement of osteons ii) impaired mineralization and iii) Appreciable pyrophosphate accumulation, which might cause the impaired mineralization. Taken together, these changes may be responsible for the focally reduced bone strength in AFF.

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PP418 Myricetin suppress LPS-induced MMP expression in human periodontal ligament fibroblasts and inhibit osteoclastogenesis by downregulating NFATC1 in LPS-induced RAW 264.7 cells
Seon-Yie Ko & Young-Joo Jang
Dankook University, Cheonan, Republic of Korea.

Periodontitis is an inflammatory disease that affects connective tissue attachments and the supporting bone that surrounds the teeth. Periodontal ligament fibroblasts (PDLF) induce the overexpression of matrix metalloproteinase (MMP), which is involved in inflammatory progression in periodontitis. Osteoclasts are responsible for skeletal remodeling and remodeling but may also destroy bone in several bone diseases, including osteoporosis and periodontitis. This study examined the anti-destructive effects of myricetin on human periodontal ligament fibroblasts (PDLF) under lipopolysaccharide (LPS)-induced inflammatory conditions, and the anti-osteoclastogenic effect of myricetin on the LPS-induced RAW264.7 cells was also investigated. The effects of myricetin on PDLF were determined by measuring the cell viability and mRNA expression and enzyme activity of tissue-destructive proteins, including MMP-1, MMP-2, and MMP-3. The effects of myricetin on osteoclasts were examined by measuring the following: i) the cell viability, ii) the formation of tartrate-resistant acid phosphatase (TRAP+) multinucleated cells, iii) MAPK signaling pathways, iv) mRNA expression of osteoclast-associated genes, v) nitric oxide (NO) and interleukin 6 (IL6) secretion and v) mRNA expression and enzyme activity of MMP-8. The myricetin had no effects on the cell viability of the PDLF and decreased the mRNA expression and enzyme activity of MMP-2 and MMP-3 in the PDLF. Myricetin inhibited the formation of LPS-stimulated TRAP(+) multinucleated cells. Myricetin also inhibited the LPS-stimulated activation of ERK signaling in the RAW264.7 cells. The LPS-stimulated induction of NFATc1 transcription factors was abrogated by myricetin. Myricetin decreased the mRNA expression of osteoclast-associated genes, including TRAP and cathepsin K in the RAW264.7 cells. Myricetin inhibited the secretion of LPS-induced NO and IL6 in the RAW264.7 cells. In addition, myricetin decreased the mRNA expression and enzyme activity of

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MMP-8 in the RAW264.7 cells. These findings suggest that myricetin has therapeutic effects on bone-destructive processes, such as those that occur in periodontal diseases.

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PP419

Analysis of clinical assessment and efficacy of once-yearly i.v. zoledronic acid for osteoporosis

Ye-Soo Park1, Hong-Sik Kim1, Jung-Hwan Lee1, Ye-Yeon Won2 & Byung-Moon Kang3
1Department of Orthopaedic Surgery, Guri Hospital, Hanyang University College of Medicine, Guri, Kyunggi-do, Republic of Korea; 2Department of Orthopaedic Surgery, Ajou University Hospital College of Medicine, Suwon, Kyunggi-do, Republic of Korea; 3Department of Obstetrics and Gynecology, Asan Medical Center, Ulsan University College of Medicine, Seoul, Republic of Korea.

Introduction
To analyze clinical assessment and efficacy of once-yearly i.v. zoledronic acid for osteoporosis.

Materials and methods
The subjects were 322 osteoporotic patients who received more than single infusion of zoledronic acid in our hospital from October 2008 to March 2011. On clinical assessment, the adherence was evaluated by measuring the rate of reinfusion. Adverse events were recorded for safety assessment. For efficacy assessment, the bone mineral density (BMD) and bone turnover marker were measured before and after infusion.

Results
Excluding the patients lost to follow-up after 1 year, 107 patients (47.6%) received the second infusion, continuously. For patients with second infusion, 41 patients (51.3%) permanently received the third infusion, except the patients lost to follow-up. The economic strain was the most common reason for non-adherence which accounted for 43.4%, and the incognition or the indifference was the second most common reason for non-adherence making up 30.8%. The adverse events were reported for 122 patients (38.2%), but the serious adverse events were not reported BMD at baseline was mean – 3.24 ± 0.63 by T-score. Mean BMD was measured at – 2.98 ± 0.65 and – 2.82 ± 0.59 in 1 and 2 years follow-up, respectively, and significantly increased (P value < 0.001). C-telopeptide at baseline was mean – 0.54 ± 0.34. Mean C-telopeptide was measured at – 0.20 ± 0.11 and – 0.23 ± 0.11 in 1 and 2 years follow-up, respectively, and significantly decreased (P value = 0.003).

Conclusion
In this study, the infusion of once-yearly i.v. zoledronic acid for osteoporotic patients decreased bone resorption and improved bone mineral density. Serious adverse events were not reported. The adherence was higher than the most published studies of adherence to oral bisphosphonates, but lower than optimal. Because incognition or indifference was major cause of non-adherence, the physicians should explain the efficacy and adverse effect of this agent.

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PP420

Subsequent hip fracture in Inchon and Bucheon area of Korea
(Cohort study)

Kyoung Ho Moon1, Ju Young Kim2 & Kee Haeng Lee2
1School of Medicine, Inha University, Incheon, Republic of Korea; 2College of Medicine, The Catholic University of Korea, Bucheon, Republic of Korea.

Introduction
A significant number of patient who have experienced previous surgical treatment for an osteoprotic hip fracture, experienced a subsequent hip fracture (SHF) on the opposite side. The incidence of asynchronous bilateral hip fractures is 1.7–14.8%. All hip fracture patients treated at five university hospitals in the Inchon and Bucheon area of Korea, were reviewed. The patients were divided into two groups, a group that had experienced subsequent hip fractures, and a group that had not. The authors analyzed the incidence of subsequent hip fracture (SHF) and its risk factors.

Materials and methods
We analyzed 2748 hip fracture patients from January 2000 to December 2010 at five university hospitals. Unilateral hip fracture patients who received no osteoporosis treatment at the time of the incident were included. Patients with history of a traffic accident, who had fallen from a height higher than the patient’s height, or with a history of pathologic fracture were excluded. Patient identification was cross checked between university hospitals in order to prevent double counting overlapping patients and to obtain an accurate count of incidence. Medical records were reviewed and presence of SHF, alcohol history, marriage status, dementia, dizziness, ASA score, osteoporosis treatment after fracture, BMI and BMD (initial and last 10 years) were analyzed.

Results
The average follow-up period was 12 months (range: 1–130 months). A total of 2546 patients (F: 1769, M: 777) who had experienced unilateral hip fractures were included. Of these, subsequent hip fractures were found in 202 patients (7.4%); (F: 169, M: 33). Mean age at the time of the first fracture was 79.2 years old (range: 50–100 years). The average interval between the first fracture and the SHF was 30.2 months (4 days–154 months). Female gender, a BMI under 22 kg/m², and being unmarried were revealed as the risk factors for subsequent fracture by multivariate analysis.

Conclusions
In this large-scale, retrospective, multicenter study, overall incidence of subsequent hip fractures was 7.4%. Independent risk factors of subsequent fracture were female gender, low BMI (<22 kg/m²), and being unmarried.

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PP421

The efficiency of bisphosphonates (alendronate, risedronate, ibandronate) for postmenopausal osteoporosis after 1 year of therapy

Diana Paun1,2, Nicoleta Totolici1, Monica Chirita1, Rodica Petris1 & Constantin Dumitrache1,2
1C.I. Parhon National Institute of Endocrinology, Bucharest, Romania; 2Carol Davila University of Medicine and Pharmacy, Bucharest, Romania.

Introduction
Bisphosphonates are drugs of first choice in the treatment of postmenopausal osteoporosis; they inhibit bone resorption. This study evaluates the efficiency of bisphosphonates (alendronate + cholecalciferol 70 mg/5600 IU per weekly vs risedronate acid 35 mg/weekly vs ibandronate acid 150 mg/monthly) after 1 year of therapy.

Methods
We present the results of a retrospective study which included 40 women with postmenopausal osteoporosis treated with one of these bisphosphonates. We evaluated (at the beginning and after 1 year): T-score, markers of bone formation (osteocalcin) and bone resorption (cross-laps), vitamin D, calcemia, phosphate-muria, the presence of fracture, and risk factors.

Results
Alendronate + cholecalciferol (A), 14 patients; risedronate (R), 11 patients; ibandronate (I), 15 patients. The average age was: 64.07 ± 5.7 s.d. years (A), 63.27 ± 6.57 s.d. years (R), 63.06 ± 11.89 s.d. years (I), in postmenopausal for 14.21 ± 4.24 s.d. years (A), 17 ± 5.56 s.d. years (R), 17 ± 7.12 s.d. years (I). The values of parameters at (the beginning and after 1 year) were: T-score (s.d.): –3.4 ± 0.47 (A); –2.83 ± 0.54 (A); –3.43 ± 0.33 (R); –3.36 ± 0.47 (R); –3.24 ± 0.51 (I) –2.97 ± 0.54 (I). Osteocalcin (ng/ml): 15.55 ± 8.02 (A) ± 14.21 ± 8.45 (A); 15.33 ± 7.06 ± 13.95 ± 4.87 (R); 16.24 ± 9.37 ± 14.52 ± 9.71 (I). Cross-laps (ng/ml): 0.63 ± 0.45 ± 0.51 ± 0.5 (A); 0.72 ± 0.67 ± 0.59 ± 0.56 (R); 0.7 ± 0.29 (I) –0.63 ± 0.58 (I) Vitamin D (ng/ml): 16.98 ± 10.36 ± 23.77 ± 11.08 (A); 19.66 ± 8.66 ± 27.37 ± 10.67 (R); 14.18 ± 6.09 ± 20.12 ± 9.44 (I). Calcemia (mg/dl): 9.54 ± 0.62 ± 9.52 ± 0.6 (A); 9.73 ± 0.49 ± 9.66 ± 0.37 (R); 9.27 ± 0.47 ± 9.48 ± 0.55 (I) Phosphate (mg/dl): 3.43 ± 0.45 ± 3.4 ± 0.48 (A); 3.33 ± 0.41 ± 3.18 ± 0.44 (R); 3.54 ± 0.46 ± 3.4 ± 0.4 (I) Conclusions
All three bisphosphonates had beneficial effects on bone reflected by an improvement of odontosistometry score and turn-over markers (reducing their level); vitamin D deficiency was noticed for most women.

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**PP422**

**Bisphosphonates poisonous action**
Buyko Maria & Atrushkevich Victoria
Moscow State University of Medicine and Dentistry, Moscow, Russia.

**Introduction**
Bisphosphonates gain increasingly greater significance in treatment oncologic diseases with bone metastasis. However, a lot of articles have lately been published in dental and oncological journals on jaw osteonecrosis (ONJ) associated with long-term ingestion of bisphosphonates. Besides extension of clinical recommendations for zolendronate use, most commonly referred to in connection with ONJ, for treatment of Paget’s disease and other disturbances of bone metabolism calls for further research to determine a poisonous dosage of bisphosphonates causing ONJ.

**Materials and methods**
30 Wistar female rats (age 0.5–1 year, average weight 399 ± 0.2 g) were randomly taken for investigation.

Rats were divided in three groups: 1st group (average weight 431 ± 0.23 g), 15 rats were subject to ovariectomy; 2nd group (average weight 389 ± 0.3 g), 15 rats, surgery wasimitated; 3rd group (average weight 379 g), controls. Rats were kept in big cages at 20°C, in good hygienic condition and were isolated from any infection that could interfere with experiment results.

1st and 2nd groups were given an i.v. injection of Aclasta (zoledronate), 0.04 ml. In 10 months i.v. injection of Aclasta was repeated to 1st and 2nd groups. Saline was injected to controls.

**Results**
In 2 months after the 2nd injection four out of ten spayed rats in 1st group had focis of osteonecrosis in ramus area. Maxilla was covered with fibrous pellicle in necrotic area. Other six rats didn’t have signs of necrosis. 2nd group and controls didn’t have any signs of ONJ.

**Conclusion**
Necrosis rate in spayed rats composed 25% which corresponds to epidemiological evidence. Besides in our study we determined an i.v. dosage of Aclasta – 0.4 ml – which causes spontaneous development of ONJ in female rats.

**PP424**

**Acute phase response and zoledronic acid therapy**
Svetlana Yureneva, Oksana Yakusheskaya, Vera Smetnik & Gennady Sukchich
Research Center of Obstetrics, Gynecology and Perinatology, Moscow, Russia.

**Introduction**
We aimed to study acceptability of zoledronic acid in the treatment of postmenopausal osteoporosis within 3 years.

**Methods**
Clinical, biochemical, shipping registration at poll by phone and on the subsequent visits.

**Results**
We studied 225 patients with postmenopausal osteoporosis. The patients were treated with zoledronic acid (Zol) 5 mg as a once-yearly infusion within 3 years and 2500 mg of calcium carbonate + 800 ME vitamin D3 daily. 110 (48.9%) patients received paracetamol (1000 mg – three times a day, 3 days) for prevention of side effects on the day of the first infusion and the next 2 days.

We found out that symptoms of acute phase response (SAPR) developed within 12–24 h after the infusion of Zol. SAPR were observed in 39% (n = 43) of patients, who received paracetamol as prevention. Among those patients without treatment with paracetamol, SAPR developed at the rate of 65.3% (OR = 0.34, 95% CI (0.2–0.59)). After the 2nd infusion symptoms of SAPR were reported in 27.9% (n = 26) (< 0.05) out of 93 patients, after the 3rd in 6.6% (n = 2) out of 30 patients. Symptoms of light (77.3%, n = 58) and moderate severity (16%, n = 12), and up to 3 day duration (68%, n = 51) were observed after the 1st infusion. Bone and muscular manifestations and a flu-like syndrome were the most frequent symptoms (P < 0.05).

**Conclusions**
Symptoms of acute phase response developed within the first 12–24 h after the infusion of zoledronic acid. SAPR were effectively prevented and treated with paracetamol. The reduction of quantity, severity and duration of SAPR was observed after each subsequent infusion.

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**PP423**

**Three years’ experience of zoledronic acid use in the treatment of postmenopausal osteoporosis**
Svetlana Yureneva, Oksana Yakusheskaya, Sergey Kuznetsov, Tatyana Ivanets, Vera Smetnik & Gennady Sukchich
Research center of obstetrics, gynecology and perinatology, Moscow, Russia.

**Introduction**
We aimed to study efficacy of zoledronic acid (Zol) in the treatment of postmenopausal osteoporosis within 3 years.

**Methods**
Clinical, bone mineral density (BMD) by DEXA (L1-L4, femoral Neck) (baseline, 12, 24, and 36 months); biochemical; immunoenzymase assay of bone turnover markers (BTM) – osteocalcin (OK) β-terminal telopeptides of type I collagen (CTX) (baseline, 1, 3, 6, 9, and 12 after one, two, three infusions). Results
225 women with postmenopausal osteoporosis (using T-score DEXA) were treated with Zol 5 mg as a once-yearly infusion for 3 years + 2500 mg of calcium carbonate + 800 ME vitamin D3 daily. 107 (47.5%) patients had previous atraumatic fractures. After the 1st infusion of Zol we observed a significant decrease in CTX – 88% (P = 0.008), −84, −82, −75, and −63%; in OK – 28% (P = 0.026), −49, −51, −46, and −42%. After the 2nd and 3rd infusions of Zol the reaction of BTM was similar, though in a lesser degree after each subsequent infusion. At the L1-L4 BMD increased by 7%, at the femoral neck by 5.9% after 36 months (P < 0.05).

**Conclusions**
Zol has a powerful antiresorptive effect on bone turnover. Preferential suppression of bone resorption led to a positive balance of bone turnover and increase in BMD in the lumbar spine and the femoral neck. Measurement of CTX, after 1 month of infusion provides for evaluation of a patient’s response to therapy with Zol and reveal ‘poor’ responders.

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**PP425**

**Evaluation with densitometry of patients with breast cancer and low bone mineral density after 2 years of treatment**
Sonia Muñoz Gil1, Tomás Muñoz Díaz2, Belén García López1, M D Torregrosa Maicas1, R Girones Sarrió1, P López Tendero2, M D García Armario3 & Pascual Muñoz Mira1
1Hospital de Manises, Manises, Valencia, Spain; 2Hospital de La Ribera, Alzira, Valencia, Spain; 3Hospital Lluís Alcanyís, Xàtiva, Valencia, Spain; 4Hospital General de Ontinyent, Ontinyent, Valencia, Spain.

**Aim**
Evaluate the differences with densitometry after 2-year treatment in patients with breast cancer and LBMD.

**Materials and methods**
A 2 year duration longitudinal study was done in patients diagnosed with breast cancer sent to the Rheumatology Osteoporosis Unit in Hospital d’Ontinyent, who required supplements of calcium and vitamin D+ bisphosphonates after a risk fracture study. Socio-demographic data, breast tumor characteristics, risk factors for osteoporosis and fragile fractures, definite diagnosis and the treatment initiated were required supplements of calcium and vitamin D+ bisphosphonates after a risk fracture study. Socio-demographic data, breast tumor characteristics, risk factors for osteoporosis and fragile fractures, definite diagnosis and the treatment initiated were registered. Differences between mean values obtained in BMD of lumbar, total femoral, and femoral neck, were evaluated with student’s t-test study.

**Results**
61 patients were studied, with an average age of 59 years old (37–79 years). All had unilateral breast cancer, while none had metastases. Treatments received were: radical mastectomy (56%), radiotherapy (64%), chemotherapy (71%), hormonotherapy (30%), tamoxifen (41%), GnRH analogues (13%), and aromatase inhibitors (90%). High-risk osteoporosis was diagnosed in 6 patients, osteoporosis in 19 patients and osteopenia in 26 patients. In spinal X-rays, 26 patients had ≥ 1 vertebral collapse and 4 of them ≥ 1 vertebral fracture. Treatment with supplements of calcium and vitamin D was initiated in 85.2%, and bisphosphonates (oral or i.v.) in 41 patients, as follow: ibandronate (15), risedronate (16), alendronate (8), and zoledronate (2).

After a 2-year follow-up, only one patient had developed metastases, 75.4% continued with aromatase inhibitors and only two had abandoned treatment. None suffered new vertebral collapse or fracture, only one suffered from other fractures.

**Conclusions**
Patients with breast cancer that require initiating treatment for fragile risk fracture present good treatment compliance. Treatment including supplements of calcium.
and vitamin D and bisphosphonates during two years improves the mineral bone density, finding statistically significative differences in femoral neck (BMD, T-score, Z-score) and in all localizations (lumbar, femoral neck and total femoral) worsening Z-score.

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PP426
Characterization and incidence on acute phase reaction in Paget’s disease after zoledronic acid infusion
A Conesa Mateos, D Rotés Sala & J Carbonell Abelló
Rheumatology, Hospital del Mar, Parc de Salut Mar. Barcelona, Spain.

Zoledronic acid (AZ), is considered first-line treatment for Paget’s disease (PD) of bone. The most common adverse event is flu-like syndrome, described between 10 and 50% of patients. Nowadays, there is not known exactly the molecular basis of this syndrome yet. Statins play an important role in the mevalonate pathway, blocking the production of proinflammatory cytokines secreted by T cells γδ. Objectives
Characterization and incidence of adverse events (AEs) secondary to treatment with AZ in patients with PD. Evaluate the impact of statins on AZ-induced flu-like syndrome.

Methods
A prospective open-label study was conducted in 50 patients with active PD after 2 years period. Each patient received a single 5 mg i.v. infusion of AZ over a 15-min period.

Results
Baseline characteristics of patients with active PD: gender: 50 (23F/27M). Mean age at diagnosis (years): 59±12.5. Mean duration of the disease (years): 14.5±8.5. Regarding to AE, there was not hematological, renal, gastrointestinal or liver toxicity detected after infusion of 5 mg AZ nor during the following period. The most frequent side effect was flu-like symptoms, observed in 54% of patients. The incidence of fever was detected in 100% of the patients affected. There was not statistically significant correlation between the presence of flu-like syndrome and a gender, scintigraphic distribution, duration of the disease, number of locations, serum alkaline phosphatase at diagnosis. Instead, it was observed a statistically significant correlation between age at diagnosis, baseline plasma calcium, 1,25-dihydroxyvitamin D3 at baseline and prolonged therapy (>3 m) with statins, with the presence of flu-like syndrome. Patients presenting flu-like symptoms had a lower serum 1,25-dihydroxyvitamin D3 at baseline and 1,25-dihydroxyvitamin D3 above average, and did not maintain statin therapy.

Conclusions
Nearly 30% of patients with osteoporotic VF treated with VP had a new VF after the procedure. Age, especially over 80 years, the presence of inferior disk cement leakage after VP, the number of cemented vertebrae and low 25OHD serum levels were related to the development of new VF; the latter indicating the need to correct vitamin D deficiency prior to performing VP.

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PP428
Persistence with different anti-osteoporosis medications: a population-based cohort study.
Ana Pages-Castella1, Cristina Carbonell-Abella2, Xavier Nogues2,3, M Kassim Javaid4, Nigel K Kendal5, Cirus Cooper4, Adolfo Diez-Perez3,4 & Daniel Prieto-Alhambra1,4
1GREMPAL Research Group (USR Barcelona), IDIAP Jordi Gol, Universitat Autònoma de Barcelona, Barcelona, Spain; 2URFOA, Institut Municipal d’Investigacions Mèdiques, Parc de Salut Mar-Universitat Autònoma de Barcelona, Barcelona, Spain; 3RETICEF (Red Temática de Investigación Cooperativa en Envejecimiento y Fragilidad), Instituto Carlos III, Barcelona, Spain; 4Oxford NIHR Musculoskeletal Biomedical Research Unit, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, Oxford, UK; 5MRC Lifecourse Epidemiology Unit, Southampton, UK.

Objective
Several reports suggest very low persistence with oral bisphosphonates, but there is a scarcity of data on persistence with other anti-osteoporosis medications. We therefore compared rates of early discontinuation (in the first year of therapy) between all available outpatient anti-osteoporosis drugs in Catalonia, Spain. Study design
Population-based retrospective cohort study.

Participants and source of data
The data in this study were obtained from the SIDIAP database (www.sidiap.org). We included all SIDIAP participants starting an OP drug at anytime between 1/1/2007 and 30/06/2011. We modelled time between the first prescription and the date of therapy discontinuation using Fine and Gray survival models with competing risk for death.

Results
We identified 127,722 participants. The most commonly prescribed drug was weekly alendronate (n=55,399). Discontinuation in the first year of therapy was very common, ranging from 50.3% (monthly risedronate) to 77.6% (raloxifene). Only monthly risendronate (RIS) had better persistence (adjusted SHR 0.89 (0.86–0.92)), whilst daily drugs had the worst: daily Alendronate (ALN) SHR 1.67 (1.54–1.80), daily Risedronate (RIS) 1.86 (1.74–1.99), Raloxifene 1.43 (1.40–1.45), Bazedoxifene 1.41 (1.29–1.54), and Strontium Ranelate 1.51 (1.48–1.53). Persistence with PTH analogues was similar to that of weekly ALN (SHR 1.02 (0.98–1.07)).

Conclusions
Early discontinuation with available therapies for Osteoporosis is very common. Monthly RIS and Weekly ALN are the drugs with best persistence. There are significant differences in risk of discontinuation in the first year of treatment: daily drugs have a 40–60% higher discontinuation risk than weekly ALN.

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PP429
Risk factors for the development of vertebral fractures after percutaneous vertebroplasty
Angels Martinez-Ferrer, Jordi Blasco, Laia Gifre, Ana Monegal, Nuria Guanabens & Pilar Peris
Hospital Clinic, Barcelona, Spain.

We recently observed an increased risk for vertebral fractures (VF) in a randomized controlled trial comparing the analgesic effect of vertebroplasty (VP) vs conservative treatment (CT) in symptomatic VF. The aim of the present study was to evaluate the risk factors related to the development of VF after VP in these patients.

Methods and results
We evaluated risk factors including age, gender, bone mineral density, the number, type and severity of vertebral deformities at baseline, the number of vertebral bodies treated, the presence and location of disk cement leakage, bone remodeling (determining bone turnover markers) and 25-hydroxyvitamin D (25OHD) levels at baseline in all the patients (57 with VP and 61 with CT). Twenty-nine radiologically new VF were observed in 17/57 patients undergoing VP (72% adjacent to the VP) and 11 new VF in those receiving CT (27% adjacent to previous VF). Patients developing VF after VP showed an increased prevalence of 25OHD deficiency (<20 ng/ml) and higher PINP values than patients without new VF. 25OHD levels <20 ng/ml were the principal factor related to the development of VF after VP on multivariate analysis (RR 15.47; 95% CI 2.99–79.86, P < 0.0001), whereas age >80 years (RR 3.20; 95% CI 1.70–6.03, P = 0.0007) and glucocorticoid treatment (RR, 3.64; 95% CI, 1.61–8.26, P = 0.0055) constituted the principal factors in the overall study population. Increased risk of VF after VP was also associated with cement leakage into the inferior disk (RR 6.14; 95% CI, 1.65–22.78, P = 0.044) and >1 vertebral body treated during VP (RR 4.19; 95% CI, 1.03–15.43, P = 0.044).

Conclusions
Nearly 30% of patients with osteoporotic VF treated with VP had a new VF after the procedure. Age, especially over 80 years, the presence of inferior disk cement leakage after VP, the number of cemented vertebrae and low 25OHD serum levels were related to the development of new VF; the latter indicating the need to correct vitamin D deficiency prior to performing VP.

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Bone Abstracts (2013) Vol 1
PP430
First in man study of a novel and highly selective cathepsin K inhibitor miv-711 – safety, pharmacokinetics and pharmacodynamics of single ascending oral doses in healthy subjects
Aim
To determine the safety, tolerability, pharmacokinetics and pharmacodynamics of the cathepsin K inhibitor MIV-711.
Methods
A double-blind, placebo-controlled, randomized study in 27 healthy subjects of both genders. Single ascending doses 20-600 mg were investigated for adverse events, clinical chemistry, vital signs, ECG parameters, pharmacokinetics, and serum levels of CTX-I.
Results
MIV-711 was well tolerated with no apparent effect on clinical chemistry, vital signs, or ECG parameters. Adverse events included skin reactions at ECG electrode sites which appeared both after active drug and placebo, headache and muscle pain. Drug exposure increased linearly with dose. MIV-711 reduced serum CTX-I levels in a dose-dependent manner compared to placebo. At 24 h after single dose, plasma CTX-I levels were similar in placebo-treated subjects compared to baseline levels before dose (pre-dose baseline: 0.59±0.16 ng/ml vs 24 h post-dose: 0.61±0.19 ng/ml, 1±4% increase, n=10). In subjects receiving 20 mg of MIV-711, serum CTX-I levels 24 h after dose were 20±2% lower than baseline (n=7). Subsequent doses of 100 mg, 200 mg, 400 mg and 600 mg of MIV-711 reduced serum CTX-I levels by 51±4%, 61±4%, 75±3% and 79±4% respectively (n=7 in each group). Efficacy was sustained for 24 h despite a short elimination half-life of MIV-711. 48 h After dose, serum CTX-I levels were back to initial baseline levels in most groups indicating reversible efficacy. Conclusions
Single doses of MIV-711 up to 600 mg were safe and well tolerated and displayed linear pharmacokinetics over the investigated dose range. Serum CTX-I levels were suppressed by up to 79% at 24 h after dose. A single 100 mg dose of MIV-711 reduced serum levels of CTX-I by more than 50% at 24 h post dose.
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PP431
Bisphosphonate treatment of painful vertebral fractures due to osteoporosis in five boys with Duchenne muscular dystrophy
Eva Aström1,2
1Department of Woman and Child Health, Karolinska Institutet, Stockholm, Sweden; 2Br3, Karolinska University Hospital, Stockholm, Sweden.
Introduction
Duchenne muscular dystrophy (DMD) is caused by mutation in the dystrophin gene on the X-chromosome, leading to progressive deterioration in muscle function from early childhood. Corticosteroid treatment prolongs the time to loss of walking ability and improves life span. The combination of muscular weakness, reduced mobility and steroids increases the risk of secondary osteoporosis. Subjects and methods
In this prospective observational study, monthly intravenous pamidronate infusions were initiated (initial dose 10 mg/m²), during 6 months increased to 30 mg/m²) to five boys with DMD, due to intensive back pain and multiple vertebral fractures. Their age was 11.6–16.4 years (median 12.7 years). They previously had steroid treatment during 3.5–10.6 years (median 5.5 years). Four of them continued the steroid treatment during the observation time. Two boys had experienced an extremity fracture with no or minor trauma. All but the youngest had growth arrest before baseline.
Results
At baseline they all recorded intensive back pain every day of the month, but at the latest recording (after 2–3 years of bisphosphonate treatment, median 2 years) pain had resolved completely in four and almost completely in one boy (VAS 1, 1/day/month). At baseline the median bone density z-scores of the whole body and spine were −3.5 and −2.2 respectively. At the latest recording the corresponding median z-scores were −3.9 and −2.9. Radiographs after 2 years showed slightly increased vertebral height in the youngest boy, but the other four had unchanged vertebral height and even slight progress of compressions of 2–3 vertebral bodies. No other fractures occurred.
Conclusions
Intravenous pamidronate is an effective treatment of back pain due to vertebral fractures in osteoporotic boys with DMD. Larger studies are needed to assess the treatment effects and optimal time of treatment start.
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PP432
Effects of a mutated sclerostin peptide on bone and lean mass in mice
Maude Gerbaix1, Dominique Pierroz1, Nicolas Bonnet1, Verena Boschert2, Thomas Mueller2 & Serge Ferrari1
1Division of Bone Diseases, Faculty of Medicine, Geneva University Hospital, Geneva, Switzerland; 2Lehrstuhl für Pflanzenphysiologie und Biophysik, Würzburg, Germany.
Sclerostin, a product of osteocytes, is known to inhibit Wnt signaling by binding to the LRP5/6 receptor. We investigated the effects of a mutated mouse sclerostin protein (muScl, R118A/R144A) with potential sclerostin antagonistic activity. In vitro, muScl fully competed with wild type sclerostin for binding to LR6. Whereas its IC50 for Wnt3a activity was 4-fold higher than sclerostin (i.e. 600 nM). Moreover, serum osteocalcin increased in mice after short-term administration of muScl at 0.1 mg/kg, but not at 1 mg/kg.
Experiment 1: 3 month-old mice received muScl by minipumps (0.01 mg/kg per day and 0.05 mg/kg per day) or vehicle for 2 weeks. The left tibia was simultaneously subjected to axial compression (12 N, at 0.1 Hz, during 7 min, 3 days/week), whereas the right tibia served as non-loaded control. Compared to vehicle, muScl increased total body BMD at both doses (1.23 mg/cm² vs −0.10 mg/cm²; P<0.05; 1.27 mg/cm² vs −0.10 mg/cm²; P<0.05, respectively). However, neither dose affected bone microarchitecture in vertebrae, non-loaded or loaded tibia. In the non-stimulated lower limb, muscle mass weight tended to increase with muScl (P=0.09 vs vehicle).
Experiment 2: 2 month-old mice received muScl s.c. (0.05 mg/kg per day) or vehicle for 3 weeks, with/without axial compression for 2 weeks. MuScl did not significantly increase bone mass, however it increased the appendicular lean mass in the non-stimulated lower limb (11% vs 96%). Tibia trabecular BV/TV and cortical BV were lower in the muScl vs vehicle group (8 vs 13.3%; 0.41 vs 0.46 mm³, respectively, both P<0.05). No additive or synergistic effect was observed between mechanical stimulation and muScl. In conclusion, at the dose of 0.01 mg/kg, muScl improved bone mass without microarchitecture changes, whereas at a higher dose it seemed to display inhibitory (sclerostin-like) activity on bone. Interestingly, muScl improved the appendicular lean and muscle mass, which would suggest a role of sclerostin in bone–muscle interactions.
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Denosumab (DMAb) significantly improves bone strength at the hip, estimated by FEA from QCT scans, from baseline (B/L) and vs placebo (Pbo) (Keaveny ASBMR 2010). We determined the extent and distribution of mass and thickness changes at the proximal femur, a key skeletal site for fracture risk, using a novel cortical bone mapping technique on the same serial QCT scans. A FREEDOM substudy included 80 women who underwent hip QCT scanning at B/L and months 12, 24 and 36 during DMAb (60 mg Q6M) or Pbo treatment. For each femur, distributions of cortical mass and thickness were measured in a blinded-to-treatment manner. Each individual femur was registered to an average femur, then distributed measures were transferred to this surface. The significance of DMAb or Pbo effects at each time point, vs B/L and between treatments, was calculated using statistical parametric mapping. In DMAb-treated women, cortical mass increased progressively over time, reaching a difference vs Pbo of 5.4% at 3 years (P<0.0001) (Table). Approximately one-third of this increase was attributed to an increase in cortical density of 2.1±2.7 mg/cm³ (P<0.0001), compared with no change in Pbo-treated subjects (P=0.58). Cortical thickness was also significantly increased with DMAb, which may represent in-filling of the cortical compartment, while average cortical mass and thickness decreased with Pbo. The distribution of increases in cortical mass with DMAb was significant over an increasingly large area of the proximal femur. In postmenopausal women with osteoporosis, DMAb significantly and progressively increased cortical mass and thickness in regions of the proximal femur associated with hip fracture.

**Table 1**

<table>
<thead>
<tr>
<th>Mean change in cortical mass, % baseline (confidence)</th>
<th>DMAb (N=47)</th>
<th>P vs B/L</th>
<th>Pbo (N=35)</th>
<th>P vs B/L</th>
<th>P DMAb vs Pbo</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 months</td>
<td>2.38 (0.55)</td>
<td>&lt;0.05</td>
<td>-0.31 (0.98)</td>
<td>NS</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>24 months</td>
<td>3.01 (0.66)</td>
<td>&lt;0.05</td>
<td>-1.31 (0.84)</td>
<td>&lt;0.05</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>36 months</td>
<td>4.18 (0.53)</td>
<td>&lt;0.05</td>
<td>-1.20 (0.96)</td>
<td>&lt;0.05</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

*Not all subjects had scans at each study time point.

**PP435**

Denosumab’s dynamic CTX profile is maintained over 6 years of treatment: first 3 years of the FREEDOM extension study

C Roux1, MR McClung2, N Franchimont3, S Adami4, PR Ebeling5, IR Reid6, H Resch7, G Weryha8, N Daizadeh7, A Wang9, RB Wagman1 & R Eastell1

1Paris Descartes University, Paris, France; 2Orem Osteoporosis Center, Proctland, Oregon, USA; 3Amgen Inc., Thousand Oaks, California, USA; 4University of Verona, Verona, Italy; 5University of Melbourne, Melbourne, Victoria, Australia; 6University of Auckland, Auckland, New Zealand; 7St Vincent Hospital, University of Vienna, Vienna, Austria; 8Hôpitaux de Brabois, CHU de Nancy, Vandoevere, France; 9University of Sheffield, Sheffield, UK.

Denosumab (DMAb) has a unique profile of bone resorption inhibition: CTX decreases rapidly by 3 days and inhibition is released at the end of the 6-month dosing interval, when DMAb serum levels decrease (McClung NEJM 2006). The dynamic CTX inhibition profile is not curtailed by continued treatment. In the 3-year FREEDOM study, CTX values at 6 months were influenced by baseline CTX values and days since the 1st injection (Eastell JBMR 2011). With 3 additional years of data in the FREEDOM extension study, we explored whether CTX inhibition follows the same profile, and whether the relationship persists between CTX levels with pre-treatment CTX values and by the days since last injection. In the ongoing FREEDOM extension, subjects receive 60 mg DMAb every 6 months and daily supplemental calcium/vitamin D. The dynamic profile of CTX was evaluated in 50 subjects from the long-term group (3 years DMAb in FREEDOM, 3 years in extension) who had CTX measurements (in fasting serum samples by ELISA: Nordic Bioscience) at 10 days and 6 months following the 1st DMAb dose in the extension. Whether pre-treatment CTX values and time since last injection continued to predict CTX values over time was determined in 79 subjects in the long-term group who had CTX measurements available at FREEDOM and extension baseline and year 6. A Tobit-style model was used to account for censoring due to the quantifiable limit at year 6 and to evaluate its relationship with days since last injection, and the FREEDOM and extension baseline CTX values. CTX values were decreased by 10 days after the 1st DMAb dose in the extension, with a median reduction of 91%; by 6 months after DMAb administration, CTX values were reduced by 77% (n=50). Median reduction in CTX at the end of the dosing interval at year 6 was 57% (n=79). CTX values at year 6 were significantly correlated with CTX values at FREEDOM baseline (P<0.001), time since the last DMAb dose at year 5.5 (P<0.0001), and CTX values at the extension study baseline (after 3 years of DMAb in FREEDOM, P<0.0001). In conclusion, long-term DMAb treatment is associated with a dynamic profile of CTX reduction. Pre-treatment CTX values and time since the last DMAb injection continue to be significant predictors of CTX values at year 6.

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**PP434**

Bone histology and histomorphometry: effects of 5 years of denosumab in the FREEDOM Extension

Jacques P Brown1, Rachel Wagman2, David W Dempster3,4, David Kendall5, Paul Miller6, Michael Bolognese7, Ivo Valter8, Jens-Erik Beck Jensen9, Cristiano Zerbini10, Jose R Zanchetta11, Nadia Daizadeh2 & Ian Reid1

1Laval University and CHUQ, Quebec City, QC, Canada; 2Amgen Inc., Thousand Oaks, CA, USA; 3University of Cambridge, Cambridge, UK; 4CHU-Q-CHUL Research Centre, Quebec City, QC, Canada; 5Oregon Osteoporosis Center, Portland, OR, USA; 6Columbia University, New York, NY, USA; 7Bethesda Health Research Center, Bethesda, MD, USA; 8Center for Clinical and Basic Research, Tallinn, Estonia; 9University of Verona, Verona, Italy; 10Hôpitaux de Brabois, CHU de Nancy, Vandoevere, France; 11University of Sheffield, Sheffield, UK.

DMAb increases BMD and reduces bone resorption and risk of vertebral, nonvertebral and hip fractures in women with PMO. Transiliac crest bone biopsies in 47 subjects treated with DMAb for 1–3 years showed reduced bone turnover vs 45 Pbo-treated subjects, which reversed on treatment cessation. Since bone turnover reduction is sustained and fracture incidence low over 6 years’ DMAb treatment, we evaluated DMAb’s effects on tissue-level remodelling in the FREEDOM Extension. Demographics for the 13 cross-over (CO) and 28 long-term (LT) subjects in the bone biopsy substudy to 5 years were comparable with those of the overall Extension population. Mean (so) time from last DMAb dose to first tetracycline dose was 5.7 (0.5) months. Qualitative bone histology in all samples showed normalized mineralized lamellar bone. Of five LT subjects without visualizable osteoid, four had intact samples showing normal mineralization. Structural indices were similar between CO and LT groups. Median (Q1, Q3) eroded surface/bone surface (i.e., resorption) was decreased in both CO (0.15% (0.44%)) and LT subjects (0.1% (0.25%)) vs FREEDOM Pbo-treated subjects (1.04% (0.55%, 1.88%)). Ten CO and 14 LT subjects had specimens with double-tetracycline label in trabecular and/or cortical compartments. Median (Q1, Q3) dynamic remodelling indices were low in the five LT subjects: mineral apposition rate 0.59 (0.51, 0.65) and 0.40 (0.30, 1.05) μm/d; bone formation rate 1.20 (0.66, 1.26) and 2.18 (0.20, 4.67)/μm/year; activation frequency 0.02 (0.01, 0.02) and 0.03 (0.07)/year; mineralizing surface 0.28 (0.22, 0.37% and 0.66 (0.28, 0.170)%. DMAb treatment over 5 years results in normal bone quality with reduced bone turnover, consistent with its mechanism of action. Bone histomorphometry in FREEDOM and its study extension (Table 1)
**PP436**

Bone mineral density changes in patients with prior fracture suboptimally treated with a bisphosphonate: results from denosumab (DMAb)/ibandronate and DMAB/risedronate trials

Christopher Recknor\(^1\), Christian Roux\(^2\), Pei-Han Ho, Jesse Hall\(^3\), Henry Bone\(^4\), Sydne Bonniek\(^5\), Joop van den Bergh\(^6\), Irene Ferreira\(^7\), Rachel Wagman\(^8\) & Jacques P Brown\(^9\)

\(^1\)United Osteoporosis Centers, Gainesville, GA, USA; \(^2\)Paris Descartes University, Paris, France; \(^3\)Amgen Inc., Thousand Oaks, CA, USA; \(^4\)Michigan Bone and Mineral Clinic, Detroit, MI, USA; \(^5\)Clinical Research Center of North Texas, Denton, TX, USA; \(^6\)VieCuri Medical Centre, Maastricht University, Maastricht, The Netherlands; \(^7\)Amergin Ltd., Cambridge, UK; \(^8\)CHUQ-CHUL Research Centre, Quebec City, QC, Canada.

In osteoporosis, poor adherence to bisphosphonate (BP) therapy is common, and is associated with poor outcomes and increased treatment costs (Sris 2006; Recker 2005). Although compliance is improved with monthly vs weekly dosing (Register 2008), no evidence suggests cycling through BP agents offers therapeutic benefit, assessed by bone mineral density (BMD). In two randomized, open-label studies in postmenopausal women aged $\geq 55$ years previously treated with, but suboptimally adherent to, BP therapy, subjects received denosumab (DMAb) 60 mg SC Q6M, ibandronate (IBN) 150 mg PO QM or risendronate (RIS) 150 mg PO QM for 12 months; DMAB treatment was associated with greater increases in BMD than either IBN or RIS (Recknor 2012; Roux 2012). We assessed if these differences were consistent in a subset of subjects who had BMD data recorded at baseline and month 12 stratified by prior fragility fracture. In the IBN and RIS studies, 237/767 (31%) and 280/809 (35%) subjects, respectively, had a prior fracture. There were no significant differences in baseline BMD by treatment group or prior fracture. BMD increases were greater with DMAB than IBN or RIS at all sites independent of prior fracture (Table). In subjects suboptimally adherent to an oral BP, switching to DMAB provided greater gains in BMD at all key skeletal sites measured than transitioning to either IBN or RIS. These findings suggest that the magnitude of treatment effect is not significantly influenced by classification of high risk, as defined by prior fragility fracture.

**PP437**

The spatial relationship between bone formation and bone resorption in healthy and ovariectomized mice treated with PTH, bisphosphonate or mechanical loading

Davide Ruffoni, Claudia Weigt, Elissa Fattorini, Alina Levchuk, Friederike Schulte, Gisela Kuhn & Ralph Müller

Institute for Biomechanics, ETH Zurich, Zurich, Switzerland.

Bone is continuously remodeled to remove damage, to adapt to changes in mechanical demands and to regulate calcium homeostasis. The first aim is accomplished by coupled bone formation and resorption whereas adaptation requires sites of formation to differ from those of resorption. The regulation of circulating ions is achieved by a stochastic exchange of bone packets. Here, we investigated these different aspects of remodeling in healthy and ovariectomized (OVX) mice treated with PTH, bisphosphonate or mechanical loading. 15-week old C57BL/6J female mice were divided into the following groups: untreated OVX (OVX, n=17); treated daily with PTH (PTH, n=9); treated once with zolendronate (BIS, n=9); treated with cyclic mechanical loading (8 N, 10 Hz, 3000 cycles) at the 6th caudal vertebra (CML, n=17); and sham operated mice (SHM, n=8). Treatment started 11 weeks after ovariectomy and micro-CT measurements were performed at start of the treatment and after 2 and 4 weeks. Registration of three consecutive scans allowed estimating the amount of coupled bone formation (i.e., bone formed at the locations where it was previously resorbed) and coupled bone resorption (i.e., bone resorbed at the locations where it was previously formed). Considering that it is biologically irrational that newly formed bone gets immediately removed, coupled resorption could be interpreted as stochastic untargeted remodeling. OVX significantly increased the amount of coupled resorption by 44% when compared to SHM (P<0.001) whereas PTH, BIS and CML decreased it by 61, 22 and 39% when compared to OVX (P<0.001). Coupled formation was significantly decreased following OVX (~35%, P<0.001) while it increased following the three treatments by 126% (PTH), 90% (BIS) and 46% (CML) (P<0.001). The proposed analysis allowed measuring the coexisting types of remodeling in living bone and indicated that PTH caused the strongest increase in coupled bone formation and the highest reduction of untargeted remodeling.

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**PP438**

Optimizing fracture prevention: the fracture liaison service, an observational study

Danielle Eekman\(^1\), Sven van Helden\(^2\), Margriet Huisman\(^3\), Harald Verhaar\(^4\), Irene Baltink\(^5\), Piet Geusens\(^6,7\), Paul Lips\(^8\) & Willem Lems\(^9\)

\(^1\)VU University Medical Center, Amsterdam, The Netherlands; \(^2\)Isala Clinics, Zwolle, The Netherlands; \(^3\)Sint Franciscus Gasthuis, Rotterdam, The Netherlands; \(^4\)University Medical Center Utrecht, Utrecht, The Netherlands; \(^5\)University Hospital, Maastricht, The Netherlands; \(^6\)University Hasselt, Hasselt, Belgium.

Objective

Increase the percentage of elderly fracture patients undergoing a dual energy X-ray absorptiometry (DXA) measurement, and investigate why some patients did not respond to invitation to our fracture liaison service (FLS).

Materials and methods

In four Dutch hospitals, fracture patients ≥50 years were invited for a DXA measurement and visit to our FLS. Patients who did not respond, were contacted by telephone. In patients diagnosed with osteoporosis, treatment was started. Patients were contacted every 3 months during 1 year to assess drug persistence and the occurrence of subsequent fractures.

Results

Of the 2207 patients that were invited: 50.6% responded. Most frequent reasons for not responding included: not interested (38%), already screened/under treatment for osteoporosis (15.7%), physically unable to attend the clinic (11.5%) and death (5.2%). Hip fracture patients responded less frequently (29%) while patients with a wrist (60%), or ankle fracture (65.2%) were more likely to visit the clinic. In 337 responding patients, osteoporosis was diagnosed and treatment was initiated. After 12 months of follow-up, 88% of the patients were still persistent with anti-osteoporosis therapy and only 2% suffered a subsequent clinical fracture.

Conclusion

In elderly fracture patients, the use of a FLS leads to an increased response rate, a high persistence to drug treatment, and a low rate of subsequent clinical fractures. Additional programs for hip fracture patients are required, as these patients have a low response rate.

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**PP439**

The direct and indirect costs of an osteoporotic fracture: a prospective evaluation of elderly patients with a clinical fracture

Danielle Eekman, Mariève ter Wee, Veerle Coupé, Seher Erisek-Demirtas, Mark Kramer & Willem Lems

VU University Medical Center, Amsterdam, The Netherlands.

Objective

The aim of this study was to gain insight into all the current overall costs (direct medical, direct non-medical and indirect costs) of clinical fractures in osteoporotic patients aged 50 years and older in the Netherlands.

Materials and methods

This prospective study was part of a larger study in which the effect of a fracture nurse on diagnosis and subsequent treatment of elderly osteoporotic patients with
PP440
Strontium potently inhibits mineralisation in bone-forming osteoblast cultures while osteoclast formation from marrow mononuclear cells is moderately reduced
Daniel Wornham, Mark Hajjawi, Isabel Orriss & Timothy Arnett
University College London, London, UK.

Strontium ranelate (SrR) is now widely used for the prevention of osteoporotic fractures. The mechanisms by which this occurs, however, remain unclear. We investigated the actions of Sr2+ salts in bone-forming cultures of primary osteoblasts from rat calvariae. Osteoblasts were treated continuously with either SrR or SrCl2 for 14 days. Abundant, discretely mineralised ‘trabecular’ bone structures formed in alizarin red-stained control cultures. Surprisingly, SrR at 10, 100 and 1000 μM inhibited mineralisation, assessed morphometrically, to 75, 16 (P<0.01) and 1% (P<0.001) of control values, respectively. SrCl2 at the same concentrations caused similar inhibitions. Collagen deposition and soluble collagen were unaffected by SrR or SrCl2 at any concentration up to 1 mM. Osteoblast cell number and alkaline phosphatase activity were also unaltered. The selective inhibitory action of Sr2+ salts on mineralisation was confirmed by inspection of unstained osteoblast cultures, revealing numerous unmineralised collagenous trabeculae. To study the effects of Sr2+ salts on osteoclast function, we cultured mouse marrow cells on ivory discs with 1 mM SrR or SrCl2 for 7 days in the presence of MCSF and RANKL. SrR dose-dependently reduced the number of multinucleated osteoclasts formed, with a 50% inhibition occurring at 1 mM; SrCl2 was somewhat less effective, eliciting a maximal 30% inhibition. Corresponding decreases in total resorption pit formation were observed, suggesting Sr2+ salts affect osteoclast formation rather than resorptive activity. Our osteoblast findings are consistent with the documented physicochemical inhibitory action of Sr2+ on mineralisation but contrast with reports that Sr2+ increases osteoblast activity and number in vitro. Osteoblast data fit with previous findings that showed modest reductions in osteoblast numbers by Sr2+ in vitro. Our results suggest that rather than acting as an agent that ‘uncouples’ bone formation and resorption, Sr2+ acts as a global inhibitor of bone cell function, with particularly marked effects on mineralisation.

PP441
Reducing the risk of hypocalcaemia with parenteral antiresorptive therapies: an audit
Wei Xu, Kenneth Baker, Rachel Reavley, Emily Oates & Terry Aspray
Department of Rheumatology, Freeman Hospital, Newcastle upon Tyne, UK.

Introduction
Intravenous bisphosphonates (IB) and subcutaneous denosumab (SD) are potent antiresorptive agents widely used in the treatment of osteoporosis, Paget’s disease and metastatic malignancy. Several case reports have identified the risk of life-threatening hypocalcaemia with these treatments, particularly in the context of vitamin D deficiency and further highlighted by recent UKMHR advice.

Design
To optimise vitamin D status and decrease hypocalcaemia risk, a two-step approach was taken: i) Clear written instructions provided to GPs to check serum 25(OH) vitamin D (25OHD) levels and start oral supplementation (colecalciferol 100 000 units total over 5 days) if 25OHD<50 nmol/l. ii) Provision of standard administration protocol with clear thresholds for 25OHD (>50 nmol/l), corrected calcium (>2.00 mmol/l) and renal function (eGFR>30 ml/min per 1.73 m2 for IB).

Results
Prior to the introduction of this protocol (October-December 2011), four (5%) of 84 patients had 25OHD tested before treatment. However, subsequent testing found 41 (49%) with 25OHD<50 nmol/l and 21 (25%) had 25OHD<25 nmol/l. One patient was hypocalcaemic (adjusted calcium 1.77 mmol/l), requiring treatment. Following introduction of the protocol (August-September 2012), 78 patients were reviewed: 66 (85%) had 25OHD checked within guideline recommendations, representing an improvement (95%CI) of 80 (77–93)% (P<0.0001). Within this group, 25 patients (32%) had insufficient vitamin D levels of which only 4 (5%) had levels below 25 nmol/l, representing improvements of 17 (2–32) % (P<0.05) and 20 (10–30)% (P<0.001). Conclusion
Vitamin D deficiency was significantly reduced in our patient population, which may have been explained, partially, by season. However, we demonstrate a significant improvement in the monitoring of vitamin D levels and appropriate oral vitamin D supplementation in line with current guidance.

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PP442
Design of a prospective observational study to evaluate persistence and adherence during denosumab treatment, and patient characteristics in postmenopausal women with osteoporosis in routine clinical practice
Maurille Feudjo-Tepie¹, Gerd Möller², Peyman Hadji³, Irene Feireira³, Suresh Siddhantı, Stephen Boonen⁴, Astrid Fahrleitner-Pammer⁵ & Nikos Papaioannou⁶
¹Amgen Ltd., Uxbridge, UK; ²Amgen Europe GmbH, Zug, Switzerland; ³Philips University, Marburg, Germany; ⁴Amgen Ltd., Cambridge, UK; ⁵Amgen Inc., Thousand Oaks, California, USA; ⁶Leuven University, Leuven, Belgium; ⁷Medical University of Graz, Graz, Austria; ⁸University of Athens, Athens, Greece.

Treatment of postmenopausal osteoporosis (PMO) has been traditionally hampered by poor persistence and adherence to short-term (≤1-monthly) medications. The efficacy of 6-monthly (Q6M) denosumab treatment has been proven in clinical trials, but effectiveness will be dependent on persistence and adherence in routine clinical practice. This study is designed to evaluate real-world persistence and adherence to denosumab, and to establish how this is best assessed in long-acting injectable medications (LAsIs). This will be an international, non-interventional, observational study in women with PMO. Treatment will be assigned prior to, and independent of, study enrolment considerations, and all subsequent information recorded as in routine clinical practice. The only deviation from routine care will be the completion of 2 patient-reported outcome questionnaires at enrolment: the 8-item Morisky Medication Adherence Scale and the 12-item Short Form 12 Generic Health-related Quality of Life instrument. Persistence will be assessed by whether injections are separated by no more than 6 months +8 week ‘time window’, and adherence assessed by whether injections occur within 6 months ±4 week time window of the previous injection. Other time windows will be considered as part of sensitivity analyses. Medication coverage ratio will be defined by the percentage of days the patient was covered with denosumab treatment (according to prescription records). All outcomes will be evaluated at 12 and 24 months. The results of this study will provide clinicians with insight into risk factors for patient non-persistence with, and non-adherence to, denosumab therapy, and determine optimal methods of evaluating these factors with Q6M denosumab treatment. This appropriately designed study will give further insight on potential measures of persistence and adherence in LAsIs, inform clinical practice by providing information on these measures with denosumab, and evaluate patient risk factors for non-persistence and non-adherence to LAsIs.

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**PP443**
Zoledronic acid vs alendronate in the management of osteoporosis

Lyn Ferguson1, Maurizio Panarelli2 & Rosemary Dargie1,2

1Department of Biochemistry, Glasgow Royal Infirmary, Glasgow, UK; 2Department of Bone Mineral Metabolism, Glasgow Royal Infirmary, Glasgow, UK.

Zoledronic acid has been shown to reduce the risk of fractures and improve bone mineral density (BMD) in osteoporosis vs placebo. This study compared changes in BMD in patients with osteoporosis treated with zoledronic acid vs alendronate. BMD at the lumbar spine and total hip pre- and post-bisphosphonate were recorded for 65 patients with osteoporosis (T score \( \leq -2.5 \)) from retrospective analysis of DEXA scans. 35 patients received annual 5 mg IV zoledronic acid infusions over 3 years; 30 patients received 70 mg once weekly oral alendronate over mean duration of 3 years. Data were analysed using Mann-Whitney-tests in Miniatub 15 statistical software. The number of fragility fractures post-bisphosphonate was recorded as was the reason for choosing zoledronic acid over alendronate.

The median percentage improvement in lumbar spine BMD with zoledronic acid was 5.5% (Interquartile range (IQR) = 0.2 to 9.1%) and with alendronate was 6.45% (IQR 1.8 to 10.4%). Whilst there was a trend towards greater improvement with alendronate compared to zoledronic acid, this was not statistically significant (\( P = 0.45 \)). The median percentage improvement in total hip BMD with zoledronic acid was 0.3% (IQR = -2.3 to 6.4%) and with alendronate was 0.8% (IQR = -2.7 to 3.4%). However, this did not reach statistical significance (\( P = 0.37 \)). 8 patients (23%) suffered fragility fractures post zoledronic acid compared to 11 (37%) post alendronate. However this was not statistically significant (odds ratio 0.5, 95% confidence interval 0.2 to 1.5, \( P = 0.2 \)). The most common reasons for prescribing zoledronic acid were oral bisphosphonate intolerance and fragility fractures/decreased BMD despite alendronate use. This study showed while both zoledronic acid and alendronate improved lumbar spine BMD, there was no statistically significant difference between them. Zoledronic acid use therefore in those who have failed to respond to alendronate is questionable; however it may be a reasonable alternative in those intolerant of oral alendronate.

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**PP444**
Correction of vitamin D deficiency in women with postmenopausal osteoporosis

Vladyslav Povoroznyuk & Nataliya Balatska
D.F. Chebotarev Institute of Gerontology NAMS Ukraine, Kyiv, Ukraine.

The aim of the research
To investigate the effect of combined calcium and vitamin D therapy (calcium 1000 mg, vitamin D 400 IU) on 25(OH)D level and concentration of bone turnover markers in patients with systemic postmenopausal osteoporosis.

Methods
20 women with systemic postmenopausal osteoporosis were examined. The average age of the patients was \( 63.0 \pm 9.0 \) years. The study was performed during winter season to exclude the influence of seasonal factors on 25(OH)D level in the blood serum. Before and the end of the sturdy it was performed during winter season to exclude the influence of seasonal factors on 25(OH)D level in the blood serum. Before and the end of the study it was evaluated the intensity of vertebral pain syndrome in the thoracic and lumbar spine and quality of life by EuroQol-5D and ECOS-16. 25(OH)D iPTH and bone turnover markers were evaluated by Elecsys 2010 analyzer (Roche Diagnostics, Germany).

Results
• Three month therapy didn’t significantly change the intensity of vertebral pain syndrome in the thoracic and lumbar spine and didn’t significantly influence quality of life by EuroQol-5D and ECOS-16.
• Combine therapy with calcium and vitamin D increased 25(OH)D level from \( 35.86 (29.40; 54.14) \) to \( 38.85 (21.91, 54.98) \) ng/ml (\( P < 0.05 \)). Bone formation marker decreased from \( 49.67 (29.40; 54.14) \) to \( 46.07 (33.75; 52.54) \) ng/ml (\( P < 0.05 \)). Markers of bone resorption were recorded as was the reason for choosing zoledronic acid over alendronate.

Conclusions
Prescriptions of combined therapy of calcium and vitamin D in patients with systemic postmenopausal osteoporosis during three winter months leads to significant improvement of 25(OH)D level in blood serum (\( P < 0.05 \)) and do not significantly influence the bone formation and resorption markers (\( P > 0.05 \)).

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**PP445**
Effectiveness of the active metabolite of vitamin D in the treatment of postmenopausal osteoporosis

Fedir Klimovitskiy1, Tetyana Povoroznyuk & Nataliya Balatska2
1M. Gorky Donetsk National Medical University, Donetsk, Ukraine; 2D.F. Chebotarev Institute of Gerontology NAMS Ukraine, Kyiv, Ukraine.

The aim of the research was to determine the efficacy of alfalcacidol (Alpha D3 Teva) in the treatment of women with postmenopausal osteoporosis and vitamin D deficiency.

Methods
20 women with systemic postmenopausal osteoporosis were examined. All patients had vitamin D deficiency (the average level of 25(OH)D in blood serum was \( 37.16 \pm 24.9, 45.11 \) nmol/l). Alfalcacidol was prescribed for 12 months in doses \( \leq 1 \mu g \) duration of observation was 12 months. Bone mineral density was examined by dual-energy X-ray absorptiometry 'Prodigy' (GE Medical systems, Lunar). 25(OH)D, iPTH and bone turnover markers were evaluated by Elecsys 2010 analyzer (Roche Diagnostics, Germany).

Results
• Alfalcacidol therapy leads to a significant reduction of iPTH level, inhibits bone resorption and leads to improvement of bone mineral density.

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**PP446**
Effects of odanacatib on BMD and safety in the treatment of osteoporosis in postmenopausal women previously treated with alendronate—a randomized placebo-controlled trial

Roland Chapurla1, Tobias De Villiers2, Sydney Bonnick3, Alberto Odo4, Santiago Palacios5, Boyd Scott5, Celine Le Bailly De Tilleghem6, Carolyn DaSilva7, Albert Leung8 & Deborah Gunner2,3,4,5,6

1INSERM, Lyon, France; 2Mediclinic Panorama, Cape Town, South Africa; 3Clinical Research Center of North Texas, Denton, USA; 4Alta California Medical Group, Simi Valley, USA; 5Instituto Palacios, Madrid, Spain, 6Merck Sharp and Dohme, Whitehouse Station, USA.

Odanacatib (ODN) is an orally-active cathepsin K inhibitor being developed for the treatment of postmenopausal osteoporosis. This study evaluated the effects of ODN 50mg once weekly on BMD, bone turnover markers and safety in patients previously treated with alendronate (ALN).

This was a randomized, double-blind, placebo-controlled, 24-month study. The primary endpoint was % change from baseline at month 24 of femoral neck (FN) BMD. Postmenopausal women (n = 243) aged 50 to 75 years old started low BMD at the total hip, FN or trochanter but no history of hip fracture and who had taken ALN for \( \geq 3 \) years were randomized to receive ODN or placebo. Patients received vitamin D3 and calcium supplementation. BMD was assessed by DXA at baseline, 6, 12 and 24 months. Biochemical markers of bone turnover (sCTX, uNTx, sBSAP and sP1NP) were measured at baseline and 3, 6, 12, 18 and 24 months. In the ODN group, BMD changes from baseline at 24 months were significantly increased from placebo at the femoral neck, trochanter, total hip and lumbar spine (1.7, 1.8, 0.8, and 2.3%, respectively). In the placebo group, BMD at the femoral neck, trochanter and total hip declined significantly from baseline by month 24 (−0.9, −1.4, and −1.9% respectively). ODN significantly decreased bone resorption marker, collagen cross-linked N-telopeptide, and significantly increased bone formation markers, sP1NP and sBSAP, vs. placebo. The increase observed for the bone resorption marker s-CTX with ODN treatment was unexpected. Adverse events were comparable between the two treatments arms. The overall safety profile appeared similar between ODN and placebo.

In this study ODN provided incremental BMD gains in osteoporotic women following ALN treatment. Biomarker results suggest that ODN decreases bone resorption while preserving bone formation.

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PP447
Effects of sclerostin antibody and maintenance of new bone induced by sclerostin antibody in animal models
Xiaodong Li, Michael S Ominsky, Min Liu, Rogely W Boyce & Hua Zhu Ke
Agen Inc., Thousand Oaks, CA, USA.

Treatment with sclerostin antibody (Scl-Ab) increases bone formation and strength in animal models. Here, we aimed to i) characterize the long-term effects of Scl-Ab on bone in cynomolgus monkeys (cynos) and ovariectomized (OVX) rats and ii) test whether follow-up treatment with OPG-Fc would maintain the bone mass gains induced by Scl-Ab in OVX rats. In the cynos study, 3 to 5-year-old male cynos were treated for 6 months with weekly SC injections of vehicle (Yeh), 3, 10, or 100 mg/kg Scl-Ab. Serum osteocalcin peaked within the first 3 months of Scl-Ab treatment and returned toward baseline levels at month 6. Scl-Ab dose-dependently increased BMD, cortical thickness, trabecular bone volume, and yield load of lumbar vertebral bodies. Positive correlations between BMD and yield load were observed across all groups. In the OVX rat study, 6-month-old OVX rats (2 months post-OVX) were treated with Yeh or Scl-Ab (25 mg/kg, SC, 1x/week) for 6, 12, or 26 weeks. Another group of OVX rats was treated with Scl-Ab for 6 weeks and then was transitioned to Yeh or OPG-Fc (10 mg/kg, SC, 2x/week) for an additional 6 or 20 weeks. BMD increased progressively up to week 26 with continuous treatment. Trabecular, endocortical, and periosteal bone formation rates (BFR/BS) increased and peaked at week 6. Trabecular and endocortical BFR/BS in the Scl-Ab group gradually declined but remained significantly greater than OVX controls at weeks 12 and 26, while periosteal BFR/BS returned to the level of OVX controls at week 26. Transitioning to OPG-Fc maintained the bone mass and bone strength gains induced by Scl-Ab upon discontinuation of Scl-Ab. These data illustrate that long-term treatment with Scl-Ab progressively increased bone mass and bone strength in both monkey and rodent models. These results also support the strategy of using anti-resorptive agents to maintain Scl-Ab-induced bone gains.

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PP448
Resolution of effects on bone turnover markers and bone mineral density after discontinuation of long-term bisphosphonate use
Claude Benhamou1, Tobias De Villiers2, C Conrad Johnston3, Bente Langdahl4, Kenneth Saag5, Andrew Denker6, Annpey Pong6
1Hospital d’Orleans la Source, Orleans, France; 2Mediclinic Panorama, Western Cape, South Africa; 3Indian University School of Medicine, Indianapolis, Indiana, USA; 4Aarhus University Hospital, Aarhus, Denmark; 5University of Alabama, Birmingham, Alabama, USA; 6Merck Sharp and Dohme Corp., Whitehouse Station, New Jersey, USA.

Relatively little is known about immediate consequences of continuing vs interrupting long-term bisphosphonate treatment. This report describes changes in bone turnover and BMD in a 1-year, dose-finding trial of the calcium-sensing receptor antagonist MK-5442 in postmenopausal, BP-treated women, randomized to placebo or alendronate (ALN). Table 1 presents the pre-treatment (baseline) and post-treatment outcomes. Markers of bone turnover, such as NTX/Cr and P1NP, were measured in urine at baseline and at months 1, 3, 6, and 12. At baseline, women continued on alendronate (ALN) had lower NTX/Cr and P1NP compared to placebo (P < 0.0001). After 1 year, both markers returned to levels similar to those expected in untreated postmenopausal women, and spine and hip BMD were reduced vs continued treatment with alendronate.

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PP449
Odanacatib treatment reduces remodeling- and stimulates modeling-based bone formation in adult OVX monkeys
C Chen1, M Shih2, H Zheng2 & L Duong2
1Merck Sharp and Dohme Corp., Whitehouse Station, USA; 2PharmaLegacy Laboratory, Shanghai, China.

Odanacatib (ODN), a selective and reversible cathepsin K inhibitor was shown to histomorphometrically reduce trabecular (Tb) and intracortical (Ic) bone remodeling while preserving endocortical (Ec) and stimulating periosteal (Ps) bone formation (BF) in monkeys. Here, we investigate the bone site specific mechanism of ODN on bone modeling (Mo) versus remodeling (Re)-based osteons. Rhesus monkeys (13–19 yrs, n=8–11/group) were ovariectomized and treated with vehicle or ODN (6 or 30 mg/kg, q.d., p.o.) for 21-months. Calcein labels at 15-d interval were given around 12-m of dosing. Lumbar vertebrae (LV) and central femur (CF) were subjected to dynamic histomorphometric and cement line analyses and only newly formed hemiosteons (Ho) were evaluated. At LV Tb surface, ODN dose-dependently reduced the number of remodeling hemiosteons (Re.Ho.N) without changing the mean wall thickness (W.Tb vs. vehicle). Note that the number of newly formed hemiosteons (Mo.Ho.N) was very low at Tb surface of the aged monkeys and ODN did not change this parameter. Overall in Tb LV, ODN dose-dependently reduced mineralizing surface, mineral apposition rate, bone formation rate (BFR/BS) and activation frequency (Tb.Acf). In the CF, ODN also decreased both Ec and Ec Re.Ho.N and the high dose tended to reduce Ec BFR/BS and AcF. Similar to Tb surface, Ec.Re.W.Th was unchanged in ODN vs. Veh. Remarkably, ODN significantly increased modeling bone formation in both Ec and Ps surfaces of the CF. ODN dose-dependently increased Ec Mo parameters, including Mo.Ho.N, Mo.Acf; Mo.W.Th and BFR/BS. At Ps surface, ODN also increased all BF parameters in a dose-dependent manner. The results demonstrated that ODN reduces remodeling while stimulating modeling-based hemiosteons, and thus increased the ratio of modeling to remodeling units. These findings explain the bone site specific actions of ODN on trabecular and cortical surfaces in OVX-monkeys. Furthermore, the mechanisms of ODN on modeling-based bone formation differentiate this agent from the standard anti-resorptives.

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PP450
Transdermal delivery of BA058, a novel analog of hPTHrP (1-34), with a short wear time patch in preclinical and clinical studies
Gary Hattersley1, Kris Hansen2, Amy Determan3, Ken Brown4, Kate McKay5, Jonathan Guerriero6, Dan McCarthy1, C Richard Lyttle1 & Louis St L. O’Dea7
1Radius, Cambridge, Massachusetts, USA; 23M Drug Delivery Systems, St Paul, Minnesota, USA.

BA058 is being developed as an anabolic therapy for the treatment of osteoporosis. Daily BA058 SC injection has produced promising safety and efficacy results in early clinical studies, and is currently enrolling in a Phase 3 fracture prevention study. There is, however, a significant need for an alternative to injection that improves patient convenience and compliance. We have investigated the use of a solid Microstructured Transdermal System (3M) for transdermal (TD) delivery of BA058. The pharmacokinetics of BA058 TD were similar to both rats and monkeys, with an early Tmax, short T1/2, and a Cmax comparable to SC injection. Efficacy of BA058 TD was evaluated in OVX rats. Following a bone depletion period, rats were treated daily for 14-days with months of placebo, mean concentrations of NTX/Cr and PINP rose to 42.2 nmol BCE/mmol Cr and 40.1 mg/ml, both markers unchanged with continued ALN (Table). After 12 months, there were also significant treatment-differences in BMD (Table). In conclusion, discontinuation of bisphosphonate treatment after a median of 5 years resulted in increases in NTX/Cr by 1 month and PINP by 3 months. After 1 year, both markers returned to levels similar to those expected in untreated postmenopausal women, and spine and hip BMD were reduced vs continued treatment with alendronate.

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BA058 TD or BA058 SC. Femur BMD was increased (+4.8%) with BA058 TD, similar to the increase with BA058 SC (+4.2%). Trabecular bone microstructure were also improved in the femur metaphysis. Three Phase 1 clinical studies were also conducted to determine the PK, safety and tolerability of transdermal BA058 in post-menopausal women. Peri-umbilical application of BA058 TD (100, 150 and 200 mcg) resulted in a desirable PK profile, with rapid delivery, early peak concentration, fast elimination of BA058, and a Cmax that matched or exceeded SC injection (80 mcg). BA058 patch wear times up to 24 h were evaluated, with a 5-minute wear time optimal for complete BA058 delivery; wear times longer than 5-minutes resulted in no further BA058 release. Seven consecutive days of BA058 TD resulted in a marked increase in serum PINP, consistent with retention of pharmacological activity and bone anabolism. After more than 300 patch applications to more than 100 subjects, BA058 TD demonstrated a favorable safety profile. Transdermal BA058 delivery using a short wear time patch potentially represents a new approach for osteoporosis treatment.

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PP451

Estimation of vertebral and femoral strength during the first three years of denosumab therapy using an alternative smooth non-linear finite element methodology
Philippe Zysset1, Dieter Fehr2, Klaus Engelke3,4, Harry Genant5, Michael McClung6, David Kendler7, Christopher Recknor7, Michael Kinzi7, Jakob Schwiedzrik1, Oleg Museyko2, Andrea Wang10 & Cesar Libanati10
1University of Bern, Bern, Switzerland; 2Vienna University of Technology, Vienna, Austria; 3University of Erlangen, Erlangen, Germany; 4Synarc GmbH, Hamburg, Germany; 5UCSF and Synarc, San Francisco, California, USA; 6Osteoporosis Center, Portland, Oregon, USA; 7University of British Columbia, Vancouver, British Columbia, Canada; 8United Osteoporosis Centers, Gainesville, Georgia, USA; 9University of Erlangen-Nürnberg, Erlangen-Nürnberg, Germany; 10Amgen Inc., Thousand Oaks, California, USA.

Denosumab subcutaneous administration every 6 months reduced the incidence of new fractures in postmenopausal women with osteoporosis by 68% at the spine and 40% at the hip over 36 months compared with placebo in the FREEDOM study (Cummings et al., NEJM, 2009;361:756). This efficacy was supported by differential improvements from baseline in vertebral and femoral strength at 36 months (18.2 and 8.6%, respectively) estimated by an established voxel-based finite element (FE) methodology (Keaveny et al., ASBMR, 2010; OIP1099). Since FE analyses rely on the choice of meshes, material properties, and boundary conditions, the aim of this study was to independently confirm and compare the effects of denosumab on vertebral and femoral strength during the FREEDOM trial using an alternative smooth FE methodology.

QCT data for two lumbar vertebrae and the proximal femur were obtained at baseline, 12, 24, and 36 months from 51 treated (denosumab) and 47 control (placebo) subjects from FREEDOM. The QCT images were segmented and converted into smooth FE models to compute bone strength. L1 and L2 were virtually loaded in axial compression and the proximal femora in both fall and stance configurations.

For L1 and L2, strength of the denosumab group increased on average by 11.3, 14.4, and 17.6% from baseline at 12, 24, and 36 months, respectively (P < 0.0001). Femoral strength of the denosumab group increased significantly in the fall configuration to 4.3, 5.1, and 7.2% above baseline at 12, 24, and 36 months, respectively (P < 0.0001). Similar improvements were observed in the stance configuration. Differences with the decreasing strengths of placebo were highly significant after 12 months (P < 0.0001).

We confirmed the significant improvements in vertebral and femoral strength previously observed with denosumab therapy using an alternative smooth FE methodology. The estimated increases in strength with denosumab and decreases with placebo were highly consistent between both FE techniques.

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PP452

Curbing our enthusiasm when prescribing strenuous exercises in osteopenia/osteoporosis, when fracture may occur under good intentions
Mehteshad Sinaki
Mayo Clinic College of Medicine, Rochester, Minnesota, USA.

Exercise can prevent or mitigate musculoskeletal challenges of aging. To prescribe an effective/ostrogenic exercise program the individual’s muscle strength, bone mineral density, and cardiovascular status would need to be considered. Osteoporotic vertebral fractures and resulting mal-posture create musculoskeletal challenges that cannot be met with pharmacotherapy alone. Bone loss, disequilibrium along with pain can increase inactivity, and further bone and muscle loss. Even in healthy persons, predisposition to falls increases with age-related neuromuscular changes. Muscle strength decreases about 50% from age 30 to 80. Furthermore, the amount of body sway increases with reduction of proprioception. Therefore, measures that can decrease disequilibrium can reduce the risk of falls and fracture.

Kypnotic posture can contribute to propensity to fall and fear of falls in osteoporotic individuals; it can also contribute to back pain due to ligamentous overstrain. Yoga is used to improve an individual’s balance, but some yoga positions have contributed to vertebral compression fractures and pain. Through implementation of SPEED (Spinal Proprioceptive Extension Exercise Dynamic) program, significant improvements were achieved in gait parameters, computerized dynamic posturography score (P < 0.003), risk of falls at obstacles (P < 0.02), and fear of falls score (P < 0.001). SPEED decreased back pain (P < 0.001) and increased level of physical activity (P < 0.001).

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PP453

Factors influencing levels of bone resorption during denosumab dosing
Richard Eastell1, Ethel Siris2, Christian Roux3, Dennis M. Black4, Nathalie Franchimont5, Graham Jang6, Nadia Daizadeh5, Rachel B. Wagman7 & Matt Austin8
1University of Sheffield, Sheffield, UK; 2Columbia University Medical Center, New York, NY, USA; 3Paris Descartes University, Paris, France; 4University of California, San Francisco, San Francisco, CA, USA; 5Amgen Inc., Thousand Oaks, CA, USA.

Denosumab treatment is associated with low fracture incidence, sustained BMD increases, and reduced sCTX. The decrease in median sCTX is at the quantifiable limit (0.049 ng/ml) one month post-dose, remains low, and attenuates at the end of the 6-month dosing interval. Using 7 years of data from the FREEDOM study and its extension, we characterized changes in sCTX over time and the influencing factors. In the bone turnover marker and pharmacokinetic substudies, serum was collected after an overnight fast and prior to denosumab dosing. Post-dose sCTX values within 7 months of denosumab dosing were included. sCTX values obtained after a subject experienced an on-study fracture or received bone-active medication were excluded. A mixed model was constructed using a cubic polynomial to estimate the attenuation of sCTX while allowing for individual subject fluctuation in the rate of attenuation. sCTX values below the quantifiable limit were assigned half the limit (0.0245 ng/ml). With each denosumab dose, there was a rapid decrease in sCTX that was not influenced by duration of denosumab exposure or other factors. Mean sCTX begins to increase after ~5 months in the first year, reaching 0.11 ng/ml at the end of the 6-month dosing interval. In the third and subsequent years, mean sCTX begins to increase after ~4 months reaching 0.18 ng/ml 6 months post-dose. The increase was greater in subjects with higher baseline sCTX, PINP, body weight, spine BMD, and older age. We conclude that up to 7 years of denosumab administration consistently resulted in post-dose sCTX reduction, with increasing attenuation at the end of the dosing interval during the first 3 years of treatment. This attenuation did not increase further with subsequent denosumab treatment, and was affected by several baseline subject characteristics. Understanding sCTX dynamics while receiving denosumab may help understand the sustained BMD increases over time.

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Other diseases of bone and mineral metabolism

**PP454**

**Long bone fragility in NF1 is due to deficiency of architecture, micro-structure and matrix mineralization**

Jirko Kühnisch1,2,3,4, Jong Seto3,4, Claudia Lange1,2, Susanne Schröd6, Sabine Stumpf7, Karolina Kubis1, Julia Grohmann1, Nadine Kossler2, Peter Varga2, Monika Osswald5, Sigrid Tischert1, Wenke Seifert1, Thaiqul El Khassawna5, David Stevenson2, Florent Elettrehour10, Uwe Kornak1,2, Kay Raum1, Peter Fratzi1,2, Mateusz Kolanczyk1,2 & Stefan Mundlos2,1

1Institute for Medical Genetics and Human Genetics, Charité, Universitätsmedizin Berlin, Berlin, Germany, 2FG Development & Disease, Max Planck Institute for Molecular Genetics, Berlin, Germany, 3Department of Biomaterials, Max Planck Institute for Colloids and Interfaces, Potsdam, Germany, 4Institut für Physiologische Chemie, MTZ, Medizinische Fakultät Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany, 5Department of Chemistry, Universität Konstanz, Konstanz, Germany, 6Julius Wolff Institute & Brandenburg School of Regenerative Therapies, Charité – Universitätsmedizin Berlin, Berlin, Germany, 7Institut für Klinische Genetik, Medizinische Fakultät Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany, 8Institute for Vegetative Anatomy, Charité, Universitätsmedizin Berlin, Berlin, Germany, 9Shriners Hospitals for Children Salt Lake City, Salt Lake City, Utah, Salt Lake City, USA, 10Center of Bone Biology, Vanderbilt University – Medical Center, Nashville, USA, 11Berlin-Brandenburg Center for Regenerative Therapies (BCRT), Berlin, Germany.

Neurofibromatosis type I (NF1) is a monogenic disorder caused by mutations in the NF1 gene encoding neurofibromin, a Ras-GAP protein. Apart from benign tumour development NF1 is frequently associated with skeletal manifestations such as osteopenia or debilitating focal skeletal dysplasia. To assess a function of NF1 in osteocytes we here apply a combinatorial approach of biophysical, histological and molecular techniques allowing differential analysis of two conditional mouse models, Nf1Prx1 and Nf1Col1, as well as cortical bone samples from NF1 patients.

Humer of Nf1Prx1 mice appear dwarf, bowed and show severe disorganization at muscle to bone insertion sites suggesting diminished mechanical resistance. Within diaophysis Nf1Prx1 humeri demonstrate massive local defects of mineralization and organic matrix maturation. These changes were confirmed to a lesser degree also in Nf1Col1 humeri. Interestingly, mineralization lesions are associated with blood vessels that persist throughout postnatal bone development. Mechanical testing revealed severe impairment of Nf1Prx1 bone tissue strength. Reduced mechanical potency is partially caused by increased osteocyte volume in Nf1Prx1 and Nf1Col1 bone tissue. Osteocytes further show Ras hyperactivation inducing amplified pMEK1 and pERK1 signalling. Expression analysis detects increased levels of Tem7, Mgp and Phex. Importantly, Nf1Prx1 mice show only inducing amplified pMEK1 and pERK1 signalling. Expression analysis detects

**Methods**

We compared 14 nonosteoporotic patients (43.7 ± 19.2 years) with atrumatic BME of lower limb to 35 age-matched healthy controls (HC). HR-pQCT examinations of distal tibia as well as DXA measurements of spine and hip, and serum examinations of BTM were performed.

**Results**

Areal BMD/RT: BMD was in osteoporotic range. All subjects presented no differences between the groups.

**PP455**

**Cortical and trabecular alterations in patients with bone marrow edema of the lower limb**

Afrodite Zendeli1, Christian Muschitz1, Roland Kocjancic1, Lukas Fischer1,2, Daniela Sues1 & Heinrich Resch1

1The VINFORCE Study Group, St. Vincent Hospital, Medical Department II, 1060 Vienna, Austria; 2CILab-Department of Radiology Medical University of Vienna, 1090 Vienna, Austria.

Background

Bone marrow edema (BME) is a localised bone lesion. We hypothesize that structural bone alterations increase the susceptibility to BME. Aim of this study was to analyse bone micro structure, bone mineral density (BMD) and serum fasting bone turnover marker (BTM) values in patients with BME.

Methods

We compared 14 nonosteoartritic patients (43.7 ± 19.2 years) with atrumatic BME of lower limb to 35 age-matched healthy controls (HC). HR-pQCT examinations of distal tibia as well as DXA measurements of spine and hip, and serum examinations of BTM were performed.

**Results**

Areal BMD/RT: BMD was in osteoporotic range. All subjects presented no differences between the groups.
Dipeptidyl peptidase 4 (DPP4) modulates activity of proteins by removing two aminoterminal amino acids. DPP4 inhibitors are currently being used to improve glucose tolerance in type 2 diabetes patients by increasing the half-life of DPP4 substrates. It has been shown that these substrates do not only increase pancreatic insulin secretion, but also influence bone cell activity. The potential therapeutic effect of DPP4 inhibition on bone metabolism is thus worth being investigated.

In the present study, we evaluated the effect of the DPP4 inhibitor sitagliptin (SG) on bone in the streptozotocin (STZ)-induced diabetic rat. This study included 64 male Wistar rats, divided into four groups (n=16): two diabetic and two control groups. One diabetic and one control group received sitagliptin through drinking water (2 g/l). Rats were scanned every 3 weeks using an in vivo micro-computed tomography scanner. After 6 and 12 weeks, rats were sacrificed after tetracyclin labeling for bone histomorphometric analysis of both static and dynamic bone parameters.

STZ-treated (diabetic) rats had significantly increased blood glucose compared to controls and reduced body weight, which was not influenced by SG. SG however significantly decreased diuresis and food consumption in diabetic rats. In vivo DPP4 inhibition of 89% was achieved in both SG-treated groups. Trabecular bone volume and bone over tissue volume ratio in the tibia was significantly lower in SG-treated rats compared to untreated rats, but was normalized through SG treatment (significant in weeks 9 and 12). Trabecular thickness was decreased and trabecular spacing was increased in diabetic rats. SG treatment resulted in partial but significant recovery of trabecular parameters in diabetic rats. Cortical bone parameters and bone histomorphometry are currently assessed.

Results show an attenuation of diabetic bone loss through DPP4 inhibition. The effect of DPP4 inhibitor treatment on bone turnover is to be confirmed further through bone histomorphometric analysis.

**Conclusions**

The effect of DPP4 inhibition on bone metabolism is thus worth being investigated. Further studies are required to confirm the effect of DPP4 inhibition on bone turnover.

**References**

Belgium.

**Methods**

To evaluate biochemical and densitometric features of 14 patients with RTH (RTHG: 7 females (4 children) and 7 males (3 children)) in comparison to 24 control subjects (CG, 14 females (8 children) and 10 males (4 children)).

**Results**

The RTH patients exhibited higher concentrations of TCa (P<0.04) and corrected serum levels of calcium for albumin concentrations (CG=9.3±0.5, RTHG=9.8±0.4 mg/dl; P=0.01), lower concentrations of iP (CG=4.5±1.2, RTHG=3.7±0.9; P=0.04) and lower Tmp/GFR (CG=4.3±1.4, RTHG=3.4±1.2; P=0.03) than the CG. The FGF-23 concentrations were significantly higher in children with RTH than in CG (CG=65.3±18.6, RTHG=74.3±34.1; P=0.03). The bone mass was lower among adults in RTHG, in whole body (CG=1.15±0.07, RTHG=1.07±0.08; P=0.02), lumbar spine (CG=1.04±0.12, RTHG=0.94±0.11; P=0.05), and femoral neck (CG=0.91±0.11; RTHG=0.76±0.16; P=0.05) than in the corresponding CG. The Ca scores were lower in the RTHG than in CG in total hip (P=0.04) and femoral neck (P=0.05).

**Conclusions**

These data indicate alterations in bone mineral metabolism in RTH. The higher concentrations of calcium and lower bone mass in RTHG than in CG associated with the results of studies using animal models with mutant mice suggest that RTHG may exhibit thyrothroph bone phenotype. However, it was not possible to point out a single pathophysiological mechanism that justifies simultaneously all changes observed.

**References**

PP459

**Correlates of tissue mineral density of bone samples from total hip arthroplasty patients with type 2 diabetes: an ex vivo study**

Janet Pritchard1, Alexandra Papaioanou1, Mark Hurtig2, Lora Giangregorio1, Stephanie Atkinson1, Karen Beatte1, J.D. Adachi1, Justin DeBee1, Mitchell Winemaker1, Victoria Avram1 & Henry Schwarzkopf3

1McMaster University, Hamilton, ON, Canada; 2University of Guelph, Guelph, ON, Canada; 3University of Waterloo, Waterloo, ON, Canada.

**Introduction**

Fracture risk is greater for adults with type 2 diabetes (T2D), despite normal or higher areal bone mineral density (aBMD) compared to controls. Tissue mineral density (TMD), measured by microCT, is more representative of actual mineral density than in vivo aBMD. The aim of this study was to determine whether TMD is greater in adults with T2D, and to investigate the correlates of TMD in adults with T2D.

**Methods**

Using proximal femur bone sections from elective hip replacement patients, we assessed TMD and bone mineralization density distribution (BMDD) in adults ≥ 65 years with (n=14) and without T2D (n=20). A microCT system (GE, London, Canada) was used to obtain images (voxel size 21.2 μm3) of 5 mm thick bone sections. MicroView ABA 2.1.2 (GE, London, Canada) was used to determine TMD (mg HA/cm3). BMDD analysis was performed using scanning electron microscope (Vega, TESCAN USA), which yielded C_mean, C_peak, C average. Between-group differences were determined using a Student’s t-test. Bivariate linear regression was used to determine correlates (determined a priori) of TMD. A P-value of 0.05 was considered significant.

**Results**

TMD was not significantly different between adults with T2D (324.18±94.14 mg HA/cm3) compared to those without T2D (309.22±41.26 mg HA/cm3, P=0.541). Table 1 shows the correlates of TMD in adults with T2D.

**Table 1**

<table>
<thead>
<tr>
<th>Standardized β-coefficient</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T2D diagnosis ≥ 15 years</td>
<td>0.614*</td>
</tr>
<tr>
<td>Participant taking biguanide</td>
<td>0.470</td>
</tr>
<tr>
<td>CR_AEAN</td>
<td>0.427</td>
</tr>
<tr>
<td>CR_NONDIETIC</td>
<td>-0.859*</td>
</tr>
</tbody>
</table>

**Conclusions**

TMD was not different between groups. Lower mineralization heterogeneity and greater number of years with T2D were associated with TMD in adults with T2D. These findings provide exploratory evidence that disease duration and mineralization heterogeneity may be linked to low bone turnover in adults with T2D, which could explain greater fracture risk.

**References**

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PP460

**Osteopontin ASARM peptide binding to crystal faces of hydroxyapatite – computational simulations**

Ahmad Mansouri1,2, David Musica1,2, Jeffrey Gray1,2 & Marc McKee1,2

1McGill University, Montreal, QC, Canada; 2Johns Hopkins University, Baltimore, MD, USA.

**ASARM peptide (acidic, serine- and aspartate-rich motif) and osteopontin (OPN) fragments accumulate in X-linked hypophosphatemia patients and/or in the Hyp mouse model and, when phosphorylated, potently inhibit mineralization in osteoblast cultures. To investigate this inhibition, we modeled the binding to hydroxyapatite of the human OPN-ASARM peptide (DDSHQSDESHHS-DESDL) using RosettaSurface computational simulations. Peptide binding to**
hydroxyapatite atomic planes constructed to have different chemical terminations was computed using a structure-prediction algorithm for peptide–solid surface interactions. {100}, {010} and {110} monoclinic hydroxyapatite planar surfaces were built having different calcium-to-phosphate ratios. Miller indices (hk1) planes (surfaces) were created with mixed–charge to reflect surfaces likely occurring during crystal growth, and leaving intact interfacial phosphate and hydroxyl ions since P–O and O–H bonds are strong and their breaking is energetically unfavourable. Binding affinities, specificities and structure were determined for ASARM-Sp0 (without phosphoserine) and two phosphorylated forms of ASARM (ASARM-Sp3 and ASARM-Sp5, with 3 or 5 phosphoserines). Energy-minimized peptide conformations in solution and adsorbed to mineral were predicted by RosettaSurfaces. Adsorption data revealed highly significant, phosphorylation-dependent differences in binding energies for the peptides. All peptide conformers were generally unstructured both in solution and upon adsorption. Adsorbed peptides showed a degree of crystal lattice matching via the phosphate and carboxylate groups coordinating with surface calcium, binding to the (100) and (101) terminations showed the highest binding energies. In conclusion, peptide–mineral binding modeling has provided mechanistic data on how OPN and its phosphorylated peptides act as potent inhibitors of mineralization.

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PP641
QTc interval in hypercalcemic kidney transplant recipients
Ruzica Smalcelj1 & Anton Smalcelj1
1University Hospital Center Zagreb, Department of Internal Medicine, Zagreb, Croatia; 2University Hospital Center Zagreb, Department of Cardiovascular Diseases, Zagreb, Croatia.

Bone metabolism disorders and hypercalcemia occur often in kidney transplant recipients. In 59 kidney recipients (aged 22–74 years, creatinine clearance > 50 ml/min) who were hypercalcemic on more than three consecutive previous visits, the following serum parameters were estimated: 1–147 months posttransplant: iPTh, Ca total and ionized, Pi, total and bone alkaline phosphatase, crosslaps, 25(OH)D3, cyclosporine/tacrolimus trough levels. Urine creatinine and Ca were also measured, and creatinine clearance and Ca:creatinine clearance ratios were estimated. According to the Ca:CrCl ratio patients were divided into three groups: i) < 0.01-0.02, normal range ii) 0.01-0.02, usually found in hyperparathyroidism. After blood sampling, ECG was performed and QTc interval estimated. None of the patients received medications known to prolong the QT interval (i.e., amiodarone). Results (median with range): Ca total 2.76, 2.54–3.27 (reference range 2.14–2.53 mmol/l), Ca ionized 1.40, 1.33–1.69 (reference range 1.18–1.32 mmol/l), iPTh 14.2, 5.1–97.6 (reference range 1.0–6.0 mmol/l), QTc 0.393, 0.347–0.443, below the reference range in one patient (reference range 0.35–0.45 s). No significant correlation between the QTc interval length and total and ionized calcium, Pi, bone turnover parameters and 25(OH)D3 levels was found. The QTc interval length did not differ significantly among groups of patients according to Ca:creatinine clearance ratios. In patients with Ca:creatinine clearance ratios > 0.02 (n = 24) QTc interval length correlated significantly negatively with cyclosporine A trough levels. Conclusions: In hypercalcemic kidney recipients, QTc intervals were not shortened and no relationship to calcium metabolism disturbances was found. Cyclosporine A might have an impact on the QTc interval.

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PP642
Expression of RANKL/RANK/OPG in colon during experimental inflammatory bowel disease
Ivana Maric1, Ivana Smoljan1, Tamara Turk Wensveen1, Andrica Lekic1, Sanja Zoricic Cvek1, Tanja Celic1, Zeljka Crncic Orlic2 & Dragica Bobinac1
1Department of Anatomy, School of Medicine, University of Rijeka, Rijeka, Croatia; 2Psychiatric Hospital Rab, Rab, Croatia; 3Department of Internal Medicine, Clinical Hospital Rijeka, Rijeka, Croatia; 4Department of Physics, School of Medicine, University of Rijeka, Rijeka, Croatia.

Introduction
The RANKL/RANK/OPG system has a key role in bone metabolism. Beyond its role in bone loss, its importance was also documented during inflammation which occurs in inflammatory bowel disease (IBD). The aim of this study was to investigate the expression of the receptor activator of NF-κB ligand (RANKL) and its receptors RANK as well as its decoy receptor osteoprotegerin (OPG) in the colon during experimental IBD and following BMP7 or corticosteroid therapy.

Methods/design
IBD was induced by intrarectal administration of trinitrobenzenesulfonic acid (TNBS). After the IBD induction, the rats were treated with BMP7 (100 μg/ml) and sacrificed on the 2nd, 5th, 14th, and 30th day after the TNBS induction. The presence of RANKL, RANK and OPG in inflammatory bowel disease was determined by the continuous monitoring of the expression level in rat colons during different phases of experimental IBD as well as after BMP7 treatment by RT-PCR. Additionally, to investigate the influence of corticosteroid therapy on RANKL/RANKL/OPG expression, we treated the diseased animals with 2 mg/kg of dexamethasone for 5 days.

Results
During IBD the expression of RANKL/RANKL/OPG system was found in all colon samples. The expression level of OPG increased with disease duration and showed the largest expression on the 30th day of colitis which is opposite to the expression level of RANK whose expression decreased according to disease duration. BMP7 therapy and control animals showed no significant difference in their expression levels compared with diseased animals. Immunohistochemical analysis revealed the presence of OPG in epithelial cells and in lymphocytes. RANKL expression was also detected in colon samples with increased expression after corticosteroid therapy which is opposite to the expression of OPG.

Conclusion
The expression pattern of components of the RANKL/RANKL/OPG system during IBD suggests their important role in inflammation and probably on bone loss associated with IBD.

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PP643
Bone morphogenetic protein-7 reduces kidney cold ischemic injury by maintaining epithelial phenotype of tubular cells
Tanja Celic1, Josip Spanjol2, Ivana Maric1, Olga Cvijanovic1 & Dragica Bobinac1
1Department of Anatomy, School of Medicine Rijeka, Rijeka, Croatia; 2Department of Urology, Clinical Hospital Rijeka, Rijeka, Croatia.

Deceased donor kidneys are exposed to cold ischemic insult, which makes them particularly susceptible to the effects of cold ischemic injury during hypothermic preservation resulting in high rates of delayed graft function. Although cold storage reduces cellular oxygen demand, ischemia causes the rapid depletion of adenosine triphosphate and accumulation of toxic substances leading to cell death. BMP-7 is a valuable reagent in a field of tissue regeneration and preservation under ischemic conditions. Following these insights, we investigated the effect of rhBMP-7 on graft preservation during cold ischemia. The study was conducted on experimental model of kidney cold ischemia in rats. Kidneys were perfused with saline, University of Wisconsin (UW), rhBMP-7 or rhBMP-7 + UW and exposed to cold ischemia for 6, 12 and 24 hours. Using PCR method the expression of mRNA BMP-7, TGF-β1, Smad1, Smad2, Smad3, Smad5 and Smad8 was analyzed. Immunohistochemical analysis was used to show expression and localization of BMP-7, TGF-β1, E-cadherin and α-SMA. In tubular epithelial cells of the kidneys perfused with rhBMP-7 and rhBMP-7 + UW solution the expression of BMP-7 and E-cadherin was observed after 24 hours of cold ischemia. In the kidneys not perfused with rhBMP-7 high expression of TGF-β1 and α-SMA was found. Also, in the kidneys perfused with rhBMP-7 solution level of mRNA BMP-7 expression was increased. In the same tissue higher level of mRNA Smad1, Smad5 and Smad8 expression, molecules of intracellular BMP-7 signal pathway, was proved. The levels of mRNA BMP-7, Smad1, Smad5 and Smad8 expression were equaly present during whole time of cold ischemia.

BMP-7 maintains the morphology of the kidney tissue better than UW solution during 24 hours of cold ischemia. BMP-7 prevents epithelial to mesenchymal transformation and consequently maintains epithelial phenotype of tubular cells.

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**PP464**

**Effects of add-on parathyroid hormone (PTH(1-84)) substitution therapy in hypoparathyroidism: results from 2.5 years of PTH treatment**

Tanja Sikaer1, Emil Moser1, Lars Rolighed2, Leif Mosekilde1 & Lars Rejnmark2

1Department of Internal Medicine and Endocrinology MEA, Aarhus University Hospital, Aarhus, Denmark; 2Department of Surgery P, Aarhus University Hospital, Aarhus, Denmark.

Conventional treatment of hypoparathyroidism with calcium and active vitamin D analogues causes a high renal calcium excretion and over-mineralized bone. We studied 62 patients with hypoPT randomized to 6 months of treatment with either parathyroid hormone (PTH(1-84)) 100 µg/d s.c. or similar placebo, administered as an add-on therapy. Forty-two of the patients had follow-up test performed after 2.5 years. 9 patients had continued daily PTH treatment (group 1), 15 had PTH for 6 months followed by 2 years of conventional treatment (group 2) and 18 had only received conventional treatment (controls).

PTH for 2.5 years kept p-calcium within the physiological range. We found no change in renal calcium excretion in group 1 or group 2 after 2.5 years. We have previously reported a decrease in BMD z-score at the hip, lumbar spine and whole body after 6 months of PTH treatment. Interestingly we found a significant increase in z-score at the hip, spine and a tendency towards an increase at the whole body, but not the forearm in group 2, resulting in a higher increase in z-score values after 2.5 years in group 2 compared to controls.

Continuous treatment for 2.5 years compared to controls resulted in a decrease at the forearm and borderline increase at the spine. A total of 2.5 years of treatment with PTH substitution therapy is capable of maintaining normal p-calcium levels, but not capable of reducing urinary calcium excretion.

Long-term PTH therapy is safe regarding BMD, the previously shown initial in BMD reverses.

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**PP465**

**Post surgical hypoparathyroidism and the risk of fractures**

Line Underbjerg, Tanja Sikaer, Leif Mosekilde & Lars Rejnmark

Department of Endocrinology and Internal Medicine, Aarhus University Hospital, Aarhus C, Denmark.

Background

Hypoparathyroidism (HypoPT) is a rare disease, characterized by low plasma levels of Parathyroid hormone and calcium. Furthermore it is characterized by high BMD and very low bone turnover.

Aim

We studied risk of fracture in patients with postsurgical HypoPT due to non-malignant diseases compared with an age- and gender-matched control group.

Method

We performed a controlled cohort study. Patients diagnosed with HypoPT due to neck surgery for non-malignant causes from 1988 to 2012 were identified using the National Patient Registry on hospital discharge diagnoses. In addition, patients were identified through regional prescription databases, by identifying patients on treatment with active vitamin D analogues. Case status of all identified patients was subsequently validated by review of their medical charts. For each patient, we received three age-(±2 years) and gender-matched controls, randomly selected from the general background population. Risk of fracture was calculated by Cox regression analyses.

Results

Within a population of 5,336,394 persons, we identified 688 patients with chronic HypoPT due to non-malignant disease (prevalence 22/100,000). Risk of any fracture did not differ between cases and controls (crude HR 0.97, 95% CI 0.77–1.21). Adjustment for a history of fracture did not change results (HR 0.95, 95% CI 0.73–1.21). Risk of fracture due to non-malignant disease (prevalence 22/100,000). Risk of any fracture did not differ between cases and controls (crude HR 0.97, 95% CI 0.73–1.21). Adjustment for a history of fracture did not change results (HR 0.95, 95% CI 0.73–1.21).

Conclusions

Risk of fracture in patients with postsurgical HypoPT due to non-malignant causes was not increased compared with healthy age- and gender-matched controls.

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**PP466**

**Bone cross-sectional geometry in adult patients with hypophosphatemic rickets: a hip structural analysis study**

Charlotte Ejersted, Signe Beck-Nielsen, Jeppe Gram & Kim Brixen

Department Endocrinology, Odense University Hospital, Odense, Denmark.

Introduction

FGF3-associated hypophosphatemic rickets (HR) is a rare disorder caused by excessive renal phosphate wasting. Patients may suffer from limb deformities and low turnover femoral fractures have been described. The aim of this study is to evaluate DXA derived hip geometry of adult HR patients using hip structure analysis (HSA).

Materials and methods

Cross sectional study of HR patients (n=21) at Odense University Hospital compared to age- and sex-matched controls (CON; n=38). Proximal larp DEXA scans were analyzed for bone geometry by use of the HSA programme developed by Beck et al. The analysis included three locations: the narrow neck (NN), the intertrochanteric region (IT), and the femoral shaft (FS).

Results

In NN cross-sectional area (CSA) were: (mean±SD): HR: 3.60±1.06, CON 3.28±0.70 cm², cross-sectional moment of inertia (CSMI) HR: 4.06±1.88, CON 3.63±1.42 cm⁴, section modulus HR: 2.04±0.75, CON 1.86±0.58 cm³, buckling ratio (BR) HR: 9.92±2.52, CON 10.50±2.41, BMD HR: 1.06±0.26, CON 0.98±0.16 g/cm². Results were similar for IT and FS. The shaft neck angle were lower in HR patients: HR: 124.0±5.8, CON 130.1± 5.2° (P<0.001); the hip axis length similar: HR: 113±11, CON 114±12 mm. HR and CON patients were at similar age (HR: 40.7±2.4, CON 42.6±2.3 years) and weight (HR: 85.7±5.5, CON 84.2±2.3 kg). HR patients were shorter than controls: HR: 159.2±5.5, CON:173.1±1.8 cm (P<0.004).

Conclusion

The HSA analysis of the hip revealed no major differences in geometry between the groups. BMD, CSA, CSMI, section modulus, and buckling ratio were similar between HR patients and sex- and age-matched controls. The shaft neck angle and height were lower in HR patients.

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**PP467**

**Bone marrow fat is metabolically distinct fat depot**

Riku Kiviranta1,2,3, Tan Pham2, Jarna Hannukainen4, Juho Jarvelin1, Anna Kärni5, Minna Seinö2, Pauliina Salminen5 & Pirjo Nuutila1,2

1University of Turku, Turku, Finland; 2Turku University Hospital, Turku, Finland; 3Turku PET Centre, Turku, Finland.

In adults, majority of bone marrow (BM) space of long bones is filled with fat tissue. Adipocytes are also present within trabecular bone areas such as vertebreal bone marrow. Despite its prevalence the roles of BM fat in energy and bone metabolism have been largely overlooked. To characterize bone marrow metabolic activity we measured regional glucose uptake in femoral and vertebral bone marrow during fasting and insulin stimulation in normal weight healthy subjects.

Nine healthy adults (age 47±6 years, BMI 23.7±1.9 kg/m²) volunteered for the study. The subjects were imaged with positron emission tomography (PET) using F18 fluorodeoxyglucose ([18F-FDG] tracer to measure glucose uptake (GU) in skeletal muscle, abdominal subcutaneous fat, abdominal visceral fat and vertebral and femoral bone marrow. PET imaging was performed at fasting state and during hyperinsulinemic euglycemic clamp to measure basal and insulin-stimulated GU. Fasting GU in femoral BM was significantly higher than in subcutaneous fat (4.93±1.58 vs 2.82±0.38 μmol/min per g, respectively, P<0.05) but did not significantly differ from visceral fat. Skeletal muscle GU was 56% higher than that of femoral BM (P<0.01). Interestingly, glucose uptake in vertebral BM that contains bone and hematopoietic cells and adipocytes, was five-fold higher than in femur (P<0.001). Insulin stimulation during clamp induced a four-fold increase in femoral BM GU (20.43±6.00 μmol/min per g, P<0.001 vs fasting state), which remained higher than that of sc and visceral fat. Surprisingly, insulin did not stimulate glucose uptake in vertebral bone marrow (25.98±3.46 clam vs 24.78±4.39 μmol/min per min at fasting).

This study shows that glucose metabolism differs significantly between vertebral and femoral BM. GU in vertebral BM cells appears to be insulin independent. Conversely, insulin stimulates GU in the mainly fatty femoral BM to similar extent as in brown fat. Moreover, the overall GU in femoral BM both in fasting state and during hyperinsulinemic euglycemic clamp is higher than in other fat depots. Thus, our data supports the hypothesis of bone marrow fat as functionally distinct ‘yellow fat’.

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Phosphorylated ASARM peptide inhibited SHED differentiation, with no mineralized nodule formation, decreased odontoblast marker expression, and upregulation of MEPE. When implanted in a tooth pulp injury model, this peptide impairs reparative dentin formation and mineralization, and increased MEPE immunohistochemical staining was detected. In conclusion, using original models to study tooth dentin abnormalities observed in XLH, we show that the MEPE-derived ASARM peptide inhibits both odontogenic differentiation and matrix mineralization, while increasing MEPE expression. These results provide a partial mechanistic explanation of XLH pathogenesis; that direct inhibition of mineralization by ASARM peptide leads to the mineralization defects observed in XLH teeth. This process appears to be positively reinforced by the increased MEPE expression induced by ASARM. The MEPE-ASARM system should be considered as a potential therapeutic target for treatment of XLH.

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PP470

Determinants of bone loss in cystic fibrosis

Déborah Gensburger, Roland Chapurlat, Raphaële Nove-Josserand, Muriel Rabilloud, Isabelle Durieu

1INSERM 1033, Hospices Civils de Lyon, Lyon, France; 2Cystic Fibrosis Adult Center, Université de Lyon et Hospices Civils de Lyon, Lyon, France; 3Département de Biostatistique, Université de Lyon et Hospices Civils de Lyon, Lyon, France.

Objectives

Bone disease is now well described in cystic fibrosis adult patients. CF bone disease is multifactorial but many studies suggested the crucial role of inflammation and chronic pulmonary infection. The objectives of this study were to assess the prevalence of osteoporosis in a current adult CF population and to examine its relationship with infections and inflammation.

Methods

Patients were recruited in the adult CF Lyon Centre and assessed in clinically stable period, later during a respiratory infection, and finally 14 days after the end of antibiotic therapy. At each time points, we performed a clinical evaluation, lung function tests and biochemical tests: markers of inflammation (CRP, IL6, and TNFa), serum markers of bone turnover (serum CTX), and serum RANK-L and OPG. Absorptiometry and dorso-lumbar radiographs were also performed. We enrolled 56 patients (29 men, mean age of 26).Bone mineral density (BMD) values indicated osteopenia in 41% and osteoporosis in 14% of patients. We found one or two vertebral fractures on radiographs in two patients without any history of previous fracture. After infections treated with antibiotics, serum RANK-L and OPG were increased (+24%, P = 0.08 and +13%, P = 0.04 respectively), with a stable ratio. This increase was delayed in comparison to the increase of inflammation markers. Serum CTX were stable during pulmonary infections. No significant correlation was found between serum inflammation markers, CTX and RANK-L.

Conclusion

In this study, bone disease among adult CF patients was less severe than previously described. We found a mild increase of serum RANK-L levels, delayed compared with the pulmonary infections, and independent from the bone resorption level.

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PP471

Long-term energy deficiency in mice induces bone alterations reversed by long-term recovery

Sara Zghieb, Stéphanie Lucas, Mathieu Mequinion, Odile Broux, Damien Leterme, Pierre Hardouin, Odile Vilariñas & Christophe Chauveau

1Physiopathologie des Maladies Osseuses Inflammatoires, EA4400, ULCO-Lille 2, Boulougnes Mer, Lille, Nord-Pas de Calais, France; 2Développement et Plasticité du Cerveau Postnatal, UMR637 Inserm, JPARC, Lille, Nord-Pas de Calais, France; 3Université de Lille 1, Lille, Nord-Pas de Calais, France.

Anorexia nervosa (AN) a condition of profound undernutrition, is characterized by alterations in neuroendocrine and metabolic functions. Among the serious pathological consequences of this eating disorder, osteoporosis is often observed and persists after recovery, leading to a high fracture risk. To study particularly bone alterations and recovery, a long term mouse model has been developed. In this model named separation-based anorexia (SBA) – a
chronic stress induced by separation is associated with a restricted-time feeding schedule. Eight-week-old C57Bl/6J females were separated and their food access was gradually reduced from 6 to 2 h/day (SBA). After 10 weeks mice were housed again in standard conditions for 10 more weeks (recovery). During the first 2 weeks of the SBA protocol, mice lost 25% of their initial body weight and then maintained this underweight while eating only 10% less than control mice. Fat and lean masses were quickly decreased and bone mineral acquisition mass was disrupted. Cortical and trabecular bone mineral densities of the tibia were significantly reduced. Reproductive functions were also rapidly and strongly altered and mice were hypoleptinemic. The recovery phase allowed a rapid normalisation of body weight, fat and lean masses as well as reproductive functions. After 10 weeks of the recovery phase, all the mice had similar bone mineral content, but SBA mice still exhibited low leptinemia despite their recovered fat mass.

We hypothesised that the high capacity of bone normalization of recovered mice could be linked to this specific context of persisting hypoleptinemia associated with normalization of the other parameters.

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PP472

Monocytic expression of osteoclast-associated receptor is induced in atherosclerotic mice and regulated by oxidized low-density lipoprotein in vitro

Kathrin Sinninger1, Martina Rauner1, Nadia Al-Fakhri2, Michael Schoppet1 & Lorenz Hofbauer1,4

1Division of Endocrinology, Diabetes, and Bone Diseases, Department of Medicine III, Technical University, Dresden, Germany; 2Department of Clinical Chemistry and Molecular Diagnostics, Philippus-University, Marburg, Germany; 3Department of Internal Medicine and Cardiology, Philippus-University, Marburg, Germany; 4DFG Research Center and Cluster of Excellence for Regenerative Therapies, Technical University, Dresden, Germany.

The osteoclast-associated receptor (OSCAR), primarily described as a co-stimulatory regulator of osteoclast differentiation, represents a novel link between bone metabolism and vascular biology. Previously, we identified OSCAR on endothelial cells responding to the proatherogenic factor oxidized low-density lipoprotein (oxLDL). Additionally, OSCAR expression was increased in the aorta of atherogenic apoE-knock-out (apoE-KO) mice, where it was further induced by feeding a high-fat diet. Because monocytes play an important role in the progression of atherosclerosis, we assessed whether atherosclerosis also regulates the expression of OSCAR on monocytes and whether it is regulated by oxLDL or other inflammatory mediators in vitro. Four weeks old male wild-type (WT), apoE-KO and idlr- (LDLR receptor) KO mice were fed a high-fat diet or normal chow for 6 weeks. Thereafter, peripheral blood mononuclear cells (PBMC) were isolated from the spleen by Biocoll density centrifugation to stain the cells with antibodies against CD14 and OSCAR for subsequent flow cytometric analysis. OSCAR surface expression on CD14-positive monocytes was increased twofold in PBMCs from apoE-KO mice compared to WT mice. Feeding a high-fat diet further increased OSCAR surface expression up to 1.5-fold in apoE-KO mice compared to apoE-KO mice fed a normal chow. Similarly, PBMCs from idlr-KO mice fed a high-fat diet showed a 1.7-fold increase in OSCAR expression compared to WT receiving the same diet. Additionally, we exposed the murine macrophage cell line RAW 264.7 to oxLDL and TNFα. OSCAR mRNA expression levels were induced by TNFα about threefold whereas oxLDL expression levels were induced by TNFα about threefold whereas oxLDL treatment increased expression by about sixfold after 48h. Signaling experiments revealed that oxLDL-dependent induction of OSCAR expression can be prevented by blocking the oxLDL receptor LOX-1 and inhibiting the NFκB-pathway. In conclusion, OSCAR expression in RAW 264.7 cells and primary murine CD14-positive cells is regulated by proatherogenic stimuli further confirming its function in the development of atherosclerosis.

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PP473

Evidence of increased bone resorption in early post menopausal women with idiopathic hypercalciuria: study with biochemical markers and pQCT of the Tibia

Konstantinos Stathopoulos1, Ilias Bournazos1, Pelagia Katsimbris2, Andonis Partisinevelos1, Arideis B Zoubos3, Panagiotis Papaggelopoulos1, Erato Atsali1 & Grigoris Skarandavos1

1Bone Metabolic Unit, 1st Department of Orthopedics, School of Medicine, University of Athens, ‘Attikon’ University General Hospital, Athens, Greece; 24th Department of Internal Medicine, ‘Attikon’ University General Hospital, Athens, Greece.

Aim

We explored the hypothesis that idiopathic hypercalciuria (IH) causes increased bone loss in early post-menopausal women.

Materials and methods

We studied 41 postmenopausal women with IH. Inclusion criteria: i) recently (<6 months) diagnosed and untreated IH, ii) postmenopausal status >3 years, and iii) normal renal function. Exclusion criteria: i) all causes of hypercalciuria other than IH and ii) use of any medication for osteoporosis 1-year prior study. All patients were assessed for serum and urine 24h calcium, phosphorus, 25(OH) vit D, PTH, bone ALP, serum NTX, and CTX. We studied three age groups: 48–59 years (n=15), 60–69 years (n=21), and 70–79 years (n=5). Patients underwent tibia pQCT (XCT 2000 scanner, Stratec), three slices obtained at the 4% (trabecular bone), 14% (subcortical), and 38% (cortical) of tibia length. For each site we estimated bone mineral content, bone areas, cortical thickness, periostal and endosteal circumference, then compared results with our published tibia pQCT database of 219 age-matched healthy postmenopausal women. We performed statistical analysis: data expressed as mean ± s.d.

Results

73% of patients in the 48–59 years group (11/15) showed evidence of increased bone turnover (≥ 1 bone marker). They also had lower cortical bone mineral mass (256.54±39.95 vs 282.63±38.63 mg/cm, P=0.019), cortical area (220.4±33.34 vs 246.85±32.85 mm2, P=0.005), cortical thickness (3.90±0.34 vs 4.53±0.57 mm, P=0.005), and greater endosteal circumference (45.27±8.11 vs 40.34±4.51 mm, P=0.001) than age-matched individuals.

Conclusions

Our results suggest that early post menopausal women with IH present increased bone resorption and bone loss than healthy age-matched women. These effects of IH on bone appear to be lost later in life.

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PP474

Insertion of the elcn7 gene mutation pG213R in mouse induces autosomal dominant osteopetrosis type II

Andrea Del Fattore1, Amie K Gray 2, Shoji Ichikawa2, Kang Chu2, Michael J Econs2 & Imranul Alam2

1Bambino Gesù Children’s Hospital, Rome, Italy; 2IUPOI, Indianapolis, Indiana, USA; 3University of L’Aquila, L’Aquila, Italy.

Autosomal dominant osteopetrosis type II (ADO2) is a rare osteosclerotic disease due heterozygous missense mutations of the CLC7 gene encoding the type seven chloride channel. Our two labs independently generated the first C57 black (6 B6) mouse model of ADO2 by inserting the pG213R- elcn7 mutation. Homozygous mice showed lack of tooth eruption and died within 30 days of age with severe osteopetrosis and central nervous system degeneration. Compared to WT, heterozygous B6 ADO2 mice showed increase of whole body aBMD (4%, P<0.05) and much greater change at distal femur for BV/TV and Trab.N (75 and 65%, P<0.01). Histomorphometric analysis revealed twofold increase of osteoclast number in the proximal tibia compared to WT mice. Bone marrow monocytes from B6 ADO2 mice showed twofold increase of osteoclast formation, and 80% reduction of resorption pits, confirming cell autonomous impairment of bone resorption. Since the penetrance of the disorder in humans is ~66% and severity varies considerably, we crossed-bred B6 ADO2 with mice of different genetic backgrounds (129, D2, Balb/c and CD1). Compared to WT, the whole body aBMD and BMC at 12 weeks of age were very high in ADO2 mice on 129 background (8 and 12%, P<0.01). ADO2 mice on D2 background also had significantly higher whole body aBMD (4%, P<0.002). The BV/TV was significantly higher at distal femur in ADO2 mice on 129, D2 and Balb/c backgrounds. CTX/TrAlP ratio was significantly lower in all ADO2 backgrounds, except the D2. Our results demonstrate that we have generated the first animal model of ADO2 that will help us to study the mechanisms of incomplete penetrance and test innovative therapies to treat this incurable disease.

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Multidisciplinary studies of ancient calcified tissues: renal stones from mummies

Mattia Capulli1, Lorenzo Arrizza2, Nadia Rucci1, Sara Gemmini Piperni1, Raimondi Quagisina1, Valentina Giulia2, Gino Fornaciari1, Anna Teti1 & Luca Ventura1
1Department of Biotechnological and Applied Clinical Sciences, University of L’Aquila, L’Aquila, Italy; 2Centre of Microscopies, University of L’Aquila, L’Aquila, Italy; 3Department of Chemistry and Chemical Engineering, University of L’Aquila, L’Aquila, Italy; 4Division of Paleopathology and History of Medicine and Bioethics, Department of Oncology, Transplants and Advanced Technologies in Medicine, University of Pisa, Pisa, Italy; 5Department of Pathology, San Salvatore Hospital, L’Aquila, Italy.

The renal stones found in the mummies of Pandolfo III Malatesta, Lord of Fano (1370–1427) and an anonymous nobleman from Popoli (XVII century) were investigated using different techniques. Both specimens were examined with binocular stereomicroscopy (BSM) and scanning electron microscopy (SEM), also with energy dispersive X-ray analysis (EDX). Multiple tiny fragments from surface and inner portions were submitted to X-ray diffraction (XRD) analysis. Subsequently, the calcui were imaged with microcomputed tomography (micro-CT). The stone from Pandolfo had a mulberry-like surface with honey brown colour and measured 12 mm in largest diameter. Along with the organic constituents (C, O, and N), the following chemical elements were detected: K, Si, Cl, Ca, P, Na, and Ba. The calculus was composed of ammonium acid urate (95%) and calcium oxalate dihydrate (weddelite) (5%). Internal structure consisted of aggregated large spheroidal crystals with different density values. In the case from Popoli, the ovaloid mass with small superficial spherical buds measured 22×16×15 mm. The cut surface showed a central nucleus of sharp-edged crystals and concentric laminations. Detected chemical elements were: C, O, Na, Ca, P, K, Si, Cl, and N. The stone composition was calcium oxalate monohydrate (weddelite: 90%) and calcium phosphate (hydroxyapatite: 10%). Internal structure detail revealed concentric laminations and aggregates of similar density values. These observations enabled us to propose an ideal protocol for the examination of stones that can be found in mummies and in osteoarcheological material. After preliminary observation with BSM, the specimen should be imaged with microCT, in order to trace a detailed map of the external surface and the whole calculus and guide the following SEM-EDX measurements for elemental distribution analysis. Matching the results from these methods avoids destructive XRD analysis and may allow to obtain an affordable evaluation of chemical composition on the entire stone, following a conservative approach.

A OPTN variant (rs1561570) interacts with TNFRSF11A polymorphism (rs1805034) on the clinical phenotype of sporadic Paget’s disease of bone

Daniela Merlotti1, Luigi Gennari1, Fernando Gianfrancesco2, Domenico Rendina3, Marco Di Stefano4, Teresa Esposito5, Giuseppina Divisato2, Giovanna Morello2, Riccardo Mucciaroli5, Giancarlo Isaia4, Pasquale Strazzullo2 & Ranuccio Nuti1
1Department of Medical Surgical Sciences and Neurosciences, University of Siena, Siena, Italy; 2Institute of Genetics and Biophysics, CNR, Naples, Italy; 3Department of Clinical and Experimental Medicine, University of Naples Federico II, Naples, Italy; 4Surgical and Medical Disciplines, Section of Gerontology and Bone Metabolic Diseases, University of Turin, Turin, Italy.

Despite mutations in QSTMI1 gene have been detected in up to 50% of patients with familial Paget’s disease of bone (PDB), their prevalence is low in sporadic PDB, likely due to the presence of additional predisposition genes. Recently, at least seven genes were associated with PDB in genome-wide-association studies, including polymorphic variation in OPTN, encoding for optineurin. In particular, a single OPTN variant (rs1561570) was highly associated with PDB in our Italian replication cohort of 205 QSTMI1-negative patients. In this study we evaluated whether this OPTN variant is associated with PDB and the severity of phenotype in a larger population of 735 cases previously screened for QSTMI1 mutations. 200 age and sex-matched controls were also genotyped for comparison. Potential interactions with a TNFRSF11A polymorphism (rs1805034) previously associated with PDB severity were also explored. In the overall population we observed an increased prevalence of rs1561570 T allele in PDB patients than in controls (OR 1.6; P<0.01). This association was higher in sporadic than familial cases. In contrast to the TNFRSF11A C variant, which was associated with increased disease severity in both QSTMI1 negative or positive patients, the OPTN variant did not appear to interact with QSTMI1. In fact, the presence of the OPTN risk allele (T) was significantly associated with an early onset and an increased number of affected sites only in QSTMI1 negative patients, and particularly in sporadic cases. Haplotype analysis showed a higher prevalence of haplotype CC–TT (containing the homozygous risk alleles for both TNFRSF11A and OPTN, respectively) in sporadic than familial cases or controls (11 vs 7% vs 3% in sporadic, familial PDB and controls, respectively; P<0.01). In summary, this study provides evidence that this OPTN variant affects the susceptibility to develop PDB and interacts with TNFRSF11A polymorphism to cause the severity of the disorder in sporadic cases.

Circulating sclerostin level in patients with ossification of the posterior longitudinal ligament of the spine

Masafumi Kashii1, Yohei Matsuo1, Tsuyoshi Sugita1, Taka-hito Fujimori2, Yukitaka Nagamoto1, Hirotosu Honda3, Takashi Kaito1, Motoki Iwasaki1 & Hideki Yoshikawa1
1Osaka University Graduate School of Medicine, Suita, Osaka, Japan; 2Osaka National Hospital, Osaka, Japan.

Ossification of the posterior longitudinal ligament (OPLL) is characterized by pathological ectopic ossification of the posterior longitudinal ligament. Development of OPLL induces compression myelopathy or radioculopathy due to spinal stenosis and the loss of spinal flexibility by ankylosing spinal hyperostosis (ASH). Although the etiology of OPLL has not been fully elucidated, systemic
and local bone formation factors may play a role in the pathogenesis of OPLL. The SOST gene encoding sclerostin is an osteocyte derived negative regulator of bone formation. Sclerostin is a Wnt/β-catenin signal antagonist necessary for bone formation. There is no reports regarding the relationships between OPLL and sclerostin.

Objective
This study aim to compare serum sclerostin levels between OPLL patients and control patients, and to identify the relationship between serum sclerostin level and bone turnover markers, OPLL localization and numbers of ossified vertebra.

Methods
Seventy-eight OPLL patients were studied and compared with age and sex matched 39 control patients with spinal canal stenosis without OPLL. Serum sclerostin and Dickkopf-1 (Dkk1) levels were measured by ELISA.

Results
Serum sclerostin levels in OPLL patients is significant higher than controls (OPLL: mean 64.1, s.d 39.3 pmol/l; control: mean 44.9, s.d 17.7 pmol/l; P=0.005). On the other hand, serum Dkk1 level in OPLL patients is significant lower that controls (OPLL: 2016±836 pmol/l, control 2394±959 pmol/l; P=0.03). In OPLL patients, the positive correlation between age and sclerostin levels was found in male OPLL patients (r=0.43, P=0.002). There are no relationship between serum sclerostin levels and bone turnover markers, OPLL localization and numbers of ossified vertebra.

Conclusion
Systemic secretion of sclerostin by osteocytes increased in OPLL patients with advancing age, and there will be a negative feedback system to suppress progression of OPLL and hyperostosis by sclerostin in OPLL patients.

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**PP479**

The activation of RANK/RANKL/OPG system in normal pregnancy and pre-eclampsia

Dorota Darmochwal-Kolarz
Medical University of Lublin, Lublin, Poland.

Objectives
The purpose of our study was to investigate RANK/RANKL/OPG system and the concentrations of other markers of bone turn-over in normal pregnancy and pre-eclampsia.

Materials and methods
Forty five patients with pre-eclampsia, 78 healthy pregnant women and twenty non-pregnant women were included in the study. Sera concentrations of the markers of bone turn-over: osteoprotegerin (OPG), sRANKL, osteocalcin and CrossLaps – degradation products of type I collagen were determined using the ELISA method. Statistical analysis was performed using Mann-Whitney U test.

Results
The concentrations of sRANKL and OPG were significantly higher in the second trimester of normal pregnancy when compared to the first and the third trimester. The concentrations of osteocalcin and CrossLaps were significantly higher in pre-eclampsia when compared to the patients in the third trimester of pregnancy.

Conclusion
The alterations in the bone metabolism are the most intense in the second trimester of normal pregnancy. These results could suggest that there are alterations in bone metabolism in pregnant women with pre-eclampsia.

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**PP480**

Effect of polyphenolic compounds from Aronia melanocarpa berries on cadmium accumulation in the bone tissue

Malgorzata M Brzóska, Malgorzata Galazyn-Sidorczuk & Maria Jurczuk
Department of Toxicology, Medical University of Bialystok, Bialystok, Poland.

Cadmium (Cd) is a toxic heavy metal characterized by strong cumulative properties in the human and animals’ organism. Although cadmium accumulation in the bone tissue is lower than in soft tissues such as liver and kidney, the bone-accumulated metal, even at low concentrations, can damage the bone tissue directly. Polyphenols are compounds possessing hydroxyl groups capable of binding divalent metals, including toxic metals, preventing their absorption from the gastrointestinal tract and retention in the body. Thus, the aim of this study was to investigate whether consumption of polyphenolic compounds may protect from cadmium accumulation in the bone tissue under low and moderate chronic exposure to this metal. For this purpose cadmium concentration in the bone tissue at the distal femoral end (trabecular bone region) of the female Wistar rats administered as the only drinking fluid 0.1% water extract of polyphenols from the berries of Aronia melanocarpa or/and cadmium in diet (1 and 5 mg Cd/kg) for 3, 10, 17 and 24 months was determined (by atomic absorption spectrometry with an electrothermal atomization in a graphite furnace). The low and moderate exposure to cadmium alone (1 and 5 mg Cd/kg respectively) increased this metal concentration in the bone tissue compared to the control group. The administration of polyphenolic compounds from Aronia melanocarpa berries during the exposure to 5 mg Cd/kg, but not at the treatment with 1 mg Cd/kg, decreased this toxic metal concentration in the bone tissue. Based on the results, it can be concluded that consumption of polyphenolic compounds present in the berries of Aronia melanocarpa may provide protection from cadmium accumulation in the skeleton under moderate exposure to this metal. This study was financially supported by the grant (no. N 405 051140) from the National Science Centre (Poland).

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**PP481**

Single nucleotide polymorphisms identification and functional analysis in PDB6 locus: a target locus for Paget's disease of bone

Iris Silva1, Natércia Conceição1, Elsa Vieira-Sousa1,2, Ana Rodrigues1,2, Joana Caetano-Lopes2,3, 1Department of Biomedical Sciences and Medicine, University of Algarve, Faro, Portugal; 2Centre of Marine Sciences (CCMAR), University of Algarve, Faro, Portugal; 3Centre for Marine Sciences (CCMAR), University of Algarve, Faro, Portugal.

Introduction
The etiology of Paget’s disease of bone (PDB) is not fully understood, but genetic factors play a clearly important role. Single nucleotide polymorphisms (SNPs) of OPTN gene within PDB6 locus have been highly associated with PDB, but no PDB causal mutation or functional effect on PDB development were reported to date. We aimed to identify functional SNPs associated with this bone disease.

Methods
Relevant candidate genes from PDB6 locus were selected based on their known biological function in bone. For each gene the coding region, splice sites, 5’ and 3’ UTRs and promoter were amplified, using an initial discovery sample of French–Canadian PDB patients from 38 different families. For each variant identified, we performed in silico analysis to determine its predicted functional effect.

Results
Sequence analysis of our sample allowed us to identify sixty SNPs already reported in the NCBI database and seven variants previously unknown in all our five candidate genes – OPTN, CAMK1D, PHFYH, SEP5H1, and CCDC3. The in silico analysis showed that the major of the SNPs could be related to alterations in gene expression possibly affecting bone cell function resulting in bone related diseases, as PDB. Furthermore, our in silico analysis performed on the variant rs3829923 found in the OPTN promoter, identified putative binding sites for NRF2, E47A and SAPI transcription factors (TFs) overlapping the SNP containing the G, whereas it was absent in the sequence containing the A. We hypothesized that this polymorphism may alter the binding of these TFs to this promoter, affecting OPTN expression. This possibility is now being evaluated.

Conclusion
PDB6 appears to be a good locus containing several bone related genes that may be involved in PDB pathogenesis. Further functional analysis using in vivo transient transfection assays are required to investigate the effect of rs3829923 in OPTN promoter.

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**PP482**

Zoledronate efficacy and safety in active Paget’s disease: long-term follow-up and retreatment in clinical practice

Elsa Vieira-Sousa1,2, Ana Rodrigues1,2, Joana Caetano-Lopes2,3, Susana Capela1, Filipa Ramos1, Ricardo Figueiredo1, Joaquim Polidoro-Pereira1, Cristina Ponte1,2, Raquel Campanilho-Marques1,2, Rita Barros1, José Carlos Romero1 & José Alberto Pereira da Silva1
1Rheumatology and Metabolic Bone Diseases Department, Santa Maria Hospital, CHLN, Lisbon, Portugal; 2Rheumatology Research Unit, Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa, Lisbon, Portugal.

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Background
Zoledronate, a third generation bisphosphonate, has shown high efficacy in the inhibition of bone resorption. The objective of this observational study was to assess short and long-term efficacy and safety of zoledronate in the treatment of active Paget’s disease (PD).

Methods
Patients with active PD treated with zoledronate 5 mg were consecutively recruited. Clinical and laboratory parameters were determined before, at 3 and every 6 months after treatment. Remission was defined as normalization of alkaline phosphatase.

Results
60 patients, with mean disease duration of 11 ± 9 years were included. 69% had polyostotic disease and a mean percentage of skeletal involvement of 10.8 ± 7.6%. 68% were symptomatic; 71% of those referring bone and 54% joint pain attributed to PD. 48.3% had been previously treated with parenteral pamidronate, with a cumulative dose of 234 ± 209 mg. The mean follow-up period after zoledronate infusion was of 37 ± 13 months (minimum of 12 and maximum of 60). Only four patients (6.6%) required retreatment, on average 30 months after the first zoledronate infusion. A significant reduction of alkaline phosphatase was observed at 3 and 6 months after zoledronate administration, being maximal at 12 months (P < 0.001). At 3 and 6 months, 95 and 96% of patients, respectively, achieved remission. Maximum effect was obtained at 12 months after treatment with 98% of patients being in remission. Significant reductions of the mean levels of bone specific alkaline phosphatase, procollagen type 1 N-terminal propeptide, and collagen type 1 C-terminal telopeptide (P < 0.001) were also verified at 3, 6, and 12 months after treatment. 47% of patients reported pain improvement: 89% at 3 months. Transitory side effects were registered in 15 patients, 18% referred flu-like symptoms and 10% showed asymptomatic hypocalcaemia.

Conclusions
This study confirms the efficacy and safety of zoledronate in a Portuguese population of patients with active PD. Biochemical remission was achieved in 98% of patients at 12 months and improvement of pain in 47%. These benefits were long-term sustained with only 6.6% of patients requiring retreatment during an average follow-up of 37 months.

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PP483
Did Paget’s bone disease changed over the last decade?
Susana Fernandes, Joana Borges, Inês Gonçalves, Luís Cunha Miranda, Rui Leitão, Alexandra Cardoso, Manuela Micaelo, Eugénia Simões, Augusto Faustino, Filipe Barcelos, Candida Silva, Miguel Sousa, Manuel Parente, Margarida Silva, Helena Madeira, Vera Las, Sara Cortes, José Melo Gomes & José Vaz Patto
Instituto Português de Reumatologia, Lisbon, Portugal.

Introduction
Paget’s bone disease (PBD) is the second most prevalent metabolic bone disease. Most patients present with pain or fracture but many remain asymptomatic. Evidence suggests a significant reduction both in its prevalence and clinical severity. Recent papers described differences in clinical course and therapeutic options in the last 10–15 years.

Objective
To characterize PBD differences between patients having been diagnosed before and after the year 2000.

Methods
Retropective study of 75 patients from a Rheumatology Centre, evaluating demographic, clinical and therapeutic characteristics. Statistical analysis was made using Q-square, Mann–Whitney U and Spearman’s correlation.

Results
54.7% were females, mean age of 73.4. Pain was present in 82.7%, deformity in 34.7%, hypoaucia in 17.3%, and fracture in 10.7%. Deformity was more prevalent in males (P = 0.016). A familiar story was present in 6.8% of the subjects. Bone involvement included pelvis (69.3%), skull (41.5%), axial skeleton (44%), femur (22.7%), tibia (22.7%), and humerus (20%). Skelal-localization was more frequent in females (P = 0.017) and shoulder in males (P = 0.037). 71.6% had polyostotic PBD. Concomitant osteoporosis occurred in 12.2%, more frequently in females (P = 0.02). Medications were alendronate (22.7%), risedronate (12%), pamidronate (4.8%), and zoledronate (69%). The subset of patients diagnosed after the year 2000 (n = 46) had less fractures (P = 0.002) and less umberal involvement (P = 0.031). Alendronate (P = 0.012) and Pamidronate (P < 0.0001) were more frequently prescribed before the year 2000. No differences were found for Risedronate or Zolendronate. Total serum alkaline phosphatase (ALP) and the difference between the highest and current levels were higher in subjects diagnosed before the year 2000 (P = 0.004; ALP max 887 vs 389).

Discussion
Our data suggests that in the last decade patients with PBD attain lower levels of ALP and report less fractures. That may be related to a generalized use of bisphosphonates in a context of earlier diagnosis.

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PP484
Osteocyte metabolism on post-menopausal bone loss and role of hormone replacement therapy
Ana Maria Silva1,2, Ana Carolina Moreira1,2, Maria Sancha Santos1,2, Anabela Albuquerque1, Izilda Ferreira4, Paulo Gil1, Jorge Isidoro1, Romeu Videira1, Rui Carvalho1,2 & Vilma Sardão1,2
1CNC – Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal; 2Department of Life Sciences, University of Coimbra, Coimbra, Portugal; 3SMN-CHUC – Serviço de Medicina Nuclear do Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal; 4CECAV Animal and Veterinary Research Centre, University of Coimbra, Tras-os-Montes e Alto Douro, Vila Real, Portugal.

Introduction
Osteocytes play a major role in the bone remodelling unit (BRU). Thus, we hypothesise that mitochondrial bioenergetics impairment and mitochondrial/per-oxisomal fatty acid β-oxidation unbalance is a cause of osteocytes metabolic decline during 17β-estradiol (E2) reduction. E2 and a phytochemical substitute, coumestrol (COU) were used (30 mg/kg) during 24 h in ovariectomized rats in order to compare bone loss with sham-operated animals.

Methods
Four groups of 12-week-old female Wistar–Han rats were used: i) controls; ii) ovariectomized animals, OVX; iii) OVX+E2; and iv) OVX+COU. Animals were sacrificed four weeks after ovariectomy and estrogens levels in blood serum were evaluated. Left and right posterior limbs were surgically removed and freeze-clamped. For each animal, one limb was used to extract metabolites from the femur and tibia bone-embedded osteocytes and to measure mineral content; the paired limb was used to measure bone mineral density (BMD) by Dual-energy X-ray absorptiometry (DXA). Methanol/water extracted metabolites were analyzed by high resolution 600 MHz 1H nuclear magnetic resonance (NMR) spectroscopy. Total lipids were trans-methylated to fatty acyl methyl esters (FAMES) and analyzed by gas chromatography coupled to a mass spectrometer (GC-MS).

Results
All experimental groups did not show differences regarding mineral content, despite OVX group presented a slight decrease on BMD. Higher lactate/alanine ratio in the E2 group, with increased content in palmitic acid, nαααα-14:0 and arachidonic acid, and in the OVX group, presenting a 62% decrease in tetradecenoylcarnitine and a 2.5-fold increase in indocosanoic acid, when compared with the control group. Fatty acid content of osteocytes was also measured. Fatty acid profile was altered in the E2 group, with increased content in palmitic acid, nαααα-14:0 and arachidonic acid, and in the OVX group, presenting a 62% decrease in tetradecenoylcarnitine and a 2.5-fold increase in indocosanoic acid, when compared with the control group.

Conclusions
Although no major alterations were observed in terms of BMD, the results suggest metabolic alterations in osteocytes, which are associated with the decline of estrogens. The methodology here described is promising in evaluating the cellular metabolites and lipid content in osteocytes and in understanding how modulation of estrogen levels impact bone metabolism and homeostasis.

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PP485
The effect of hormone therapy on the change of bone mineral density in women with early menopause from pelvic radiation therapy for uterine cervical cancer
Dong Ock Lee1, Hoon Choi2 & Jung Gu Kim3
1National Cancer Center, Goyang-si, Gyeonggi-do, Republic of Korea; 2Sanggye Paik Hospital, Inje University College of Medicine, Seoul, Republic of Korea; 3Seoul National University Hospital, Seoul, Republic of Korea.

Objectives
To evaluate the effect of hormone therapy on the change of bone mineral density in women who showed early menopause after pelvic radiation therapy for uterine cervical cancer.

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Materials and methods

Through retrospective chart review, the changes in bone density in 63 women with early menopause after pelvic radiation therapy for uterine cervical cancer were evaluated. After the diagnosis of early menopause which was defined as level of serum FSH higher than 40 mIU/mL before the age of 45 years with amenorrhea for 1 year, all the women were interviewed and got thorough explanation about health-impact of early menopause. Forty-five women agreed with the use of hormone therapy for their early menopause and eighteen women rejected hormone therapy. The changes of bone mineral density were compared after 3 years. For further analysis, two normal age-matched women with regular menstruation were selected and compared with the women used hormone for their early menopause.

Results

For 3 years, there were no significant changes in bone density of women treated with postmenopausal hormone therapy for early menopause but women rejected hormone therapy showed significant loss of bone mass. In inter-group analysis, there were significant differences in changes of bone density between two groups. When compared with normal women with regular menstruation, women used hormone therapy after early menopause showed no difference in the change of bone mineral density for 3 years.

Conclusion

Women treated with hormone therapy for early menopause following pelvic radiation showed normal age-related change in bone density. Hormone therapy may be effective for prevention of bone loss in women with early menopause after pelvic radiation therapy.

Key words

Early menopause, bone density, hormone therapy, radiation therapy.

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PP487

Preliminary study for the effect of PDGF or mesenchymal stem cells on tissue repair of cutaneous radiation injury

Soon Jung Hwang1,2,3, Tae Hyung Cho1, Boomseok Lee4, Ji Hye Oh5 & In Sook Kim6

1Department of Oral and Maxillofacial Surgery, School of Dentistry, Seoul National University, Seoul, Republic of Korea; 2Brain Korea 21 2nd Program for Craniomaxillofacial Life Science, Seoul National University, Seoul, Republic of Korea; 3Dental Research Institute, Seoul National University, Seoul, Republic of Korea.

Purpose

Osteoradionecrosis (ORN) of the mandible is a serious complication of radiation therapy, and preceded by 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 PDGF-BB or mesenchymal stem cells (rMSCs) on radiation-induced soft tissue injury.

Methods

Rat model was designed to irradiate the skin of SD rats while sparing the body and internal organs by utilizing a non-occlusive skin clamp along with an X-ray image guided stereostactic irradiator. All wounds were created using the 50 Gy dose level both on the right and the left flank at a 100 cm source-to-surface distance. Next day, experimental groups were randomly divided into three groups (n = 3–4, each group). Left side in a subject was administered by 8 µg PDGF-BB, rMSCs and combination of PDGF and rMSCs, while the right side was used as vehicle control. Each wound was analyzed by defining the percentage of the irradiated area ulcerated at given time points and histological observation.

Results

No systemic or lethal sequelae occurred in any animals, and all irradiated skin areas in the multi-dose trial underwent ulceration. Greater than 60% of skin within each irradiated zone underwent ulceration within 16 days. PDGF-BB treatment groups (only PDFG group or PDGF and rMSCs mixed group) improved healing quality more highly organized collagen fiber deposition in full-thickness compared with control group. Experimental groups were all reached peak ulceration above 50%, with all healing significantly but incompletely by the 56-day endpoint compared with control group.

Conclusions

These results suggest that PDGF-BB or rMSCs are an alternative as a treatment to heal soft tissue injury, highlighting future therapeutic options, particularly for patients suffering from an impaired capacity for ORN.

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PP488

Circulating RANKL is not a reliable biomarker for bone loss in primary hyperparathyroidism

Daniel Grigorie1,2, Alina Sucalnic1,2, Elena Neacsu1, Roxana Militaru1, Alina Diaconescu1 & Mirela Ivan1

1National Institute of Endocrinology, Bucharest, Romania; 2Carol Davila University of Medicine, Bucharest, Romania.

Introduction

The aim was to examine serum levels of RANKL, OPG and TNF-α before and after curative surgery (PTX) in patients with primary hyperparathyroidism, and their relationship to bone turnover and bone loss.

Patients and methods

A 46 patients with rather severe primary hyperparathyroidism (mean PTH = 196 ± 69 pmol/l, mean total Ca = 11.4 ± 0.5, mean osteopetrosis in 9 patients, 51 women/five males, had their serum RANKL, OPG and TNF-α measured at baseline and, in a subset, after curative surgery (25 patients, 14 paired data). Serum C-telopeptide (CTX) and osteocalcin, and BMD (spine and hip) were measured yearly.

Results

Baseline serum RANKL levels were extremely variable between subjects (1.1 ± 1.7 pmol/l, range: 0.04–6.24 pmol/l) and did not change after PTX. In patients having repeated measurements we noticed no difference in serum levels over

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time; a very good correlation between pre and post-surgery levels ($r = 0.99$) was found. Circulating RANKL did not correlate with PTH but did correlate with serum CTX ($r = 0.36$), serum osteocalcin ($r = 0.29$) and with the annual change in BMD at the FN (%) ($r = -0.44$). Serum OPG levels ($3.9 \pm 1.3$ pmol/l) were in our normal postmenopausal range and did not change after PTX. We noticed a good correlation ($r = -0.48$) between serum RANKL:OPG ratio and the loss at FN. Serum TNF-α were extremely variable between subjects ($29.35 \pm 48.94$ pg/ml) but highly consistent in the same patient ($r = 0.99$) and decreased non-significantly ($P = 0.08$) after PTX. It correlated weakly with serum PTH ($r = 0.3$), but not with either bone loss or CTX. There was a good correlation between serum CTX and femoral loss ($r = -0.52$).

Conclusion Circulating levels of RANKL were extremely variable between subjects and did not change significantly after surgery. The rather weak correlation with serum CTX makes it unsuitable as a sensitive marker of bone loss.

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PP491
Cross sectional study of bone mass and 25OH vitamin D levels in erythropoietic protoporphyrina
Gonzalo Allo1, Guillermo Martinez-Diaz-Guerra1, Maria del Carmen Garrido-Astray2, Rafael Enriquez de Salañanca2 & Federico Hawkins4
1Hospital 12 de Octubre, Madrid, Spain; 2European University, Madrid, Spain.

Objectives Erythropoietic protoporphryia (EPP) is a rare disease with cutaneous photo-sensitivity, in which patients avoid sun exposure and use sunscreen. Our purpose was to study bone mineral density (BMD), serum 25-OHD levels and other mineral parameters, to evaluate the impact of these measures in the follow-up of EPP patients.

Patients and methods A ten EPP patients (median age 25; range 22–55, four males and six females), were studied for clinical features, biochemical values (bone markers: serum osteocalcin, β-CTX and IP10 and 25-OHD) and lumbar and hip BMD (Hologic 4500 DQR) and serum porphyrins (total and free).

Results Median serum 25(OH)D level was 19.65 ng/ml (17.50; 24.80). Four patients had 25(OH)D in insufficiency range (20–30 ng/ml) and five patients in the deficiency range (< 20 ng/ml). Lumbar T-score median levels were in the osteopenia range in both females (−1.50 (−2.30; −1.0)) and males (−1.99 (−2.40; −0.70)). Also in the female group, femoral neck T-score were in the osteopenia range (−1.20 (−1.60; −0.60)). No correlation was found between levels of protoporphyrins and bone markers, BMD or 25OHD.

Conclusions We report that low bone mass and vitamin D deficiency are frequent in EPP. The contribution of sunlight avoidance measures to this results remains to be clarified. The monitoring of serum vitamin D levels and BMD in EPP patients seems to be mandatory, adding vitamin D and calcium supplementation to their treatment protocol.

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PP492
Allele dependent silencing of collagen type I using small interfering RNAs targeting 3’UTR indels—a novel therapeutic approach in osteogenesis imperfecta
Katarina Lindsali1, Andreas Kindmark1, Navya Laxman1, Eva Åström2, Carl-Johan Rubia3 & Östen Ljunggren1
1Department of Medical Sciences, Uppsala University, Uppsala, Sweden; 2Neuropediatric Unit, Department of Women’s and Children’s Health, Karolinska Institutet, Astrid Lindgren Children’s Hospital, Stockholm, Sweden.

Objective Of this study we investigate the factors influencing fractures in endogenous Cushing’s syndrome (CS) of various etiologies.

Materials and methods The retrospective data of patients, who had received treatment due to endogenous CS, (2001–2011 years) was evaluated. All enrolled patients underwent standard spinal radiographs in lateral positions of the vertebrae Th4-L4. Recent low traumatic non-vertebral fractures were recorded in the medical cards. Bone mineral density (BMD) was measured by DXA GE Lunar Prodigy. Serum samples on osteocalcin (OC), carboxyterminal cross-linked telopeptide of type I collagen (CTx), late-night cortisol in serum, adrenocorticotropic (ACTH) were assayed by electrochemiluminescence (ECLIA). 24 h urinary free cortisol (UFC) was measured by an immunoenchemiluminescence assay (extraction with diethyl ether).

Results Among 215 patients, 178 were females and 37 males, median age 35 (Q25–Q75 27–48); 88 patients (40.9%) had low traumatic fractures, including vertebral fractures in 76 cases (in 60 cases multiple vertebral fractures) and non-vertebral fractures in 27 cases (17 patients had ribs fractures, three fractures of metatarsal bones, two fractures of radius, two fractures ofibia and fibula, 1 – humerus, 1 – breastbone; 2 – hip fractures). Patients with fractures had higher 24 h UFC, late-night cortisol in serum, ACTH, lower OC, total hip and spine BMD, but did not differ in age, BMI, CTX and etiology of CS. After applying the logistic regression analysis (adjusted for sex, age, BMI, BMD, OC), the main predictor of fractures was 24 h UFC level ($P = 0.02$) and a separately analyzed late-night serum cortisol level ($P = 0.001$). Patients with late-night serum cortisol higher than 597 nmol/l were more likely to have low traumatic fractures (odds ratio 2.86 (95% CI 1.55–5.28) $P = 0.001$)

Conclusions The severity of hypercortisolemia is the best predictor of low traumatic fractures in patients with CS. Patients with higher levels of late-night serum cortisol might need earlier preventive treatment for osteoporosis.

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PP490
Fracture predictors in patients with endogenous cortisol excess
Zhanna Belaya, Natalia Draganova, Liudmila Rozhinskaya, Larissa Dzeranova & Galina Melnichenko
The National Research Center for Endocrinology, Moscow, Russia.

Objective Of this study was to investigate the factors influencing fractures in endogenous Cushing’s syndrome (CS) of various etiologies.

Materials and methods The retrospective data of patients, who had received treatment due to endogenous CS, (2001–2011 years) was evaluated. All enrolled patients underwent standard spinal radiographs in lateral positions of the vertebrae Th4-L4. Recent low traumatic non-vertebral fractures were recorded in the medical cards. Bone mineral density (BMD) was measured by DXA GE Lunar Prodigy. Serum samples on osteocalcin (OC), carboxyterminal cross-linked telopeptide of type I collagen (CTx), late-night cortisol in serum, adrenocorticotropic (ACTH) were assayed by electrochemiluminescence (ECLIA). 24 h urinary free cortisol (UFC) was measured by an immunoenchemiluminescence assay (extraction with diethyl ether).

Results Among 215 patients, 178 were females and 37 males, median age 35 (Q25–Q75 27–48); 88 patients (40.9%) had low traumatic fractures, including vertebral fractures in 76 cases (in 60 cases multiple vertebral fractures) and non-vertebral fractures in 27 cases (17 patients had ribs fractures, three fractures of metatarsal bones, two fractures of radius, two fractures ofibia and fibula, 1 – humerus, 1 – breastbone; 2 – hip fractures). Patients with fractures had higher 24 h UFC, late-night cortisol in serum, ACTH, lower OC, total hip and spine BMD, but did not differ in age, BMI, CTX and etiology of CS. After applying the logistic regression analysis (adjusted for sex, age, BMI, BMD, OC), the main predictor of fractures was 24 h UFC level ($P = 0.02$) and a separately analyzed late-night serum cortisol level ($P = 0.001$). Patients with late-night serum cortisol higher than 597 nmol/l were more likely to have low traumatic fractures (odds ratio 2.86 (95% CI 1.55–5.28) $P = 0.001$)

Conclusions The severity of hypercortisolemia is the best predictor of low traumatic fractures in patients with CS. Patients with higher levels of late-night serum cortisol might need earlier preventive treatment for osteoporosis.

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PP489
Homeostasis of calcium and vitamin D in patients with aggressive periododontitis
Maria Zyablitskaya, Victoria Atrushkevich & Ashot Mktumian
Moscow State University of Medicine and Dentistry, Moscow, Russia.

Aim Periodontologists all over the world are more and more interested in connection between pathogenesis of aggressive periodontitis (AP) and calcium and vitamin D metabolic disturbances. Vitamin D besides its direct effect on calcium homeostasis, has immunomodulatory action, that makes interesting the study of vitamin D effect on pathogenesis of AP.

Materials and methods We studied 102 (49 males; 53 females) patients with AP (40.32 ± 1.13), 42 patients without AP in control group (41.41 ± 0.96). The main criteria of patient selection were an early onset of the disease (18–20). Dental status was defined by clinical indexes. Laboratory assessment of mineral metabolism included: calcium total, calcium ionized, parathormone, calcitonin, vitamin D (25-OH-D), osteocalcin, β-CrossLaps. StatPlus software, descriptive statistics methods (Student criterion) were used for statistical assessment of the results. The significance level was determined to be $P < 0.05$.

Results Statistically significant differences of bone turnover indices in patients with AP in comparison with control group were detected: statistically significant increase of ionized calcium level in blood in patients with AP (1.15 ± 0.01 mmol/l, $P < 0.05$) vs control indices was observed in case of increased level of parathormone (53.91 ± 2.56 ph/ml) and decreased level of calcitonin (2.85 ± 0.22 ng/ml, $P < 0.05$). Decrease of osteocalcin level (5.89 ± 0.49 ng/ml, $P < 0.05$), which indicates inhibition of osteoblastic function and hence disturbances of osteogenesis was observed. 25-OH-D level was significantly lower in AP patients than in control (15.64 ± 1.93 ng/ml, $P < 0.05$).

Conclusion In summary, our study has shown that disturbance of calcium homeostasis characterized by increase of ionized calcium associated with imbalance of calcium-regulating hormones (increase of parathormone and decrease of calcitonin) is observed in patients with AP. Statistically significant decrease of osteocalcin level confirms inhibition of osteoblastic function and the shift of remodelling process towards osteoclastic resorption. That can be connected with the revealed lack of vitamin D in AP patients.

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PP493
Functional assessment of Paget’s disease-causing mutations in sequence-to-someone-1 (Q19STM1)
Eman Azzam, Miep Helfrich & Lynne Hocking
University of Aberdeen, Aberdeen, UK.

Abstract
Paget’s disease of bone (PDB) is characterised by focal lesions of local bone turnover driven by overactive osteoclasts, which often contain nuclear and cytoplasmic inclusion bodies. Mutations affecting the sequence-to-someone-1 (Q19STM1) ubiquitin-associated (UBA) domain have been identified in individuals with PDB. Q19STM1, also known as p62, is a ubiquitously-expressed scaffold protein of 62 kDa that functions in multiple signalling pathways important for cell survival and osteoclast activity. The mechanisms by which Q19STM1 mutations cause PDB remain unclear. Here, we report our laboratory’s recent advances in understanding the role of Q19STM1 in PDB pathogenesis. Using molecular and microscopic methods to examine Pagetic bone biopsies, osteoclast cultures and various cell lines, we have identified two isoforms of Q19STM1. In all cell types examined, four Q19STM1 transcripts were detected, differing in their 5’ untranslated region; one transcript encodes p62, while the other three encode 55 kD-Q19STM1. The newly identified isoform also contains the UBA domain mutated in PDB. Using biochemical and microscopic methods, we found that both Q19STM1 isoforms are degraded by autophagy. The isoforms interact with each other and form aggregates upon autophagy inhibition. 55 kD-Q19STM1 is ~45% more abundant in osteoclasts than Q19STM1/p62. Biochemical and microscopic methods showed that mutations in Q19STM1/p62 impair autophagic degradation. Cell lines expressing mutations in Q19STM1/p62 form paracrystalline inclusion bodies, that by immuno-transmission electron microscopy (TEM) were found to contain Q19STM1 and ubiquitin and were ultrastructurally identical to those found in PDB. As observed by TEM, these inclusions can be degraded by autophagy. The effects of mutations in 55 kD-Q19STM1 have yet to be characterised. Taken together, these data show that mutations in Q19STM1 isoforms impair protein degradation and can lead to inclusion body formation suggesting that PDB results from dysregulated protein degradation in osteoclasts. Further characterisation of the effect of mutations in 55 kD-Q19STM1 in stably transfected cell lines is ongoing.

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PP494
BMP-9 induces the calcification of vascular smooth muscle cells
Dongxing Zhu, Neil Mackenzie, Colin Farquharson & Vicky MacRae
The Roslin Institute, Royal (Dkck) School of Veterinary Studies, The University of Edinburgh, Easter Bush, Roslin, Midlothian, EH25 9RG, Scotland, UK.

The process of vascular calcification shares many similarities with that of skeletal mineralisation, and involves the deposition of hydroxypatite crystals in arteries and cardiac muscle. However, the cellular mechanisms responsible have yet to be fully elucidated. BMP-9 has been shown to exert direct effects on both bone development and vascular function. In the present study, we have investigated the role of BMP-9 in vascular smooth muscle cell (VSMC) calcification. Murine VSMCs were cultured in calcifying medium containing Na2HPO4/NaH2PO4 for 14 days. Calcium deposition was confirmed by alizarin red staining. Calcified VSMCs showed increased Runx2, Bmp2 and Pft-1 mRNA expression (P < 0.001), which are recognised osteogenic markers of vascular calcification. BMP-9 mRNA expression was significantly up-regulated by 7 days (1.4-fold; P < 0.05) in calcified VSMCs. BMP-9 treatment (50 ng/ml) caused a significant increase in VSMC calcium content (3.4-fold; P < 0.05), ALP activity (10.1-fold; P < 0.001) and mRNA expression of osteogenic markers (P < 0.001). BMP-9-induced calcium deposition was significantly reduced (68%; P < 0.001) following treatment with the ALP inhibitor 2,5-dimethoxy-N-(quinolin-3-yl)benzenesulphonamide (3 μM) confirming the mediatory role of ALP in this process. BMP receptor expression, including ALK1, ALK2, BMPR-II, ActR-IIA and ActR-IIB, was not detected in mouse VSMCs. The inhibition of ALK1 signalling using a soluble chimeric protein (ALK1-Fc) significantly reduced calcium deposition (85%; P < 0.001) and ALP activity (33%; P > 0.01), confirming that BMP-9 is a physiological ALK1 ligand. Signal transduction studies revealed that BMP-9 (0.5–50 ng/ml) induced Smad1/5/8 and Smad2 phosphorylation. Therefore, as both of these Smad proteins directly bind to Smad4, siRNA studies were subsequently undertaken to examine the functional role of Smad4 in VSMC calcification. Smad4-siRNA transfection induced a significant reduction in ALP activity (72%; P < 0.001) and calcium deposition (59%; P < 0.05). These novel data demonstrate that BMP-9 induces VSMC calcification through a Smad signalling mechanism. This may identify new potential therapeutic strategies for clinical intervention.

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PP495
Long-term effects of symptomatic vs intensive bisphosphonate therapy for Paget’s disease of bone: the PRISM-EZ study
Kirsteen Goodman1, Graeme MacLennan2, William Fraser3, Peter Selby4 & Stuart Ralston1
1University of Edinburgh, Edinburgh, UK; 2University of Aberdeen, Aberdeem, UK; 4University of East Anglia, Norwich, UK; 3University of Manchester, Manchester, UK.

Paget’s disease of bone (PDB) is a common metabolic bone disease characterised by increased and disorganised bone remodelling affecting one or more skeletal sites. Bisphosphonates are highly effective at suppressing bone turnover in PDB but it remains unclear whether greater suppression of bone turnover improves clinical outcome. In the PRISM study, we previously reported that PDB patients randomised to ‘intensive’ treatment aimed at normalising alkaline phosphatase (ALP) levels had a similar long-term outcome as those randomised to ‘symptomatic’ treatment aimed at controlling symptoms. Here, we report initial results from an extension of the PRISM study (PRISM-EZ) in which zolodronic acid was used as the bisphosphonate of first choice in the ‘intensive’ arm. We studied 502 patients who consented to take part in the extension; 270 continued to receive intensive treatment and 232 continued to receive symptomatic treatment. The treatment groups were well matched at entry to the extension for age, previous fracture, previous orthopaedic surgery, bone deformity and quality of life scores. As expected mean ± SEM ALP values at entry to the extension were lower in the intensive group: (0.85 ± 0.04 vs 1.04 ± 0.06, P = 0.012, where 1.0 is the upper limit of normal). The ALP values decreased further in the intensive group and were consistently lower throughout follow-up (0.71 ± 0.04 vs 1.01 ± 0.06, P < 0.001). There were no differences between the groups in quality of life scores or bone pain. Fractures were more common during follow up in the intensive group (8.2 vs 4.7%; hazard ratio = 1.80 (0.87–3.71) although most (82%) affected non-Pagetic bone. The difference in fractures between the groups was not significant (P = 0.11). We conclude that more profound suppression of ALP levels with bisphosphonates including zolodronic acid was not associated with clinical benefit in this group of patients with PDB.

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PP496
The miR-221/222 family regulates vascular smooth muscle cell calcification
Neil Mackenzie1, Dongxing Zhu1, Paul Genover2 & Vicky MacRae1
1The Roslin Institute, Royal (Dick) School of Veterinary Studies, Edinburgh, UK; 2The University of York, York, UK.

The process of vascular calcification shares many similarities with that of skeletal mineralisation, and involves the phenotypic trans-differentiation of vascular smooth muscle cells (VSMCs) to osteoblastic and chondrocytic cells within a calcified environment. Various microRNAs (miRs) are known to regulate cell differentiation, however their role in mediating VSMC calcification has yet to be fully understood.

Murine VSMCs were cultured for up to 28 days in calcifying medium containing phosphate. Calcium deposition and gene expression of calcification markers (aggrecan, collagen types II and X), osteoblast markers (osteocalcin, Runx2) and regulators of calcification (Ank, Enpp1, Pii) were significantly elevated by 7 days in VSMCs (P < 0.05), confirming the chondro-osseous phenotype associated with vascular calcification. miR-microarray analysis revealed the significant down-regulation of a wide range of miRs by 9 days of culture, including miR-198 (290-fold), miR-29a (168-fold), miR-221 (108-fold), miR-222 (81-fold) and miR-31 (40-fold).

Following this microarray analysis, subsequent studies investigated the specific role of the miR-221/222 family in VSMC calcification. qPCR data confirmed the down-regulation of miR-221 (30%; P < 0.01) and miR-222 (15.7%; P < 0.05). VSMCs were transfected with mimics of miR221 (50 nM) and miR222 (50 nM), individually and in combination. Interestingly, an increase in calcium deposition was observed in the combined treatment (sevenfold; P < 0.01) but not individual miR treatments. These data suggest that miR-221 and miR-222 work concomitantly to alter the trans-differentiation of VSMCs and increase the rate of calcification in vitro.

The miR-221/222 family is known to target PTEN, a phosphatase involved in cell cycle regulation, in cancer cells. Western blot analysis confirmed a reduction in PTEN expression in calcifying VSMCs following transfection with miR-221 and miR-222 mimics in combination. Increased PTEN expression through miR-221 down-regulation of miR-222 worked in concert to alter the trans-differentiation of VSMCs and increase the rate of calcification in vitro.

Taken together, these results strongly suggest that the in vitro RANKL-independent osteocalcic phenotype observed in osteocalcic cultures derived from this osteopetrosis patient can be explained by expression of C-terminal RANK causing ligand-independent activation of NF-kB.

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PP498
Toxic osteomyelitis of the jaw ones against the backgrounds of chronic intoxication
Margarita Skikevich & Liudmyla Voloshyna
Ukrainian Medical Stomatological Academy, Poltava, Ukraine.

This case study is based on the results of the clinical observation of 48 patients aged 22–40 years with toxic necrosis of the jaw bones (28 of whom had a lesion of the mandible, 10 – lesions of the upper jaw, 10 – lesions of both jaws). All patients were observed in the maxillofacial department of Poltava Regional Clinical Hospital. However, only one patient had been referred to the department with the diagnosis ‘toxic osteomyelitis’, 18 – sent with a diagnosis of ‘malignancy’, 20 – diagnosed with ‘the chronic odontogenic osteomyelitis’, 2 – with the diagnosis: ‘pathological fracture of the mandible’.

The data on the growing number of people using synthetic drugs an increase in cases of atypical osteomyelitis of the bones of the facial skeleton has been observed lately. In our study, we summarized diagnostic data and clinical data of this category of patients.

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PP499
Histological structure of the albino rats lower incisors of different ages after thyromectomy
A A Kochubev, V I Luzin & A V Yeryomin
SE “Lugansk State Medical University”, Lugansk, Ukraine.

Introduction
The purpose of this research was to study the histological structure of albino rats lower incisors of different ages after thyromectomy.

Materials and methods
The experiment was conducted on 360 white rats of three age groups: immature, mature and senile period. All animals were subjected to surgical thyromectomy.

Results
In immature rats after thyromectomy predentin layer width was less than the control from 30 till 180 days of the experiment, respectively 5.94, 4.69 and 6.89%. Also, on the 90 and 180 days experiment width dentin and mesial to distal incisor size was less control respectively by 4.67 and 4.62%, and 3.79 and 4.33%. After thyromectomy in adult animals a similar picture observed, but expressed less width predentin layer was less than the control at 90 and 180 days after surgery to 5.72 and 6.49%, and on day 180 – the width of the layer of odontoblasts and the mineralized dentin, and also mesiodistal incisor size – 3.99, 4.75 and 3.11%. In rats, old age width layers of odontoblasts and predentin and mesio-distal mandibular incisor size 90 and 180 days of the experiment were less control values, respectively, 4.15 and 4.65%, and 4.53 and 4.13%, and 2.62 and 2.93%. On day 180, and the width of the mineralized dentin layer was less than the control group – 5.74%.

Conclusion
Thyromectomy has negative effects in the structure of the tooth, especially in immature animals.

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**PP500**

**IFITM5 c. → T mutation causes variable V osteogenesis imperfecta phenotype and decreased COL1A1 expression but increased mineralization by cultured proband osteoblasts**

Aditi Reich1, Alison S Bae1, Ailette M Barnes1, Wayne A Cabral1, David Chitayat2 & Joan C Marini1

1Bone and Extracellular Matrix Branch, NICHD, NIH, Bethesda, MD, USA; 2Department of Obstetrics and Gynecology, The Hospital for Sick Children, The Prenatal Diagnosis and Medical Genetics Program, Toronto, ON, Canada.

Introduction

Osteogenesis imperfecta (OI) is a genetically heterogeneous disorder characterized by bone fragility. OI type V, with autosomal dominant inheritance, is characterized by ossification of the forearm interosseus membrane, radiodense metaphyseal bands, propensity for hyperplastic callus formation, and mesh-like lamellation on bone histology. Type V OI probands are reported to have white sclerae and normal metaphyseal bands. Recent reports identified the cause of type V OI as a unique heterozygous mutation in IFITM5 (c. → T), which encodes Bril, a transmembrane protein expressed in osteoblasts. The mutation generates a start codon in the untranslated region, adding five residues at the N-terminus of Bril.

Methods

IFITM5 was sequenced in gDNA from three patients with OI type V and 25 patients with OI of unknown etiology. Mutations were confirmed by Sanger restriction digest. Cultured osteoblasts from type V OI probands and control donors were differentiated over 15 days; cells were analyzed by qPCR, western blot and alizarin-red mineralization assay, to compare functional differences.

Results

Three patients with clinical and histological criteria of type V OI were positive for the known IFITM5 mutation. Two patients not previously classified as type V OI, a child with strongly blue sclerae and no dense metaphyseal bands and an adult with progressive deforming OI, were also found to have the same mutation. We verified expression of IFITM5 transcripts in cultured proband osteoblasts. During days 10–15 of the differentiation timecourse, type V OI osteoblasts had less than half the COL1A1 expression of control cells. During the same timeframe, type V osteoblasts displayed increased mineralization and expression of osteocalcin.

Conclusion

Patients without the well-described type V OI phenotype may also have the type V OI IFITM5 mutation. Type V OI osteoblasts demonstrated a collagen-related defect and increased mineralization during differentiation, possibly underlying overactive calcification of interosseous membrane and during callus formation.

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**PP501**

**Abnormal type I collagen glycosylation pattern and cross-linking in a cyclophilin B KO mouse model of recessive osteogenesis imperfecta**

Wayne Cabral1, Irina Perdivara2, MaryAnn Weis2, Masahiko Terajima1, Angela Blissett1, Weizhong Chang1, Elena Makareeva4, Sergey Leikin4, David Eyre1 & Mitsuo Yamauchi1

1Bone and Extracellular Matrix Branch, NICHD, NIH, Bethesda, MD, USA; 2NIEHS, NIH, Research Triangle Park, NC, USA; 3Orthopaedic Research Laboratories, University of Washington, Seattle, WA, USA; 4Section on Physical Biochemistry, NICHHD, NIH, Bethesda, MD, USA; 5North Carolina Oral Health Institute, University of North Carolina, Chapel Hill, NC, USA.

Introduction

Recessive osteogenesis imperfecta (OI) is caused by mutations in genes encoding proteins involved in post-translational interactions with type I collagen. Types VII–IX OI involve defects in the collagen prolyl 3-hydroxylation complex, which modifies N1[I]Pro986. PPB encodes CyPB, a complex component with PPIase activity and the major isomerase facilitating collagen folding. We investigated the role of CyPB in collagen post-translational modifications and crosslinking.

Methods

Ppib−/− mice were generated using a gene-trap ES cell clone with a β-gal reporter inserted into Ppib intron 1. Type I collagen modifications were analyzed by LC–MS/MS and HPLC. Bone architecture was investigated by micro-CT and DXA.

Results

Ppib transcripts and protein are absent in skin, fibroblasts, femora, and calvarial osteoblasts; only residual (10%) N1[I]Pro986 3-hydroxylation is detectable in fibroblast and osteoblast collagen. Although collagen from KO cells has delayed electrophoretic mobility, total collagen 5-hydroxy and prolyl 4-hydroxylation was normal, suggesting altered glycosylation in KO. MS analyses indicated that, except for lysyl residues involved in crosslinking, most helical residues of KO FB and OB collagen have increased diglycosylation. Total mature crosslinks (HP + LP) in KO bone were increased 1.5–1.7× vs WT. We detected a 4–5-fold increase in trivalent LP crosslinks (P<0.001), which decreased the HP/LP ratio correspondingly. Total immature cross-links (DHNL + HLNL) were also significantly increased and the DHNL:HLNL ratio decreased, implying reduced lysyl hydroxylation of helical crosslink residues in KO bone. Abnormal collagen modification is associated with 70–80% reduction of collagen deposited into KO matrix in culture, associated with smaller long bones with significantly reduced BMD, BV and TbN.

Conclusions

In Ppib KO mice, absence of CyPB delays collagen folding and alters collagen glycosylation patterns in culture; tissue investigations are ongoing to confirm these effects. Altered modification may impair collagen matrix interactions and promote abnormal bone mineralization. Collagen crosslink patterns are shifted to trivalent forms lacking helical Hyl, possibly contributing to decreased matrix deposition and bone strength.

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**Paediatric bone disease**

**PP502**

**Craniofacial consequences of high-dose zoledronic acid injections in onco-pediatric patients**

Frédéric Lezot1, Julie Chesneau1, Séverine Battaglia1, Régis Brion1, Jean-Christophe Farges2, Géraldine Lescaillle3, Beatriz Castaneda4, Perrine Marc-Berard5, Laurence Brugieres5, Nadège Corradini5, Dominique Heymann1,2 & Françoise Redlin1,2

1INSERM UMR957, Nantes, France; 2UNAM de l’université de Nantes, Nantes, France; 3Service d’Endocrinologie, Hôpital Pitié Salpêtrière, Paris, France; 4Insitut d’Hémato-Oncologie Pédiatrique, Lyon, France; 5Département de Pédiatrie, Villejuif, France; 6Service d’Hémato-Oncologie Pédiatrique, Nantes, France.

Background

High zoledronic acid (ZOL) dose protocol, one of the most potent inhibitors of bone resorption, is currently evaluated in a phase III clinical trial in Europe for the treatment of malignant pediatric primary bone tumors. The impact of such an intensive treatment on the craniofacial skeleton growth is a critical question in the context of patients with actively growing skeleton, in the light of our previous studies evidencing that endochondral bone formation was transiently disturbed by high doses of ZOL.

Methods

Two protocols adapted from pediatric treatments were developed for newborn mice (a total of five or 10 injections of ZOL 50 μg/kg every 2 days). Their impact on skull bones and teeth growth was analyzed by micro computed tomography and histology up to 3 months after the last injection. In parallel, radiographies of patients from the French OS2000 protocol were analyzed for potential orofacial consequences.

Results

In mouse, ZOL administrations induced a transient delay of skull bone growth and an irreversible delay in incisor, first molar eruption, and root elongation. Other teeth were affected, but most were erupted by 3 months. Root histogenesis was severely impacted for all molars and massive odontogenic tumor-like structures were observed in all mandibular incisors. In younger pediatric patients a significant delay of tooth eruption was observed.

Conclusion

In mouse, high doses of ZOL irreversibly disturbed tooth eruption and elongation, and delayed skull bone formation. In human, the same treatment may impact the permanent teeth eruption. These preclinical and clinical observations are essential for the follow-up of onco-pediatric patients treated with ZOL.

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**Bone Abstracts (2013) Vol 1**
High dickkopf-1 levels in sera and leukocytes from children with 21-hydroxylase deficiency on chronic glucocorticoid treatment

Giacomina Brunetti1, Maria Felicia Faienza2, Laura Piacentino1, Annamaria Venturi1, Angela Orsina1, Claudia Carboni1, Adriana Di Benedetto1, Graziana Colaianni1, Giorgio Mori1, Silvia Colucci1, Luciano Cavollo2 & Maria Grano3
1Department of Basic Medical Sciences, Neuroscience and Sense Organs, Section of Human Anatomy and Histology, University of Bari, Bari, Italy; 2Department of Biomedical Sciences and Human Oncology, University of Bari, Bari, Italy; 3Department of Biomedical Science, University of Foggia, Foggia, Italy.

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Conclusions

Children with 21-hydroxylase deficiency (21-OHD) need chronic glucocorticoid (cGC) therapy to replace congenital deficit of cortisol synthesis, and this therapy is the most frequent and severe form of drug-induced osteoporosis. In the study we enrolled 18 patients (9 females) and 18 sex- and age-matched controls. We found in 21-OHD patients high serum and leukocyte levels of dickkopf-1 (DKK1), a secreted antagonist of the Wnt/b-catenin signaling pathway, known to be a key regulator of bone mass. In particular, we demonstrated by flow cytometry, confocal microscopy, and real time PCR that monocyties, T lymphocytes and neutrophils from patients expressed high levels of DKK1, which may be related to the cGC therapy. In fact, we showed that dexamethasone treatment markedly induced the expression of DKK1 in a dose- and time-dependent manner in leukocytes. The serum from patients containing elevated levels of DKK1 can directly inhibit in vitro osteoblast differentiation and Receptor Activator of NF-kappaB Ligand (RANKL) expression. We also found a correlation between both DKK1 and RANKL or C-terminal tetrapeptides of Type I collagen serum levels in 21-OHD patients on cGC treatment. Our data indicated that DKK1, produced by leukocytes, may contribute to the alteration of bone remodeling in 21-OHD patients on cGC treatment.

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The serum levels of carboxylated and undercarboxylated osteocalcin in children with cystic fibrosis

Jadwiga Ambroszkiewicz1, Dorota Sands2, Joanna Gajewska1, Magdalena Chelchowska1 & Teresa Laskowska-Klita1
1Screening Department, Institute of Mother and Child, Warsaw, Poland; 2Department of Pediatrics, Institute of Mother and Child, Warsaw, Poland.

Introduction

Osteocalcin (OC) is the noncollagenous protein of bone matrix produced by osteoblasts which play an important role in bone metabolism. In its carboxylated form (c-OC) osteocalcin binds to hydroxyapatite in bone and plays a regulatory role in bone formation and mineralization. In contrast, undercarboxylated OC (uc-OC) binds less effectively to hydroxyapatite and a significant association has been found between fracture incidence and uc-OC in elderly subjects. Undercarboxylated OC is recognized as a functional marker of vitamin K status. Deficiency of vitamin K, observed in subjects with cystic fibrosis, may play an important role in bone health in these groups of patients. The aim of this study was to assess the serum levels of c-OC and uc-OC in prepubertal children with cystic fibrosis.

Materials/Methods

The study group consisted of 25 children aged 5–9 years (median 7.0 years) with confirmed cystic fibrosis attending the CF Clinic at the Institute of Mother and Child (Warsaw, Poland). The control group included 25 healthy children matched for age and gender without infections and diseases that might influence bone status. Serum concentrations of total OC, carboxylated OC, and undercarboxylated OC were determined by immunoenzymatic ELISA assay. Statistical analyses were performed using the Statistica software program, version 10.0 PL.

Results

Total OC levels were comparable in cystic fibrosis patients and in healthy children. However, in children with cystic fibrosis we observed lower c-OC (median values: 25.4 vs 29.8 ng/ml, P = 0.058) and significantly higher uc-OC concentrations (median values: 40.7 vs 31.2 ng/ml, P = 0.031). The ratio of c-OC to uc-OC was significantly lower in children with CF compared to healthy ones (P < 0.05).

Conclusion

Children with cystic fibrosis have significantly lower uc-OC and higher uc-OC concentrations than healthy subjects. Reduced c-OC may lead to abnormal bone formation in these patients.

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PP506

Body composition in 3- and 4-year-old preterm and full-term infants – preliminary data

Elzbieta Karczmarewicz1, Edyta Czekuc-Kryskiewicz1, Justyna Czech-Kowalska2, Maciej Jaworski1, Pawel Płotowski1, Dorota Bulisiewicz2, Maria Kornacka1, Anna Niziołka2, Agata Pleśkaczynska2, Monika Nowakowska-Ryza2, Anna Dobrzańska2 & Roman Lorenc1
1Department of Radioimmunology, Biochemistry and Experimental Medicine, The Children’s Memorial Health Institute, Warsaw, Poland; 2Neonatal Intensive Care Unit, The Children’s Memorial Health Institute, Warsaw, Poland.

Introduction

The body composition at term equivalent age of infants born preterm is different from that of infants born at term. The aim of the study was to compare the body composition in preterm and full-term infants at age 3 and 4 years.

Materials/Methods

Patients and methods

Total body bone mineral content (TBBMC, g), density (TBBMD, g/cm²), and body composition (fat mass – FM, g; lean body mass – LB, g) were measured using dual-energy X-ray absorptiometry (Prodigy, pediatric software) in 48 preterm infants (mean age: 3.12 ± 0.54 years at V1 and 4.06 ± 0.53 years at V2) and 24 full-term infants (mean age: 3.34 ± 0.58 years at V1 and 4.32 ± 0.63 years at V2). P3NP was measured in serum by radioimmunoassay.

Results

Body weight (BW) but not body height was lower in preterm in comparison with full-term infants at V1 (P = 0.012) and V2 (P = 0.004). Evaluation of body composition at V1 indicated that preterm infants have significantly higher body content of LB (PLMB/BW) and serum P3NP than full-term infants (P = 0.047 and P = 0.004, respectively). At V2 full-term infants and preterm infants have
comparable fat mass content (FM/BW) as well as bone mass to muscle mass index (TB BMC/LBM) ($P=0.057$ and $P=0.158$, respectively). On the contrary, preterm infants had higher muscle content in comparison to full term infants (LBM/BW – $P=0.039$; FM/LBM – $P=0.044$) as well as TB BMD ($P=0.045$).

Conclusions

Despite lower body weight, the body composition in 4-years-old preterm infants was better than in children born at term due to the advantage of muscle mass and BMD. It may indicate that the risk of bone and metabolic disorders is low in preterm infants at age 4 years.

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PP507

Perceived activity capability in children and adolescents with osteogenesis imperfecta

Malin Hagberg1,2 & Kristina Löwing1,2 & Eva Åström1,2
1,Karolinska Institute, Stockholm, Sweden; 2,Astrid Lindgrens Children’s Hospital, Karolinska University Hospital, Stockholm, Sweden.

Introduction

Osteogenesis imperfecta (OI) is a genetic disorder which mainly affects the collagen in the bone mass with fractures and deformities as the main symptoms. In OI there is a great variation in dysfunction related to the disease. Mobility and activities related to mobility are often most difficult. The objective for this study was to find a relevant, valid and reliable instrument to assess the children’s activity capability.

Method and participants

A total of 58 children and adolescents from 7 to 18 years answered the Activities Scale for Kids – capability, version 38 (ASK-c). ASK-c is a questionnaire with the highest score of 100. The participants were handed the questionnaire when seeing the Swedish OI team for a first or a follow-up visit. They were divided in to two groups: wheelchair users and nonwheelchair users. The data were statistically processed with Statistical Package for the Social Sciences (SPSS).

Results

The 58 participants had a mean age of 11.5 years; 39 were boys and 19 girls. Forty had OI type I, 10 had OI type III and 8 had OI type IV. 16 were wheelchair users and 42 did not use a wheelchair on a regular basis. The wheelchair users had a mean score of 64.5 and the nonusers had a score of 90.9. There was significant difference in how the two groups perceive their activity capability. The most difficult items for the children were activities related to sports, e.g. ‘I think I could run…fast’ … and ‘I think I could participate in team-sports’. There was also a difference in how children with different OI types answered the questionnaire.

Conclusion

ASK is a valid instrument for self-report and appears to be useful in assessing activity capability in children and adolescents with OI.

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PP508

Comparison of the bone densitometry and anthropometric parameters between the Ukrainian, Indian and Nigerian young male students, graduated in Lugansk State Medical University

V Luzin, L Sklyyanina, Y Usko & A Ignat’ev
Lugansk State Medical University, Lugansk, Ukraine.

Objectives

To establish the average bone mineral density (BMD) and bone mineral content (BMC) in young male population from the different ethno-geographical groups

Materials and methods

Estimations of the calcaneal BMD (g/cm²) and BMC (r), using on ALOKA-5.0 DXA machine among Indian ($n=58$) and Nigerian ($n=72$) male students (17-20 years), were done. The anthropometric program included body weight, height, shoulder and thorax width, triceps, biceps, suprailliac and calf skinfold measurements among Ukrainian ($n=200$), Indian ($n=84$) and Nigerian ($n=97$) male students (18-21 years). Total body fat percentage was calculated by the Mateigka (1921) equation, total body muscular mass by the Kuczynski R.J, Flegal K.M. Default (2000).

Results

Obtained data reveal that the Ukrainians and Nigerians have mostly similar BMD and BMC: BMD 1.05 ±0.04 in Ukrainians, 1.05 ±0.02 in Indians; BMC 77.40 ±1.49 in Ukrainians, 77.32 ±2.21 in Indians. Indians have the lowest BMD and BMC among compared groups: BMD 0.94±0.02; BMC 67.09±1.96, which are significaly (10.35% for the BMD and 13.40% for the BMC, $P<0.001$) lower, than in Ukrainians and Nigerians. Anthropometric data reveal the highest body parameters of the weight (74.58 ±1.95 kg), height (173.58 ±0.92 cm), shoulder and thorax width and lean muscular body mass (52.12 ±1.58 kg). Ukrainians show the lowest weight (55.53 ±0.69 kg), height (166.06 ±0.57 cm), thorax width, moderate muscular mass (46.08 ±1.5) and highest thickness of the skinfolds and body fat (16.54 ±0.52%). Indians expose the moderate weight (63.89 ±1.25 kg), height (109.16 ±1.05 cm), fat percentage; thorax width was same as that in Nigerians, but the muscular mass was lowest (45.78 ±3.30 kg) among participants.

Conclusions

BMD, BMC and anthropometric parameters have obvious ethno-geographical features.

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PP509

Prostate tumorigenesis in estrogen receptor $\beta$ inactivated, prostate targeted fibroblast growth factor 8B transgenic mice

Teresa Elo, Lan Yu, Eeva Valve, Sari Makelä & Pirko Härkönen
Institution of Biomedicine, University of Turku, Turku, Finland.

Prostate cancer is a commonly diagnosed malignancy in Europe. Bone is one of the common metastasis sites. There is increasing evidence for estrogen involvement in the prostate tumorigenesis. ER$\beta$ knockout (BERKO) mice have been reported to have anti-proliferative, anti-inflammatory and potential anti-tumorigenic functions of ER$\beta$. However, prostate phenotype of BERKO mice has been under debate.

The potential effect of ER$\beta$ on prostate tumorigenesis was studied by crossing BERKO mice with prostate targeted fibroblast growth factor 8b (Fgf8b-Tg) mice. Fgf8b-Tg mice develop advanced stromal and epithelial changes in the prostate that slowly progress to prostate intraepithelial neoplasia (PIN) lesions and to prostate cancer with mixed features of adenocarcinoma and sarcoma at old age. In addition, androgen receptor staining was decreased in the transformed epithelium and in the hypercellular stroma but strongly increased in the sarcoma-like lesions of Fgf8b-Tg mice. Prostate phenotypes of 1-year-old WT, Fgf8b-Tg, BERKO and Fgf8b-Tg-BERKO were analyzed. Fgf8b-Tg mice contained similar change as previously reported, including stromal aberration, PIN lesion, inflammation and cancer. The prostate of BERKO mice contained mild epithelial hypercellularity and inflammation, but not neoplastic changes. Prostate phenotype of Fgf8b-Tg-BERKO mice was mostly similar to that of Fgf8b-Tg mice. However, mucinous metaplasia was statistically significantly more frequent in the prostate that slowly progress to prostate intraepithelial neoplasia (PIN) lesions and to prostate cancer with mixed features of adenocarcinoma and sarcoma at old age.

The potential effect of ER$\beta$ on prostate tumorigenesis was studied by crossing BERKO mice with prostate targeted fibroblast growth factor 8b (Fgf8b-Tg) mice. Fgf8b-Tg mice develop advanced stromal and epithelial changes in the prostate that slowly progress to prostate intraepithelial neoplasia (PIN) lesions and to prostate cancer with mixed features of adenocarcinoma and sarcoma at old age. In addition, androgen receptor staining was decreased in the transformed epithelium and in the hypercellular stroma but strongly increased in the sarcoma-like lesions of Fgf8b-Tg mice. Prostate phenotypes of 1-year-old WT, Fgf8b-Tg, BERKO and Fgf8b-Tg-BERKO were analyzed. Fgf8b-Tg mice contained similar change as previously reported, including stromal aberration, PIN lesion, inflammation and cancer. The prostate of BERKO mice contained mild epithelial hypercellularity and inflammation, but not neoplastic changes. Prostate phenotype of Fgf8b-Tg-BERKO mice was mostly similar to that of Fgf8b-Tg mice. However, mucinous metaplasia was statistically significantly more frequent in the prostate that slowly progress to prostate intraepithelial neoplasia (PIN) lesions and to prostate cancer with mixed features of adenocarcinoma and sarcoma at old age.

Our results suggest that ER$\beta$ could have a role in differentiation of the prostatic epithelium and in the protection from inflammation, but do not provide evidence for a direct role of ER$\beta$ as a tumor suppressor.

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PP510

Glucocorticoids inhibit bone formation independent of miRNA regulation

Peng Liu1,2, Ulrike Baschant1, Marco Groth2, Mario Baumgart2, Matthias Platzer2, Hans-Martin Ja¨ck3 & Jan Tuckermann1
1Institute for General Zoology and Endocrinology, University of Ulm, Ulm, Germany; 2Leibniz Institute for Age Research – Fritz Lipmann Institute, Jena, Germany; 3Division of Molecular Immunology, University of Erlangen-Nuremberg, Erlangen, Germany.

Glucocorticoids inhibit bone formation independent of miRNA regulation

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Glucocorticoid-induced osteoporosis (GIO) is the most frequent secondary osteoporosis in patients undergoing steroid therapy. Recently we demonstrated that the inhibition of bone formation in GIO is occurring in part via the suppression of autocrine cytokines by the glucocorticoid receptor (GR) monomer in osteoblasts (Cell Metab 11, 517–531). Since emerging evidences indicate that microRNAs (miRNAs) play a critical role in the differentiation of osteoblasts, we evaluated the impact of miRNAs in GIO by conditional ablation of the miRNA-processing enzyme Dicer in osteoblasts. Runx2-Cre transgenic mice were crossed with Dicerflox mice, and the resulting Dicer<sup>Runx2Cre</sup> mice were growth retarded, accompanied with impaired bone formation and low bone density. qRT-PCR for representative miRNAs showed severe reduction of miRNA levels in femurs of Dicer<sup>Runx2Cre</sup> mice. Similarly, calvarial osteoblasts with a conditional ablation of Dicer upon 4-hydroxytamoxifen treatment derived from mice expressing a Cre-Estrogen ligand binding domain (CreERT2) fusion protein (Dicer<sup>CreERT2Rosa26CreERT2</sup>) displayed suppressed osteoblast differentiation. Importantly, ablation of dicer in primary osteoblasts did not affect dexamethasone-inhibited proliferation and differentiation in vitro. Accordingly Dicer<sup>Runx2Cre</sup> mice treated with prednisolone for 2 weeks exhibited a strong inhibition of bone formation as WT mice. Taken together, despite a strong impact on skeletal development, the conditional ablation of Dicer-dependent miRNAs in osteoblasts does not impair glucocorticoid-suppressed osteoblast differentiation and inhibition of bone formation. Our data suggest that miRNAs do not play a major role in GIO. Rather regulation of protein encoding genes or other Dicer-independently processed RNA species seem to mediate these deleterious glucocorticoid-effects.

Conclusion:
Better care for patients taking long-term corticosteroids requires regular control of systemic side effects for the optimized therapeutic compliance.

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**PP512**

**Positive regulation of osteogenesis by bile acid through FXR**

Chun Soo Shin1, Sun Wook Cho1, Hyejung Park1, Jae-Yeon Yang1, Sang Wan Kim2, Young Joo Park1, Minjung Yim2, Jung Eun Kim1, Seong Yeon Kim3 & Jee Hyun An3
1Seoul National University College of Medicine, Seoul, Republic of Korea; 2Soonkyung Women’s University, Seoul, Republic of Korea; 3Kyungpook National University School of Medicine, Daegu, Republic of Korea; *Konkuk University Hospital, Seoul, Republic of Korea.

Farnesoid X receptor (FXR) is a member of the nuclear receptor superfamily, which functions as bile acid sensor controlling the bile acid homeostasis. We have investigated the role of FXR in regulating bone metabolism in vivo. We have identified expression of FXR in calvaria and bone marrow cells, which was gradually increased during osteoblastic differentiation in vitro. Deletion of FXR in vivo has resulted in significant reduction in bone mineral density by 4.3-6.6% from 8 to 20 weeks of age compared with FXR<sup>+/+</sup> mice. Micro-computed tomography analysis of distal femur demonstrated significant reduction of trabecular bone volume, trabecular thickness and cortical thickness in FXR<sup>−/−</sup> mice compared with FXR<sup>+/+</sup> mice. Histologic analysis of lumbar spine also showed that FXR deficiency reduced bone formation rate as well as trabecular bone volume and thickness. Moreover, TRAP staining of femurs revealed that both osteoclast number and osteoclast surface were significantly increased in FXR<sup>−/−</sup> mice compared with FXR<sup>+/+</sup> mice. At the cellular level, induction of alkaline phosphatase (ALP) activities were blunted in primary calvarial cells from FXR<sup>−/−</sup> mice compared with those from FXR<sup>+/+</sup> mice in concert with significant reduction in the Col1a1, ALP, BSP, Runx2 and osterix gene expressions while treatment of C3H10T1/2 cells with bile acids (CDCA or GW4064) or synthetic FXR agonists (GW4064 or fexaramine) significantly enhanced ALP activities. Interestingly, culture of bone marrow derived macrophages from FXR<sup>−/−</sup> mice resulted in increased number of osteoclast formation and increased NFATc1 expression compared with those from FXR<sup>+/+</sup> mice. Furthermore, treatment of the macrophages with FXR agonists has potently inhibited osteoclast formation. Taken together, these results suggest that FXR positively regulates bone metabolism through both arms of bone remodeling pathways, i.e. bone formation and resorption.

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**PP513**

**Indole-3-carbinol and epigallocatechin-3-gallate for complex treatment of inflammatory periodontal diseases in female patients with gynecological pathologies**

Victoria Atrashkevich, Ella Zabalueva & Elena Zoryan
Moscow State University of Medicine and Dentistry, Moscow, Russia.

Introduction

Previous studies demonstrated that estrogen deficit in female patients causes inflammatory periodontal diseases. Sex steroids act by interacting with intracellular receptors located in odontoblasts, osteoblasts, gingival fibroblasts, and periodontal ligament cells. Inflammation in periodontal tissue is caused by excessive concentration of sex hormone receptors in oral mucosa. Aim

Studying the influence of indole-3-carbinol (I3C) and epigallocatechin-3-gallate (EGCG) on receptor status of gingival epithelium in female patients with hormone imbalance.

Materials and methods

We examined 41 female patients aged between 18 and 59 (35.5 ± 9.4) having hormone imbalance diagnosed with labatorial enzyme immunooassaay. Gingival

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status was determined with hygiene index (HI), gingival assessment (GA), papilla bleeding index (PBI), record pockets (RP), and Fuchs index. During surgical sanitation, the gingival area underwent standard tissue processing with hematoxylin-eosin technique and immunohistochemical examination both before starting complex treatment with I3C and EGCG and 2 months after the treatment. 

β-estrogen receptor detection was performed in a single-step method with epitope retrieval on paraffin sections using diagnostic kits by Dako (Denmark); proliferative activity (Ki67) of gingival epithelium cells was determined as the average of marked nuclei number in each 100 examined.

Results

Decreased histiolymphocytic infiltration of gingival epithelium, reduced edema and homogeneity of connective stroma in proper mucous plate, collagen fiber fascicles became more clearly defined and better oriented, the number of microhemorrhage foci decreased. Gingival status before and after treatment is correspondingly: HI 12.4±7.9/18.6±10.2; GA 1.2±0.7/0.5±0.3; PBI 1.9±0.7/1.5±0.5; RP 3.9±0.7/3.5±0.6; Fuchs index 0.67±0.08/0.71±0.06. The percentage of cells in gingival epithelium, expressing β-estrogen receptors (50.0±16.7%/17.5±5.8%) and Ki67 (20.0±10.7/5.2±2.6), decreased significantly.

Conclusion

A I3C and EGCG in complex treatment of gingival inflammatory diseases in female patients with gynecological pathologies improve periodontal status indices, decreasing the number of β-estrogen receptors and gingival epithelium Ki67.

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