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IS01

USING FRACTURE RISK ASSESSMENT TOOLS

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Background: The World Health Organization plans to release a fracture risk model in 2008. Implementing fracture risk assessment in the clinic involves correct use of fracture risk tools, including counseling about risk and treatment to reduce risk.

Methods: We developed a simple fracture risk tool using risk factors and fracture rates from the literature. We validated the risk findings in 2 cohorts. We tested the impact of quantitative 5- adn 10- year fracture risk data compared to bone density T-scores.

Results: A simple risk model can be used to provide quantatitve risk fro individual patietns. When provded with such data, providers are more likely to treat eldelryl women who are not obviously osteoporotic and less likely to treat younger women with osteopenia.

Conclusions: Fracture risk assessment is a practical and accurate way to make decisions regarding management of low bone mineral density. Quantitative risk data are preferred to T-scores.

Conflict of Interest: None declared

IS02

ALLIED HEALTH PROFESSIONALS DOING RESEARCH

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Aim: The care of patients with osteoporosis relies on the entire spectrum of health care providers, not just physicians. It is therefore important that Allied Health Professionals also start doing research and contribute to this scientific forum as they form an important part of the team in osteoporosis prevention, diagnosis and treatment.

Methods: Allied health professionals are health care practitioners with formal a education and clinical training who are credentialed through certification, registration and/or licensure. They collaborate with physicians and other members of the health care team to deliver high quality patient care services for the identification, prevention, treatment of diseases, disabilities and disorders. Allied Health Professionals in this setting could be nurses, physiotherapists, laboratory technicians, dieticians, occupational therapists, etc.

Currently in the health care sector, there is much concern regarding patients' low adherence to pharmacological treatments and to beneficial life style changes. The real issue is the care gap between scientific knowledge and patients' ability to cope with and handle different chronic illnesses. Lack of effective communication between patients and health care professionals and the inadequate level of patient education are important determinants of poor adherence. A multidisciplinary approach is necessary to meet the patient's different needs in handling osteoporosis in every day life.

Each of the allied health professions arises to fill a specific need. Although the professions may have common ground, each profession is developed to fill a unique gap in terms of knowledge and expertise in the osteoporosis field. During the talk there will be drawn attention to different research projects done by allied health professionals. The different disciplines will ultimately survive by documenting its value through research.

Conclusion: There are going to be an increased need and increasing opportunities in the future for allied health professionals to work on specific research projects, particularly in health promotion and treatment.

Conflict of Interest: Dorthe Nielsen; Nycomed, Novo Nordisk Foundation, Servier, Grant Research Support.

IS03

BONE REPAIR: POTENTIAL PHARMACEUTICAL MANIPULATION

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The potential for pharmaceutical manipulation of bone repair is a hot topic in orthopaedic research. With the advent of effective pharmaceutical treatments for osteoporosis and metabolic bone disease, there is an active effort to optimise the application of similar compounds to bone repair.

Obstacles to the development include the increased complexity of bone repair to that of bone remodelling. In addition to the processes that modulate bone formation and resorption, bone repair involves the recruitment of mesenchymal cells and the vasculature in a coordinated way to re-establish the bone microenvironment.

A further obstacle is the lack of a unified system to classify deficiencies and treatments. Riggs and Parfitt have suggested classifying osteoporosis drugs according to their principle action, ie anabolic or anti-catabolic. It is similarly useful to expand on this concept and think of bone repair as a system of coordinated anabolic and catabolic responses. The anabolic response can be further divided into non-specific (wound repair) and specific (bone) anabolism. One must also remember that agents can effect both anabolic and catabolic processes, and the relative effects are situation and time dependent.

Thus potential pharmaceutical agents can be classified according to their effects on the various parts of the process. For example, PTH is a very specific anabolic, ie it acts on mature bone cells. PTH has no known effect on non-specific anabolism (ie wound repair phenomena of cell recruitment and angiogenesis). PTH also indirectly increases catabolism. BMPs have broad effects on non-specific and specific anabolism which is perhaps why they are successful agents in fracture repair and spinal fusion. However it must also be remembered that they can be pro-catabolic. VEGF and PDGF are non-specific anabolics which can increase mitogenesis and angiogenesis but have no specific bone anabolic effect. These non-specific effects may still be useful in the presence of other specific bone formation signalling. Bisphosphonates are anti-catabolic but eventually have indirect effects which reduce bone formation via remodelling.

This system can be applied to novel therapies which are emerging such as RANKL or SOST inhibition such that the likely effective scenarios for their application in bone repair can be deduced and then tested.

Conflict of Interest: D Little, Novartis, Grant/Research Support, Consultant
D Little, Stryker Biotech, Grant/Research Support, Speakers Bureau

IS04

BRIDGING THE GAP - SKELETAL REGENERATION USING OSTEOPROGENITOR POPULATIONS - AN INTERDISCIPLINARY APPROACH

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Given the demographic challenges of an ageing population combined with rising patient expectation and the growing emphasis placed on cost containment by healthcare providers, economic regenerative medicine approaches for skeletal regeneration is a major clinical and socio-economic need. Human bone marrow stromal stem cells or mesenchymal stem cells are defined as multipotent progenitor cells

with the ability to generate cartilage, bone, muscle, tendon, ligament and fat. These primitive progenitors exist postnatally and exhibit stem cell characteristics, namely low incidence and extensive renewal potential. These properties in combination with their developmental plasticity have generated tremendous interest in the potential use of these progenitor populations to replace damaged tissues. To date, relatively little is known concerning the phenotypic characteristics, whether from a morphological or biochemical standpoint whilst direct in vivo confirmation of the lineage potential and plasticity or interconversion potential that exists of mesenchymal stem cells and osteogenic progenitor cells remains limited. Nevertheless, strategies harnessing tissue engineering approaches offer much promise for skeletal regeneration using mesenchymal populations. Studies centered, in the first instance, on isolation, expansion and translational studies of human fetal and adult mesenchymal populations for skeletal repair will be covered. Areas to be reviewed using data from the group include: i) isolation, expansion and characterization of the plasticity of fetal and adult mesenchymal populations, ii) combination of progenitor cells with tailored growth factor containing polymer scaffolds in an attempt to modulate the phenotype of the mesenchymal populations to generate mineralized bone tissue and iii) translational studies to examine the efficacy of mesenchymal populations using impaction bone grafting as an exemplar. The challenge and opportunity for all involved will be the development of multidisciplinary approaches that integrate materials, cell, molecular and clinical techniques for skeletal tissue regeneration - the possible impact in terms of advancement, healthcare costs and, more importantly, improved quality of life are immense.

Conflict of Interest: None declared

IS05

STATISTICAL ISSUES IN CLINICAL TRIALS

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Since the first fracture endpoint trials of about 10 years ago, there have been a large number of important trials in osteoporosis which have served as the basis for approval of several new medications for osteoporosis. As the field has matured, it has become clear that many of the general statistical issues relevant to clinical trials are important issues in osteoporosis fracture trials as well. Some of these that will be discussed in include the following:

Analysis by intention to treat: There is an increasing a awareness that the most rigorous technique for conducting trials is the ITT approach. ITT is not simply a strategy for data analysis, but should be considered in the design and especially the implementation of trial. Most important, a rigorous ITT approach requires that the primary endpoint for a trial be collected on ALL randomized patients at the end of the trial, regardless of adherence to study medications. We will review the various components of ITT and how they have been applied in osteoporosis trials.

Endpoints for trials: Choice of the best endpoint involves a trade off between risk, potential benefit and ease of assessment. Trials have used vertebral, non-vertebral and hip fractures as outcomes but methods for assessments have varied, particularly for vertebral fractures.

Subgroup analyses: While it is often of great interest to examine the effect of treatment within subgroups of patients, subgroup analyses are extremely problematic due to the potential of being misled due to low power and/or multiple comparisons issues. While some subgroup findings in osteoporosis have been consistent, others may be spurious. General guidelines for subgroup analyses will be presented.

Safety evaluation: The assessment of safety in trials osteoporosis, as well as other therapeutic areas, has become more controversial. The causes for difficulties include the following: multiple comparisons, classifications of adverse experiences, evaluation of safety problems that are rare, etc. Statistical difficulties in safety assessment present problems to scientists, clinicians, patients, regulators and pharmaceutical companies and examples of problems will be presented.

Conflict of Interest: None declared

IS06

CLINICAL TRIALS: STUDY DESIGN

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There is now a wide range of therapeutic options for the prevention of fractures in postmenopausal women. These are effective, safe and offer the patient a variety of dosing regimens. This poses a problem for those who develop new treatments, because many believe that it is not ethical to give a placebo to individuals who are at high risk of fracture. What alternatives are available?

The topics to be covered in this workshop will include a discussion of the ethical issues involved in using a placebo-controlled design in Phase III studies in high-risk individuals. Studies in low risk populations, the advantages and disadvantages of comparator studies and whether acceptable primary end-points other than fracture can be developed will be discussed. In addition, designs other than the classical randomised controlled trial will be considered.

The target audience includes individuals with an interest in drug development, particularly in Phase III clinical trials, those with an interest in pharmacotherapy for osteoporosis, and scientists with an interest in in vivo measures of bone strength other than fracture. The session will be informal and interactive, structured around the questions raised above.

Conflict of Interest: None declared

IS07

ORGANIZATION OF INDUSTRY-SPONSORED TRIALS AND THE ROLE OF ACADEMIC INVESTIGATORS

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Concern has grown about the validity of results from industry-sponsored trails. Increased involvement of academic investigators in conduct of analysis and publication from trials might improve the credibility of results from such trials.

The relationship between sponsors and investigators varies considerably. At one extreme the sponsor designs, conducts, and analyzes results and then involves top recruiters or well-known researchers to author papers that are developed by marketing departments and written by contract medical writers. At the other end of the spectrum are 'collaborative' models: academic investigators collaborate with the sponsor's scientists (often as a Steering Committee) to design the trial and oversee its conduct, develop analysis plans to guide data analyses and investigators write the first drafts and revisions of articles and the Committee reviews and approves for publication. Sponsors are often concerned that sharing control over analysis and publication might allow irresponsible use of data. Publication guidelines and peer review before submission decreases the possibility of such 'rogue' misuse of data.

A few Journals require analysis or confirmation of results by academic-based statisticians. Some require descriptions of the sponsors role in the process of analysis and writing. At a minimum, I believe that analysis plans for papers should be available to journals and publically accessible and papers should report who developed that analysis plan, wrote the first draft, analyzed the results and approved the version for submission.

There is no evidence about the best organization for industry-sponsored trials. Our experience with several pivotal trials suggests that collaboration via a Steering Committee tends to produce more high impact papers. Such collaborations might also improve the credibility of results from industry-sponsored research.

Conflict of Interest: Research support and/or fees for consulting and/or speaking from Lilly, Pfizer, Novartis, Amgen, Pfizer, Kyphon, Organon, Zelos, P&G, Merck, Roche

IS08

NOTCH SIGNALING IS BONE

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Notch signaling is a central mechanism for controlling embryogenesis. However, its *in vivo* function during mesenchymal cell differentiation, and specifically, in bone homeostasis remains largely unknown. We report that osteoblast-specific gain of Notch function causes serve osteosclerosis due to increased poliferation of immature osteoblasts. Under these pathological conditions, Notch stimulates early osteoblastic proliferation by up-regulatin *Cyclin D. Cyclin E. and Osterix*. Notch also regulates terminal osteoblastic differentiation by directly binding *Runx2* and repressing its transactivation function. In contrast, loss of all physiologi Notch signaling in osteoblasts, generated by delection of *Presenilin 1 and 2* in osteoblasts, is associated with late onset, age-related osteoporosis resulting from increased osteoblast-dependent osteoclastic activity due to decreased production of osteoprotegerin. This defect in osteoclastogenesis is both evident in vivo and in co-culture studies. Together, these findings highlight the potential dimorphic effects of Notch signaling in bone homeostasis and may provide direction for novel therapeutic applications.

Conflict of Interest: None declared

IS09

ROLES OF NOTCH SIGNALLING IN OSTEOBLASTIC ACTIVATION IN THE HEMATOPOIETIC STEM CELL NICHE

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The bone marrow microenvironment, or niche, regulating hematopoietic stem cells (HSC) has been difficult to define in mammals, in part due to the anatomic complexity of bone and bone marrow, and the lack of a single, explicit marker identifying HSC. However, anatomic studies had suggested proximity of primitive hematopoietic cells to the endosteal surface, and osteoblastic cells were found to efficiently support HSC in vitro. Data from our laboratory and others have utilized genetically-altered murine models to demonstrate that osteoblasts are an essential regulatory component of this complex cellular network. We established that parathyroid hormone (PTH), through activation of the PTH/PTHrP receptor in osteoblastic cells, could alter the HSC niche expanding HSC in vivo and in vitro and improving the survival of mice receiving HSC transplants. These findings are of great clinical appeal, because they suggest that a strategy aimed at modifying supportive cells in a stem cell niche can expand HSC. Subsequently a number of molecules have been found to be important for hematopoietic/osteoblastic interactions, and the role of osteoblastic cells in the HSC niche has been confirmed in a number of experimental models. We have focused on the Jagged1/Notch signaling pathway, which has previously been demonstrated to be important for HSC self-renewal. In particular, the Notch ligand Jagged1 is expressed in human stromal cells and can improve their ability to support HSCs in vitro. In our studies in mice genetically altered to express a constitutively active PTH/PTHrP receptor in osteoblastic cells only, and in mice treated with PTH(1–34), we aimed to determine whether Notch signaling could mediate the osteoblastic effects on HSC behavior. Our data suggest that Notch signaling is activated in vivo upon osteoblastic stimulation by PTH. Moreover, PTH can rapidly regulate the Notch ligand Jagged1 in a subset of osteoblastic cells through Protein Kinase A activation. Finally, activation of gamma-secretase is required for the PTH-dependent HSC expansion in vitro. Notch ligands and receptors are expressed in both stromal/osteoblastic and hematopoietic components of the bone marrow microenvironment. Therefore, our current studies are aimed at defining the role of specific Notch signaling partners in niche and stem cells, in order to determine the cellular and molecular mechanisms mediating the osteoblastic regulation of HSC behavior.

Conflict of Interest: None declared

IS10

THE ROLE OF NOTCH SIGNALLING DURING CHONDROGENESIS IN BONE MARROW STEM CELL

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There is great interest in the use of adult stem cells to generate cartilage for the repair of damaged and diseased joints. Bone marrow stem cells have capacity to differentiate into chondrocytes, but little is known of the signals involved in the early steps of differentiation. As chondrogenesis typically involves forming cell-cell contacts we investigated Notch signaling during chondrogenesis in human bone-marrow stem cells (hMSC) in 3D cell aggregate cultures. Expression analysis of Notch receptor and ligand genes showed that the Notch ligand, Jagged-1 (Jag-1) sharply increased in expression peaking at day 2 and then declined and a Notch target gene, HEY-1, was also activated and this preceded the rise in type II collagen expression that characterized chondrogenesis. The shut-down in Notch signaling was critical for chondrogenesis to proceed, as adenoviral hJag-1 transduction of hMSCs, which caused continuous expression of Jag-1, completely blocked chondrogenesis. In these cultures there was inhibited production of extracellular matrix and the gene expression of aggrecan and type II collagen were strongly suppressed and this may reflect the retention of a prechondrogenic state. The JAG-1 mediated Notch signaling was also shown to be necessary for chondrogenesis, as the inhibitor of Notch signaling, DAPT, inhibited chondrogenesis when present early in the process. The results thus showed that Jag-1 mediated Notch signaling in hMSC was necessary to initiate chondrogenesis, but must be switched off for chondrogenesis to proceed. The need for active Notch signaling in chondrogenesis may explain why forming cell pellets, micromasses, or 3D cultures greatly favours differentiation towards the chondrocyte lineage and as it mimics early mesenchymal condensation, it may suggest that similar Notch activation occurs during limb morphogenesis in the embryo.

Conflict of Interest: None declared

IS11

MAPPING COMPLEX TRAITS IN HETEROGENEOUS STOCK MICE

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Difficulties in fine-mapping quantitative trait loci (QTLs) are a major impediment to progress in the molecular dissection of complex traits in mice. We show that genome-wide high resolution mapping of multiple phenotypes can be achieved using a stock of genetically heterogeneous mice. Using a conservative and robust bootstrap analysis 843 QTLs were mapped with an average 95% confidence interval of 2.8 megabases. The QTLs contribute to variation in 97 traits, including models of human disease (asthma, type 2 diabetes mellitus, obesity and anxiety) as well as immunological, biochemical and haemotological phenotypes. The genetic architecture of almost all phenotypes was complex, with many loci each contributing a small proportion to the total variance.

Conflict of Interest: None declared

IS12

GENETIC DETERMINANTS OF OSTEOPOROSIS

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Many -if not all- common diseases such as osteoporosis, have strong genetic influences and therefore intense efforts are ongoing to identify the underlying genetic variants. Knowledge of these variants can help in understanding the disease process and might benefit development of interventions and diagnostics. Genome Wide Association (GWA) studies have now become the standard approach to uncover the strongest genetic effects of common variants. The GWA approach builds upon the availability of extensive data on human genetic variation, substantial improvements in genotyping technology including very high-density SNP arrays, and accessibility of biobanks of large population cohorts with DNA and phenotype information.

In the past 2 years GWA has proven to be widely successful for several complex diseases in discovering novel and common risk genes mainly in Caucasian populations. For osteoporosis several GWA studies are underway and initial results are expected to come out soon. Interestingly, experience so far has shown that GWA -in general- identifies DNA variants rather then genes, and that the effects seen for the common variants are modest, e.g., with Odds Ratios ranging from 1.1–1.7. On the one hand these features shed interesting light on how human genes are regulated and what common genetic variation is tolerated throughout evolution, but on the other hand also pose serious challenges in translating the results of GWA to clinical practice. Much effort is therefore needed to link GWA findings to actual genes and to establish the biological mechanism of associations. In addition, efforts will be shifting from analysing common variants, mostly single nucleotide polymorphisms, to the more rare DNA variants, and also include other types of DNA sequence variation.

An important part of the process of GWA analysis is the testing of identified variants in international consortia with very large collections of DNA samples with a certain phenotype. The GENOMOS consortium has played such a role in the field of osteoporosis and has in the "preGWA" era already identified (and refuted) associations of well known candidate genes. This consortium is expected to play an important role in the upcoming GWA analyses for osteoporosis. Together with genetic studies on more rare syndromes, the GWA approach is likely to help in clarifying the genetic architecture of complex traits such as BMD and -eventually- fracture risk.

Conflict of Interest: None declared

IS13

OPTIMIZATION OF TREATMENT OF OSTEOPOROSIS

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Therapies of chronic diseases should be efficacious, convenient for the patient and devoid of side effects. In daily practice, the risk of serious outcomes and the preference of patients as well as the cost of the interventions should also be considered. The pathophysiological basis of osteoporosis provides the rationale for the use of interventions that either reduce bone resorption and turnover or stimulate bone formation. Several antiresorptive treatments are used in the treatment of osteoporosis while PTH is the only anabolic therapy currently available (1–34 and 1–84 peptides). Evidence for efficacy and safety from controlled studies has been obtained for up to 10 years for antiresorptives and up to 2 years for PTH, while short-term head-to-head studies with surrogate endpoints have also been performed. Such studies illustrate the different mechanism of action of the two types of interventions but do not allow any conclusions about any potential differences in antifracture efficacy. These considerations are reflected in recommendations of several regulatory authorities. It is also frequently assumed that antiresorptives should be given mainly to patients with high bone turnover while anabolics should be reserved for patients with low bone turnover. However, analyses of the results of trials with bisphosphonates and PTH 1–34 indicated that the antifracture efficacy of these agents is probably independent of prevalent rates of bone turnover. Further analysis of the pharmacodynamic responses to these treatments, reveal distinct patterns with attainment or not of steady-states that provide the basis for the design of regimens with the use of both types of therapies, in some patients at least. Such therapeutic approaches need to be explored further and their efficacy in reducing fracture risk, their safety as well as their cost-effectiveness need to be evaluated.

Conflict of Interest: S. Papapoulos, Research Support and/or honoraria, Amgen, Eli Lilly, Merck & Co, Novartis, Procter & Gamble/ Sanofi-Aventis, Roche/GSK, Servier.

IS14

BISPHOSPHONATES AND OSTEONECROSIS OF THE JAW

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Osteonecrosis of the jaw (ONJ) is a complication of high-dose bisphosphonate use, characterized by the finding of exposed bone in the oral cavity. Almost all cases have occurred in oncology patients after several years' therapy, usually with monthly doses of pamidronate or zoledronate. In many cases, the precipitating event appears to have been a dental extraction or other invasive procedure. With local and/or systemic antibacterial therapy, most lesions stabilize and some

show healing. The limited histology data available show evidence of bone necrosis and, often, infection, but not usually of ischemia.

It has been assumed that the primary lesion lies in bone and is related to over-suppression of bone turnover, but it is unclear why such a lesion should present with loss of the soft tissue covering of the mandible or maxilla as the primary clinical feature. A possible explanation of this paradox is that bisphosphonate is accumulated in bone in concentrations sufficient to be directly toxic to the oral epithelium. This would result in the failure of healing of soft tissue lesions (such as those caused by invasive dental procedures or by subclinical trauma from dentures) leading to secondary infection of the underlying bone. This model would explain why bone resection is unhelpful in managing this problem, suggests that low bone turnover caused by non-bisphosphonate drugs should not cause the same problem, and raises the possibility that agents which reverse bisphosphonate effects in vitro might have a role in the management of ONJ.

Conflict of Interest: IRR has received research funding and consultancy fees from Merck, Novartis, adn Procter and Gamble

IS15

BONE MASS GAIN DURING GROWTH IN HEALTH AND DISEASE

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The evidence that peak bone mass (PBM) affects bone strength during later life has called attention on the development of the skeleton and the acquisition of bone mass.

Throughout childhood and adolescence, the skeleton changes in both size and shape. Bones are growing in length and width, cortical thickness is increasing, and there is a significant increase of bone mass and bone density. In healthy subjects, both girls and boys, physical (height and weight) and pubertal development exert major effects on bone mass/density, even if with gender specificity. Many hormones (sex steroids, growth hormone, and vitamin D metabolites) influence development, including that of bone mass/density. Other factors affect the accrual of bone mass/density, among them heredity, diet, physical activity. Some epidemiological studies seem to indicate that an individual's future accumulation of bone mineral is influenced during intrauterine life, especially by the maternal vitamin D status.

Studying the development of bone mass/density is still very difficult because all the involved factors have both a direct and an indirect action on bone modelling, through complex interactions.

The complexity of the growth and development processes explain also the difficulty in evaluating the acquisition of PBM in different diseases. In these cases not only the physiological factors but also some specific factors related to the disease (for example, pulmonary function in cystic fibrosis or inflammation in rheumatoid arthritis) and/or its treatment (drug, dose, duration, etc.) are major determinants of the accrual of bone mass/density and of the attainment of an optimal PBM. Often, the disease-related factors prevail over the physiological factors: for example, in many ill children, bone mass/density is more related to the severity of the underlying pathology than to the chronological age.

Presently, we use a somewhat "imprecise" way to estimate the bone mass/density accrual in children affected by various chronic conditions during the growing age: the comparison with the accrual observed in healthy children. We should make an effort to improve this evaluation not only considering the differences in the pubertal status and body-size development, but also considering the specific characteristics of bone mass/density development, at least in the main groups of chronic diseases.

Conflict of Interest: None declared

IS16

DIAGNOSIS AND EVALUATION OF SECONDARY OSTEOPOROSIS IN YOUNG ADULTS

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In the absence of fragility fractures, low bone mineral density may reflect attainment of a lower peak bone mass in comparison with the young adult mean value. It is necessary to distinguish between low peak bone mass and a systemic disorder resulting in low bone mineral density and skeletal fragility. Low peak bone mass in the absence of fragility fracture or progressive bone loss may not require pharmacological intervention. However, systemic disorders contributing to bone loss do require diagnosis and intervention. Common causes of low bone density in premenopausal women include ovulatory disturbances and low body weight. Other diseases, conditions or medications may also contribute to bone loss and these should be identified and treated if present. Fracture risk is reduced by lifestyle changes and pharmacological intervention in those with glucocorticoid-induced bone loss. Discontinuing depot medroxyprogesterone acetate use has been associated with improvements in bone mineral density. Bone mineral density alone is insufficient for the diagnosis of osteoporosis in premenopausal women or young men in the absence of fragility fractures. Bone mineral density testing should only be performed in premenopausal women or young men in the presence of approved indications.

Conflict of Interest: None declared

IS17

OSTEOPOROSIS IN PREMENOPAUSAL WOMEN: HOW AND WHEN TO TREAT

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Few data exist to guide management of premenopausal women with low BMD (Z score <-2.0) and/or fragility fractures. Therefore my recommendations are based upon opinion.

Such young women should have a complete history, physical and laboratory evaluation to identify 2o causes of bone loss. The most common 2o causes are glucocorticoid (GC) excess and causes of estrogen deficiency. In women with low BMD but no fractures, another goal is to distinguish those with low but stable BMD, likely due to low peak bone mass, from those with ongoing bone loss. Contributing conditions should be addressed specifically if possible.

Lifestyle modifications are recommended for most young women with osteoporosis: maintain normal weight; adequate calcium (1000–1200 mg); vitamin D3, 400–800 IU or sufficient to maintain serum 25OHD $>$ 70 nmol/l; regular weight-bearing exercise; discontinue tobacco and excess alcohol. Premenopausal women with osteoporosis treated only with increased dietary calcium and exercise had improved BMD and no new fractures. Pharmacological therapies available for premenopausal women include estrogen (E2), calcitonin, bisphosphonates (BPs) and parathyroid hormone (PTH). SERMs should not be used to treat osteoporosis in young women as they block E2 action and cause more bone loss. There are few studies of calcitonin in young women. However, in perimenopausal women treated with 100 IU intranasal calcitonin or placebo, there was no improvement in BMD. BPs cross the placenta and accumulate in fetal bones in animal studies; their long half-life in bone is concerning in reproductive age women. BPs are approved only

premenopausal women on GCs in some countries. PTH prevents bone loss in premenopausal women on GnRH agonists and GCs and has the advantage of not being retained in the skeleton. However, PTH effects may dissipate unless followed by BPs.

The approach to therapy should vary according to the clinical presentation. For young women with low but stable BMD, pharmacologic therapy is generally not indicated because fracture risk is generally very low. Therapy may be necessary for women with recurrent fractures and those with rapid ongoing bone loss or known secondary causes for fractures that cannot be treated specifically (GCs, GnRH or chemotherapy for breast cancer with premature menopause, osteogenesis imperfecta).

Conflict of Interest: Grant/Research Support: Novartis, Aventis P&G, Lilly, Amgen

IS18

ROLE OF CYTOKINES IN BONE HEALTH AND DISEASE

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The bone remodeling cycle is highly regulated by a variety of agents, including hormones, cytokines and growth factors. These are produced both locally and systemically and act in concert to direct bone turnover. Cytokines are locally acting proteins that were originally identified by their ability to regulate immune and hematopoietic cell function. It is now clear that multiple cytokines are produced in the bone microenvironment either spontaneously or in response to specific stimuli. Recent studies of the mechanisms regulating bone remodeling have concentrated on the role that local cytokine production has in this process because a number of cytokines have been shown to be critical for both normal and pathologic bone cell function. It is now apparent that during both health and disease, the production of cytokines by cells in the bone microenvironment and the responses of bone cells to these cytokines are regulated in a highly ordered manner. It is likely that the spectrum of cytokines, which are produced locally in bone, defines the responses of bone cells to a particular state and predicts the subsequent development of normal or pathologic bone remodeling. Diseases of bone where cytokines are believed to play an important role include osteoporosis, Paget's disease, and the effects of malignancy on bone. Studies of the production of cytokines in bone and the responses of bone cells to these cytokines are providing insights into the mechanisms that regulate the development of metabolic bone diseases and may lead to new therapies for these conditions. This talk will provide a broad overview of the actions that a number of cytokines have on bone and the mechanisms by which bone cells respond to these factors. Particular emphasis will be placed on recent work in animal models and the relevance of these findings to human metabolic bone diseases.

Conflict of Interest: None declared

IS19

T CELLS: UNEXPECTED PLAYERS IN THE EFFECTS OF ESTROGEN AND PTH IN BONE

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T cells have the capacity to secrete cytokines known to regulate osteoclast (OC) formation and activity. T cells are also known to express receptors for both estrogen and PTH, and alterations of T cell function have been described in postmenopausal women and patients with severe hyperparathyroidism. We have reported that ovariectomy (ovx) fails to induce the loss of both cortical and trabecular bone in T cell deficient nude mice while does so in WT mice and T cell reconstituted nude mice. We have now confirmed these findings in WT mice depleted of T cells via injection of anti T cells antibodies and using the novel inhibitor of costimulation Abatacept (CTLA-4 Ig). CTLA-4 Ig binds to human and murine CD80 and CD86 blocking their interaction with CD28, promoting anergy and T cell apoptosis. Therefore, CTLA-4 Ig is a potent suppressor of T cell activation in vivo. We have found that CTLA-4 prevents the increase in T cell activation induced by ovx. Attesting to the relevance of T cell activation for ovx induced bone loss, both T cell depletion and CTLA-4 Ig treatment completely prevented the loss of both cortical and trabecular bone induced by ovx. Studies were also conducted to evaluate the role of T cells in the effects of both intermittent PTH (iPTH) and continuous PTH (cPTH) treatment. We found that cPTH does not stimulate bone resorption, nor induces cortical bone loss in nude mice, TCRbeta −/− mice, WT mice depleted of T cells and WT mice treated with CTLA-4 Ig. Thus, T cells are required for PTH to induce cortical bone loss. We also found that iPTH induces a greater increase in bone formation rate, osteoblast differentiation and trabecular bone volume in WT than in T cell deficient mice. T cells mediate both ovx and cPTH induced bone loss through T cell expressed CD40L. This receptor binds to stromal cells expressed CD40 and endows SCs with the capacity to support OC formation. In contrast, T cell potentiate the anabolic activity of iPTH by activating the Wnt pathway. In summary, the data demonstrate that T cells play a pivotal role in the mechanism of ovx and PTH induced bone loss in both WT mice and genetic strains lacking T cells. The finding that T cell mediates the catabolic effect of cPTH and potentiate those of iPTH provides a new paradigm for T cells as an essential target of PTH in bone.

Conflict of Interest: None declared

IS20

REGULATION OF OSTEOCLASTOGENESIS BY INNATE IMMUNE CO-RECEPTORS

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Immunoreceptor tyrosine-based activation motif (ITAM) signaling mediated by DAP12 or Fc epsilon receptor Iγ chain (FcRγ) are critical during osteoclast differentiation and maturation to provide costimuli for RANKL-induced osteoclastogenesis under normal physiological conditions. Interestingly, we found that in the rapid bone remodeling state induced by estrogen-deficiency with ovariectomy (OVX), an ITAM adapter-independent bypass mechanism allows for enhanced osteoclastogenesis and activation in specific bony microenvironments. Following OVX, bone loss in vertebrae and cortical bone requires ITAM signaling receptors, however, significant trabecular bone loss in the tibia and femur occurs in mice deficient in ITAM adapters. Despite the general concept that ITAM signals provide an activation signal to regulate differentiation and function, recent studies have suggested that DAP12 can also mediate inhibitory signals in immune cells. Several groups recently demonstrated that DAP12-deficient macrophages show enhanced cytokine responses to TLR stimulation and that the inhibitory signal is mediated by the

DAP12 associated receptor TREM2. Following OVX, DAP12$^{-/-}$ FcR$\gamma^{-/-}$ mice and DAP12$^{-/-}$ quantitatively lose much more absolute bone compared to wild-type mice following OVX, and one possible explanation is TREM2/DAP12 also mediates an inhibitory signal in osteoclasts through DAP12 to fine-tune the resorption magnitude during estrogen-deficiency induced bone loss. The combined input of both activating and inhibitory receptor signals likely facilitates tight control over osteoclast differentiation and function. Since innate immune receptors generally sense microenvironmental changes, it is plausible that different receptors are required in distinct microenvironments. Osteoclasts are likely similar to other innate immune cells which utilize arrays of receptors to sense and regulate responses to changing local stimuli from their surrounding cellular and matrix environment. The mechanism bypassing ITAM signaling is not readily explained by our current understanding of osteoclastogenesis and likely utilizes different receptors and/or signaling pathways which suggests that osteoclastogenesis is differentially regulated dependent on the type of microenvironmental stimuli.

Conflict of Interest: None declared

IS21

ARE NON-RESORBING OSTEOCLASTS SOURCES OF BONE ANABOLIC ACTIVITY?

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In normal healthy individuals bone formation is coupled to bone resorption in a tight equilibrium. When this delicate balance is disturbed, the net result is pathological situations, such as osteopetrosis or osteoporosis. Some osteopetrotic mutations lead to low resorption, increased numbers of osteoclasts and increased bone formation, whereas other osteopetrotic mutations lead to low resorption, low numbers of osteoclasts and decreased bone formation. This suggests that the balance between bone resorption and bone formation may be shifted.

Human osteopetrosis, caused by mutations proteins involved in the acidification of the osteoclastic resorption lacuna (ClC-7 or the a3-V-ATPase), is characterized by decreased resorption in face of normal or even increased bone formation. Mouse mutations leading to ablation of osteoclasts, e.g. loss of M-CSF or c-fos lead to secondary negative effects on bone formation, in contrast to mutations where bone resorption is abrogated with sustained osteoclast numbers, such as the c-src mice. These data indicate a central role for osteoclasts, and not necessarily their resorptive activity, in the control of bone formation. In support of this, osteoclasts were recently shown to secrete factors which stimulated bone formation.

We consider the balance between bone resorption and bone formation, reviewing novel data that have shown that this principle is more complex than originally thought. We highlight the distinct possibility that osteoclast function can be divided into two more or less separate functions, namely bone resorption and stimulation of bone formation. Finally, we describe the likely possibility that bone resorption can be attenuated pharmacologically without the undesirable reduction in bone formation.

Conflict of Interest: None declared

IS22

OSTEOCLAST-OSTEOBLAST BIDIRECTIONAL SIGNALLING

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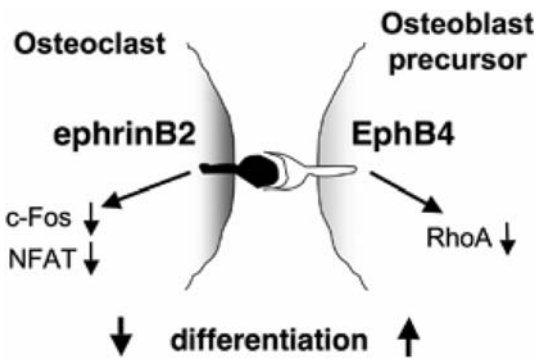
Bone remodeling is a complex process requiring 'coupling' of osteoclastic and osteoblastic activities. AP-1 refers to a collection of dimers formed between basic leucine zipper (bZIP) proteins of the Fos (c-Fos, Fra1, Fra2 and FosB) and Jun (c-Jun, JunB and JunD) families. In search of bone remodeling regulators, we have been analyzing potential target genes of the osteoclastogenic transcription factor c-Fos in the osteoclast lineage. Identified direct and indirect target genes of c-Fos during osteoclast differentiation include those encoding Fra1, interferon-β, NFATc1, and ephrinB2. Cell-surface ephrinB family molecules are transmembrane proteins, while ephrinA family molecules are glycosyl-phosphatidyl inositol (GPI)-anchored proteins. Both ephrinB and ephrinA are ligands for tyrosine kinase receptors EphB and EphA, respectively, and interaction between ephrin- and Eph-expressing cells results in bidirectional signal transduction. 'Reverse signaling' through ephrinB2 into myeloid precursors suppressed osteoclast differentiation, while 'Forward signaling' through EphB4 into mesenchymal precursors enhanced osteoblast differentiation. These findings indicate that when osteoclast activity is attenuated and osteoblasts initiate bone formation, ephrinB2-EphB4 interaction may facilitate transition from the resorption phase to the formation phase in the bone multicellular unit (BMU) or in the bone remodeling compartment (BRC).



Conflict of Interest: None declared

IS23

FRZB SIGNALING PROVIDES A MOLECULAR BASIS FOR AN INVERSE RELATIONSHIP BETWEEN OSTEOARTHRITIS AND OSTEOPOROSIS

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Human osteoarthritis is characterized by damage of the articular cartilage and loss of joint function. Osteoporotic fractures are caused by loss of cortical and trabecular bone density. They are common diseases in the elderly with important health and socio-economic impact. Clinical observations suggest an inverse relationship between these disorders. Frizzled-related protein (FRZB) can provide a molecular basis for this hypothesis. We identified FRZB in a chondrogenic/osteogenic extract from articular cartilage and documented its expression in developing skeletal elements. FRZB (also called soluble frizzled related protein-3 (sFRP3)) is a soluble antagonist of the Wnt signaling pathway. Polymorphisms in FRZB are associated with osteoarthritis, including a differential association with osteoporotic fractures of the hip. Furthermore, we show that gene-targeted

deletion of Frzb in mice (Frzb−/− mice) increases articular cartilage loss in models of arthritis triggered by instabililty, enzymatic injury or inflammation. In addition, absence of Frzb in mice reduced exercise performance in treadmill running. Mechanistically, enhanced cartilage loss in Frzb−/− mice is associated with increased Wnt signaling, Matrix Metalloproteinase-3 expression and activity.

Also, Frzb−/− mice show an increased cortical bone thickness and density. This results in stiffer bones as demonstrated by a different stress-strain relationship in Frzb−/− mice as compared to wild-type controls. Moreover, the periosteal anabolic response to mechanical loading is strikingly larger in Frzb−/− mice than in wild-type mice. We suggest that altered biomechanical properties in the absence of Frzb may protect against osteoporotic fractures but may also increase articular cartilage load and loss during locomotion and contribute to osteoarthritis.

Conflict of Interest: None declared

IS24

OSTEOARTHRITIS PROGRESSION AND GENETIC FACTORS

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A number of studies in the last few years have shown unequivocal evidence that at least 50% of the variance of OA in the hands, knees and hips is accounted for by genetic factors. These include classical twin studies of unselected populations as well as population based family studies and affected sib pair studies. A genetic effect on spinal disk degeneration, and spinal osteophytes, has also been demonstrated in twins using MRI data. We have also shown in a recent twin study that the rate of progression of knee OA has a strong heritable basis.

Reports of significant associations for candidate genes for common forms of OA of the knee and hip now include over 50 genes—and over a dozen have now been replicated independently. These include VDR, ERG, CILP, Col2A1, AACT,BMP-2, GFR-5, FRZB, ADAM12, IL-1, IL-1-RA, ASPN, LRCH1, matrillin3, COMP, and OPG. Genome—wide association studies are now showing promising results with the likelihood of many new genes.

In conclusion, OA is a strongly genetic disease, which is likely to be a complex polygenic disorder that may differ genetically by gender site and race. We now have more than a dozen replicated genes and are close to being able to use these clinically for both diagnosis and to predict progression and prognosis. Genes may well be used in combination with traditional blood biomarkers. Understanding how the individual genes influence the many intermediate processes is likely to be a fruitful avenue to provide insight into disease pathways and potential new drug targets. A large EU Framework 7 Consortium (Treat-OA) has been formed for this purpose with access to more than 40,000 samples in a dozen countries.

Conflict of Interest: None declared

IS25

ANIMAL MODELS OF AORTIC CALCIFICATION

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Vascular calcification has long been recognized as a complication of ageing and disease, yet until recently, little attention has been paid to its clinical consequences. In the last years, experimental and clinical studies have revealed important insights into the common pathogenesis of vascular calcification and bone disorders. We need to develop a greater understanding of the bone-vascular axis and the mechanisms leading to differential mineralization of these tissues in ageing and disease. However, there are still many unanswered questions that need to be pursued with animal models.

The generation of knockout mice with targeted deletions of bone-related genes, such as matrix Gla protein, osteopontin, fetuin-A, smad6, klotho or osteoporotegerin have suggested the role of these genes in the calcification process.

The induction of vascular calcifications may be also achieved using different treatments with calcitriol or warfarin. The addition of phosphate or adenine to the diet is another way for obtaining calcifications. A recent study carried out in our group has demonstrated the presence of aortic calcifications in nephrectomized rats with high phosphate-containing diet during 20 weeks. The molecular analysis of the aortic tissue revealed an important decrease in vascular smooth muscle-related genes such as elastin and tropomyosin. By contrast, an increase in osteogenic genes, such as cathepsine K, or genes related with the Wnt signalling, such as secreted frizzled-related proteins, was observed.

All the data supported the idea that animal models may display a combined skeletal and vascular phenotype. Although, the findings in rodents cannot be directly extrapolated to the human osteoporosis/vascular calcification syndrome, these data highlight the need for clinicians to think about this approach that may benefit patients with bone disorders and vascular calcification.

Conflict of Interest: None declared

IS26

MOLECULAR MECHANISMS OF ARTERIAL CALCIFICATION

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Patients with osteoporosis frequently suffer from vascular calcification which was shown to predict both cardiovascular mortality and osteoporotic fractures. Various common risk factors and mechanisms have been suggested to cause both bone loss and vascular calcification, including aging, estrogen deficiency, vitamin D abnormalities, chronic inflammation, renal insufficiency, and oxidative stress. Major breakthroughs in molecular and cellular biology of bone metabolism and animal models with targeted deletion of bone-related genes have led to the concept that common cytokines, signaling pathways, transcription factors, and extracellular matrix interactions may account for both skeletal and vascular abnormalities. For example, mice which lack the osteoclast-regulating factor osteoprotegerin display a combined osteoporosis-vascular calcification phenotype. A unifying hypothesis of vascular calcification will be presented that combines both active and passive mechanisms such as calcium and phosphate imbalances, deficiencies of systemic or local calcification inhibitors, and phenotypic transformation of vascular smooth muscle cells to osteo/chondrocytic cells with aspects of bone metabolism. Under appropriate conditions, cells either residing in the vascular wall (smooth muscle cells) or precursor cells with mesenchymal differentiation potential acquire osteogenic properties. These osteoblast-like cells deposit bone matrix proteins that subsequently become mineralized. In addition, matrix vesicles and apoptotic bodies from calcifying vascular smooth muscle cells form the nidus for calcification, unless physiological inhibitors are present. These inhibitors include fetuin-A, MGP, or osteopontin. In addition, fetuin-A forms soluble "calciproteins" and serves as an opsonin, thus facilitating

phagocytic removal of mineral precipitates. The major protective effect of OPG on the vascular system seems to be related to its potent inhibition of RANKL, thus suppressing osteoclastic release of calcium and other minerals from bone. The link between enhanced bone resorption and vascular calcification is further supported by the findings that other inhibitors of bone resorption (bisphosphonates) have similar effects in animal models of vascular calcification. In this workshop, we discuss the current data and evaluate potential mechanisms of the osteoporosis-arterial calcification syndrome.

Conflict of Interest: None declared

IS27

CLINICAL IMAGING OF ARTERIAL CALCIFICATION

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Early detection of arterial calcifications is of major importance for assessing risk of stroke, coronary heart disease and mortality in general. A large variety of imaging methods have been developed for accurate depiction of calcium load, vessel lumen and functional status. High resolution computed tomography (CT) depicts calcified sections of coronary arteries with excellent image quality. The carotid artery can also well be imaged by ultrasound. Mammography as a special purpose technique is optimized for breast arterial calcifications. Calcifications of the larger vessels, specifically the abdominal aorta can also be visualized with plain X-rays. Recently, it has been reported that Dual-X-ray Absorptiometry (DXA) devices when operated in the lateral imaging mode also allow identification of aortic calcifications. Certain technical requirements need to be met, most importantly the field of view needs to be large enough to encompass the region of interest and signal to noise needs to be adequate. In a first larger systematic comparison Schousboe et al. reported a very good intra-class correlation coefficient of 0.81 (0.66–0.90) between lateral spinal X-rays and lateral DXA spine images. This suggests that despite the much lower radiation dose of lateral DXA image quality may be sufficient. Interestingly, associations between aortic calcification as assessed on radiographs and fracture risk have been reported. Thus lateral DXA spine images can be used for vertebral fracture analysis and for the assessment of aortic calcifications and along with that, for improved fracture risk assessment. When applied to monitoring, the techniques listed above need to meet additional requirements.

A general caveat for imaging of arterial calcifications should be noted. Calcifications generally develop at a relatively late stage of arterial disorders and some of the earlier phases that already impose substantial risks will remain undetected if the focus if left on calcifications alone. The detection of vulnerable plaque (before it becomes calcified) represents one the most important goals for vascular risk assessment and a large variety of imaging methods are being developed for this goal. Still, imaging of vascular calcifications is of high value if used in the appropriate setting. And it is of particular relevance for studying the relationships between bone and vascular disorders.

Conflict of Interest: None declared

IS28

MOLECULAR IMAGING OF BIOCHEMICAL FUNCTIONS USING (SMALL ANIMAL) PET

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Molecular imaging has become a very popular term in medicine. In the literature and at scientific meetings images are presented under the term "molecular" - irrespective of the imaging method (CT, US, MRI, BLI or PET/SPET) and the information gained from the imaging method. However some methods will lead to e.g. structural images, whereas molecular imaging methods make molecular processes visible, quantifiable and track able over time in a living cell, animal or human. Understanding biology at the molecular levels needs molecules (molecular imaging probes), which are part of the biological processes underlying normal or diseased states. The choice of a certain imaging modality depends primarily on the specific questions to be addressed. Answering those questions requires methods with specific properties on spatial resolution, sensitivity and specificity.

Most interactions between physiological targets and ligands (e.g. neurotransmitter and brain receptors or of energy metabolism of metastases) can only be visualized with highest sensitivity that only PET/SPET can provide. If the question concerns monitoring drug distribution, pharmacokinetics and pharmacodynamics for most organs PET (and to a lesser extent SPET) are the only choice as nuclear imaging technique.

A few examples will be given to demonstrate the requirements for a good radiotracer, the merits and limits of the PET and SPECT methodology. One example is about the uptake of 18F-FCNT (2-carbomethoxy-3-/4-chlorophenyl)-8-(2-fluorotropane), a dopamin-transporter ligand in striatum of mice. The striatal degeneration in Parkinson's desease can be visualized non-invasively by small animal PET in a mouse model and used for drug development. A second example demonstrates the human PET imaging of metastatic disease through the metabolic uptake of 18F-FDG (2-fluoro-deoxy-glucose).

And eventually the identification of bone metastases with newly introduced 18F PET imaging compared to clinical routine 99mTc-phosphonate SPET imaging will be compared and discussed.

Conflict of Interest: None declared

IS29

2D AND 3D WHOLE BODY OPTICAL IMAGING AND ITS APPLICATIONS IN BONE RESEARCH

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Recent advances for imaging weak visible light sources using CCCD cameras, peltier cooled detectors and micro-plate channel intensifiers allow detection of photon emission from inside the tissues of small animals. Whole body Fluorescent imaging (FLI) and Bioluminescent imaging (BLI) are now applied to study cell- and tissue specific promoters but also to follow trafficking, differentiation and fate of i.e. GFP/RFP and/or luciferase expressing cells, or processes like tumor progression and metastasis, apoptosis, inflammation, protein-protein interaction, hypoxia and angiogenesis, and gene-transfer. Optical imaging and optical reporter systems are also very cost-effective and time-efficient and they are particularly well suited for small animal imaging. Until recently using firefly luciferase as a reporter, BLI was the most commonly used technology for whole body optical imaging. In Cancer Research whole body optical imaging has allowed semi-quantitative measurements of tumor progression and (bone) metastasis and treatment response.

Limitations of GFP reporter imaging include auto-fluorescence, the requirement of an external light source and the exponentially decreasing intensity of light with increasing depth of the target. However, a new class of red fluorescent proteins and its more red shifted variants (i.e. mCherry, mPlum) as well as the development of

near-infrared dyes and quantum dots that can be coupled to al kinds of ligands, antibodies etc, are providing better deep tissue imaging characteristics (cm's).

Optical imaging has been based on 2D images and, therefore, spatial resolution was poor and quantification difficult and semi-quantitative. 3D optical tomography has now made it possible to better quantify photon emission. In addition, fusing 3D optical images with images obtained from the same animal using CT allows obtaining structural anatomic information and greatly enhances spatial resolution. Furthermore, structural tissue information obtained by fast CT or MRI also allows generating a tissue atlas that can be used to correct for tissue-dependent photon scattering and absorption. This allows for the first time to obtain real quantitative data.

In the present presentation examples will be shown how this new exciting technology can be used in the field of bone research following non-invasively processes like, tumor progression and osteolytic bone metastasis, angiogenesis, bone formation, inflammation, and tissue engineering.

Conflict of Interest: None declared

IS30

CAGED HYPERPOLARIZED XENON AS A TARGETED MRI AGENT WITH HIGH CONTRAST

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The development of optical pumping of xenon by Happer and coworkers provides a high nuclear polarization and thereby a strong signal for NMR detection. Such 'hyperpolarized' signals from xenon and helium have been used for lung imaging, but it has taken some time to develop methods to exploit these noble gasses for other biological applications. We have shown that xenon in solution interacts with proteins, particularly those with internal cavities. Xenon in fast exchange among binding sites has a shift determined by the population weighted average of shifts in all binding sites, and this effect can be used to report on the conformation of a protein even in a complex mixture. However the 'detection threshold' is determined by the ability to reliably measure a small shift. To overcome this limitation we developed cage based xenon biosensors, in which the xenon is in slow exchange with dissolved xenon. Detecting the presence of the caged xenon requires detection of the shifted signal, which can be done with much higher sensitivity than measurement of shifts. The remarkable sensitivity of the xenon shift to the surroundings makes it possible to detect binding of ligands attached to the cage conjugates, increasing the sensitivity of detection. Using direct detection of the xenon signals we can detect molecules at the 100 nM level. Recently we have exploited chemical exchange of the caged xenon with bulk dissolved xenon, an approach we term HyperCEST, to further improve the detection sensitivity. We have demonstrated spatial imaging of a target protein at micromolar concentration, and detection of the presence of a target at 10 nM concentration. Principles of these approaches, and examples of how they may be applied will be presented.

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Conflict of Interest: None declared

IS31

ROLE OF FGF23 IN PHOSPHATE HOMEOSTASIS AND RELATED DISORDERS

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FGF23 was identified as a responsible gene for autosomal dominant hypophosphatemic rickets/osteomalacia (ADHR). FGF23 was also cloned as a causative humoral factor for tumor-induced rickets/osteomalacia (TIO). These diseases are characterized by impaired reabsorption of phosphate in proximal tubules and inappropriately low serum 1,25-dihydroxyvitamin D [1,25D] levels for hypophosphatemia. In accordance with these clinical characteristics, subsequent studies indicated that FGF23 suppresses proximal tubular phosphate reabsorption by reducing expression levels of type 2a and 2c sodium-phosphate cotransporter in proximal tubules. FGF23 also decreases serum 1,25D at least in part by inhibiting 1,25D production. In addition to ADHR and TIO, overexpression and high circulatory levels of FGF23 have been shown to participate in the development of autosomal recessive hypophosphatemic rickets/osteomalacia (ARHR), X-linked hypophosphatemic rickets/osteomalacia (XLH) and hypophosphatemic disease associated with McCune-Albright syndrome/fibrous dysplasia. The responsible genes for ARHR and XLH are *DMP1* (*dentin matrix protein 1*) and *PHEX* (*phosphate-regulating gene with homologies to endopeptidases on the X chromosome*), respectively, and McCune-Albright syndrome is caused by somatic mutations of the gene coding alpha subunit of Gs protein. However, the mechanism of overproduction of FGF23 in these diseases remains to be clarified. In contrast to these hypophosphatemic diseases, deficient actions of FGF23 cause familial hyperphosphatemic tumoral calcinosis characterized by enhanced tubular phosphate reabsorption and high 1,25D levels. *FGF23* and *GALNT3* encoding a protein involved in the synthesis of O-glycan have been shown to be responsible for hyperphosphatemic tumoral calcinosis. Mutations in both genes cause enhanced processing of FGF23 protein and result in low circulatory full-length FGF23 with biological activity. In addition, it has been recently shown that inactivating mutation of *Klotho* gene also cause hyperphosphatemic tumoral calcinosis and FGF23 level is quite high in the hyperphosphatemic patient caused by the mutation in *Klotho* gene. These results indicate that FGF23 works as a hormone regulating serum phosphate level and FGF23 requires Klotho for its action.

Conflict of Interest: None declared

IS32

FGF-23: BEYOND PI REGULATION

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Maintenance of physiologic phosphate balance is of crucial biological importance, as it is fundamental to cellular function, energy metabolism, and skeletal mineralization. Disturbances in mineral ion homeostasis can affect functional activity of almost any organ system, could influence the aging process, and could eventually affect survival. Fibroblast growth factor-23 (FGF-23) was recently identified as a master regulator of phosphate homeostasis. Genetic ablation of *Fgf-23* from mice ($Fgf\text{-}23^{-/-}$) results in a short lifespan with numerous biochemical and morphologic features consistent with human premature

aging. The close resemblance of the phenotype to the *klotho* null mutants suggested a common signaling pathway. In fact, Klotho has recently been found to be required as co-ligand for Fgf-23 to activate the FGFR-1c. To analyze the abnormal phenotype of *Fgf-23*$^{-/-}$ mice we performed various *in vivo* and *in vitro* experiments. Elimination of the vitamin D signaling pathway from *Fgf-23*$^{-/-}$ mice significantly ameliorated the premature-aging like features. Furthermore, we discovered that the apoptosis caused by excessive Vitamin D could be prevented by the activation of signal transduction pathways initiated by FGF-23 and Klotho. We also elucidated the pathophysiological role of upregulation of NaPi2a in renal proximal tubules in *Fgf-23*$^{-/-}$ mice. *Fgf-23*$^{-/-}$/*NaPi2a*$^{-/-}$ double mutant mice showed a complete reversal of serum phosphate levels, however, the skeletal phenotype still resembled the one of *Fgf23*$^{-/-}$ animals suggesting a local effect of Fgf-23 on bone. Our studies show that FGF-23 acts mainly as a hormone to regulate phosphate homeostasis by controlling the expression of Napi2a and by interacting with the calciotrophic hormones, parathyroid hormone and vitamin D, but it also seems to exhibit local actions on bone. Further studies are required to better understand the actions of FGF-23.

Conflict of Interest: None declared

IS33

NEW PHYSIOPATHOLOGICAL PATHWAYS OF BONE METASTASES

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Bone metastases are common complications of several cancers, including carcinomas of the breast, prostate and lung. Bone metastases from breast and lung carcinomas are generally osteolytic, whereas those from prostate carcinoma are most often osteoblastic. Metastatic cells residing in the bone marrow are, on their own, unable to destroy or form bone. Instead, they alter the functions of bone-resorbing (osteoclasts) and bone-forming cells (osteoblasts), leading to the formation of osteolytic or osteoblastic lesions. Drugs, such as bisphosphonates, which bind avidly to bone and inhibit osteoclast-mediated bone resorption, are used extensively to treat patients with bone metastases. Yet, these treatments are only palliative and do not provide a life-prolonging benefit to patients with advanced disease. A better understanding of the molecular mechanisms that precede the overt development of skeletal lesions is therefore required to identify new targets for cancer therapeutics. In this review, we highlight cellular and molecular events that precede the formation of osteolytic or osteoblastic lesions. We focus on genes that draw cancer cells to metastasize to bone. We also present evidence that cancer cells acquire bone-like properties to adapt and thrive in the bone microenvironment.

Conflict of Interest: P.Clezardin, Novartis, Galapagos, Grant Research Supports

IS34

NEW DIAGNOSTIC AND THERAPEUTIC APPROACHES FOR CANCER INDUCED BONE DISEASE

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In many patients metastatic bone disease is a chronic condition requiring multidisciplinary management to optimise individual patient care. Bone metastases result from the interactions between cancer cells in the bone marrow microenvironment and normal bone cells. This leads to stimulation of osteoclastic bone resorption, and provides the rationale for bone-targeted therapies such as bisphosphonates as an adjunct to traditional anticancer agents. New diagnostic modalities include greater use of MRI and PET, while markers of bone metabolism have been shown to provide useful prognostic and predictive information on skeletal morbidity and clinical outcome. Multiple, randomised controlled trials over the past two decades have clearly demonstrated that bisphosphonates are effective in reducing skeletal morbidity from metastatic cancer. Zoledronic acid is the most potent bisphosphonate, and has shown superior efficacy to pamidronate in the prevention of skeletal complications in breast cancer and is the only agent to show clear benefit in the management of metastatic bone disease from prostate cancer (hormone refractory), lung cancer and other solid tumours. Oral agents such as ibandronate and clodronate provide a useful alternative for some clinical situations in breast cancer and multiple myeloma. Quite appropriately, bisphosphonates are increasingly used alongside specific anticancer treatments to prevent skeletal complications. However, bisphosphonates are relatively expensive supportive care drugs, and it is simplistic to assume that all patients require the same dose or schedule of bisphosphonate treatment. Recent studies indicate that the risk of skeletal complications is strongly related to the rate of bone resorption. Additionally, clinical benefit from bisphosphonates appears to be related to the effective suppression of accelerated bone resorption. A tailored approach to therapy may be more appropriate, safer and cost-effective. It seems likely that the efficacy of bisphosphonates in metastatic disease has reached a therapeutic ceiling. Recently, the biological importance of the RANK ligand-RANK-OPG system and cathepsin K in bone has been defined. Early phase studies of an antibody to RANK ligand (denosumab) and cathepsin K (odacatinib) have been completed, and phase III development is either underway or planned.

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OC01

LOW BONE MASS AND BLUNTED RESPONSE TO PHYSICAL EXERCISE IN PERIOSTIN KNOCK OUT MICE

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Background: Periostin is an osteoblast-specific factor involved in cell adhesion and recruitment, playing a central role in odontogenesis. Whether the absence of periostin (POSTN) is associated with phenotype expression is not known. We investigated the skeletal phenotype of POSTN knock out (KO) mice and assessed whether the absence of periostin modified the response to mechanical stimulation.

Methods: We characterized bone mineral density (BMD) by in vivo absorptiometry and bone microarchitecture at several skeletal sites by ex-vivo micro-computerized tomography of 12 week-old POSTN KO and heterozygote (HET) male mice compared to wild-type (WT) littermates. In a separate experiment, these mice were submitted to treadmill exercise (EXE) (45 min/day, 5 days/week) during 5 weeks or left untrained (UN).

Results: Trabecular bone fraction (BV/TV) and number (TbN) in jaw alveolar bone was lower in POSTN KO mice compared to WT (−31% and −24% respectively)

Conclusions: These results demonstrate that the absence of periostin causes profound alterations of bone microarchitecture throughout the whole skeleton including the jaw. Furthermore periostin haploinsufficiency is associated with decreased trabecular bone volume density and completely blunts the skeletal response to physical exercise, indicating that periostin may be implicated in mechanotransduction.

Conflict of Interest: None declared

OC02

INVOLVEMENT OF TWO MATRIX PROTEINS IN BONE HEALING: OSTEOPONTIN AND BONE SIALOPROTEIN

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Bone fracture healing involves a well characterized cascade of events controlled by chemotactic factors, growth factors, direct cell communications and bone matrix proteins. Our study is focused on two major non-collagenous bone matrix proteins belonging to the Small Integrin-Binding LIgand N-linked Glycoprotein (SIBLING) family: osteopontin (OPN) and Bone Sialoprotein (BSP). OPN promotes angiogenesis, inhibits bone mineralization, and regulates osteoclast functions. BSP is involved in osteoclast adhesion and differentiation, in matrix mineralization, and mediates endothelial cell migration and angiogenesis. Recently BSP has been reported as a positive modulator of osteoblast activity. Together, these data suggest that BSP and OPN could play a role in bone fracture healing.

To investigate the involvement of these proteins during the cortical bone healing, we used mice knocked-out for the OPN or BSP genes (S.Rittling ; J-E AUBIN). We developed a new model of bone healing in mice which consists in a 0.9 mm diameter cortical defect in the femoral diaphysis. Micro-computed tomography was used to quantify mineralization at 14 and 21 days. Histology (at 14 and 21 days) and quantitative RT-PCR (at 10 days) were used to evaluate cellular functions related to ECM formation, bone formation and remodelling.

Using micro-CT, we have demonstrated in BSP−/− mice a significant delay of bone healing as shown by a slower decrease of the Bone Volume fraction (BVF) and of the Tissue Mineral Density (TMD) of new bone within the defect when compared to WT mice. At 10 days, RT-PCR analysis reveals a lower induction of osteocalcin and collagen 1a mRNA in BSP−/− mice compared to the BSP+/+ mice. These preliminary results suggest that the delay in bone healing observed in BSP−/− mice could be due to a decreased osteoblast activity.

In contrast, only the female OPN−/− mice show an advanced bone healing, at 14 days, contrary to the OPN+/+ animals. This effect could be explained by an early differentiation of OPN−/− osteoprogenitor cells. To complete these results, histomorphometric analyses are in progress.

In summary, our results reveal that during bone healing the two matrix proteins have antagonistic roles: OPN seems to be an inhibitor of cortical bone healing whereas BSP looks like an activator. The effects of the knock-out are more marked at 14 days. Furthermore, the phenotype of KO OPN during bone healing stays very limited whereas the BSP−/− mice have a marked phenotype.

Conflict of Interest: None declared

OC03

THE ROLE OF RUNX1 IN SKELETAL DEVELOPMENT

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Mammals have 3 runx genes: *Runx*-1, -2 and -3. The *Runx* genes are highly homologous, and all three recognise the same DNA binding sequence. *Runx2* is essential for osteoblast differentiation, and *Runx2*-ko mice lack bone. *Runx1* is highly expressed in chondro- and osteo-progenitor cells, and in vitro experiments have indicated that *Runx1* is important in the early stages of osteoblast and chondrocyte differentiation. However, as *Runx1*-ko mice are embryonic lethal due to failure of fetal liver hematopoiesis, the role of *Runx1* in skeletal development is currently unknown. Here we describe the bone phenotype of a *Runx1*-ko mouse model with *Runx1* expression rescued in the hematopoietic lineage.

Reactivatable *Runx1* knockout mice (Runx1-LacZ) were generated by the targeted insertion of a loxP-flanked LacZ-stop cassette that ablates the expression of *Runx1*. *Runx1* expression was reactivated in the hematopoietic lineage by crossing with mice expressing Cre under control of the TIE2-promoter (Runx1-Re mice). Gene expression was determined by in situ hybridisation, and the skeleton visualised by μCT scanning and Alcian Blue/Alizarin Red staining.

Unlike Runx1-LacZ embryos, Runx1-Re embryos survived past embryonic day 12 (E12), however, they died at birth. The skeletal cartilage was normal in E17.5 Runx1-Re embryos. Although the Runx1-Re embryos showed a predominantly normal skeleton, they lacked mineralisation centres in the sternum, and showed delayed formation of the occipital bone plates. The bone volume of these structures in heterozygote mice was intermediate, suggesting a gene-dose effect. Hypertrophic chondrocytes were absent in the sternum of Runx1-Re mice, while Sox-9 and collagen type II were highly expressed, suggesting that the cells in the Runx1-Re sternum were proliferating chondrocytes. The WT sternum showed high levels of *Runx1* expression whereas *Runx2* was undetectable, in contrast to the vertebrae and long bones which showed high *Runx2* expression.

In conclusion, *Runx1* is not essential for the development of cartilage and most bone structures. However, absence of *Runx1* results in delayed bone formation of the sternum and the occipital bone plates. *Runx2* and/or *Runx3* may compensate for the lack of *Runx1* during the early development of most skeletal structures. The delayed development of the occipital bones and the sternum suggests that *Runx1* may augment the actions of *Runx2* in these tissues.

Conflict of Interest: None declared

OC04

DLK1/FA1 IS A NOVEL MARKER FOR CHONDROCYTE DIFFERENTIATION DURING ENDOCHONDRAL BONE DEVELOPMENT AND CHONDROGENIC LINEAGE PROGRESSION OF HUMAN EMBRYONIC STEM CELLS

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Delta-like 1/fetal antigen 1 (Dlk1/FA1) is a trans-membrane protein that belongs to Notch/Delta/Serrata family and it is expressed by many mesoderm-derived tissues including cartilage. Here, we studied the stage specific expression of Dlk1/FA1 during endo-chondral bone formation in mice. Dlk1/FA1 was expressed by the mesenchyme condensation at E12.5 and throughout the proliferating zone of the epiphyseal growth plates chondrocytes at E14.5–E16.5 that co-expressed with Sox9 and type IIA procollagen. Dlk1/FA1 gene expression was completely abolished from the hypertrophic chondrocytes expressing type X collagen. This data suggest that dlk1/FA1 specifically marks the chondro- progenitor cells in vivo. We then employed dlk1/FA1 as a surface marker to identify chondro-progenitor cells during human embryonic stem cells (hESC) differentiation, into chondrogenic lineage in vitro. Similar to the

in vivo findings, dlk1/FA1 was expressed during in vitro differentiation of hESC into embryoid bodies (EBs) upon down-regulation of undifferentiated markers e.g. Oct3/4 and in parallel with the expression of mesoderm specific gene markers. In vitro treatment of EBS with activin B, a member of TGF-ß-family, markedly increased dlk1/FA1 expression in association with up-regulating the mesoderm-specific markers (e.g. FOXF-1, MEOX-1, MIXL1, TBX6, KDR and ALX4) in a dose dependent manner. FACS-cell sorted dlk1/FA1+ cells from activin B-treated cells showed a high developmental potentiality toward definitive chondrocyte when cultured as micromass pellets in xeno free system containing TGFß1. In conclusion, we identified dlk1/FA1 as a novel marker of chondrogenesis whose expression specifically marks the embryonic lineage progression of endochodral bone formation from proliferating to prehypertrophic chondrocytes. Tracking dlk1/FA1 expression as chondro-progenitor surface marker provides a novel strategy for designing clinically relevant protocols to direct the differentiation of hESC into chondrocytes in chemically-defined, xeno free culture systems.

Conflict of Interest: None declared

OC05

YOUNG DLX5 HETEROZYGOUS MALE MICE DEVELOP REDUCED CORTICAL THICKNESS ASSOCIATED WITH AN INCREASE IN BONE RESORPTION

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Dlx5 is an homeodomain transcriptional factor expressed in osteoblasts during skeletal development. Our previous studies have shown that Dlx5 deficiency in vivo affects bone formation and leads to bone defects in mouse embryo. In vitro, the absence of Dlx5 was responsible for a reduction in osteoblasts differentiation. As Dlx5-null mice exhibit perinatal lethality, the function of Dlx5 in bone modeling/remodeling is still unknown.

In this study we examined the role of Dlx5 in postnatal bone development of Dlx5 heterozygous mice. Dlx5+/− mice and wild-type littermates (WT) were analysed at 10 weeks of age. We characterized the skeletal phenotype using dual-energy X-ray absorptiometry (DXA), micro-computed tomography (μCT) and histomorphometry. Urinary deoxypyridinoline, normalised by the amount of creatinin, was used as a biochemical marker for bone resorption.

Herein, we report that Dlx5 is still expressed at high levels in bones of young adult mice. DXA study of femurs revealed that Dlx5+/− male mice exhibit lower (by 7.8%; $P < 0.05$; $n = 14$) bone mineral density than WT littermates and that females BMD was unaffected. Comparing Dlx5+/− male to WT, μCT analyses showed that the cortical thickness at the midshafts of femurs was significantly lower (14%; $P<0.01$, $n = 13$). Data obtained by histomorphometry on distal femora showed no changes in trabecular structure or remodeling and confirmed a reduction in cortical thickness of Dlx5+/− mice. Unexpectedly, the cortical decrease was the result of an increased marrow diameter with a significantly higher number of endosteal osteoclasts per bone surface. Urinary level of deoxypyridinoline was higher in heterozygous mice (29.7 ± 3.12 nmol; $P<0.05$; $n = 9$) when compared to WT (22.1 ± 1.46 nmol) confirming an increase in bone resorption. Moreover, no expression of Dlx5 was found in osteoclasts differentiated from spleen cells in the presence of MCSF and RANKL in vitro. The phenotype was not due to a deficiency in testosterone level as no significant difference was noticed between the two genotypes.

In summary, our data demonstrate that Dlx5 significantly interferes with long bones remodeling in male and lead to a bone phenotype mainly affecting the cortices. The increase in bone resorption might result from a disorder in the coupling between osteoblasts and osteoclasts. We suggest that Dlx5 may play a role in the development of sexually dimorphic skeleton.

Conflict of Interest: None declared

OC06

MUTATIONS OF SQSTM1 STRONGLY PREDICT DISEASE SEVERITY AND COMPLICATIONS IN PAGET'S DISEASE OF BONE

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Paget's disease of bone (PDB) is a common condition with a strong genetic component. Mutations affecting the Sequestosome 1 gene (SQSTM1) are a common cause of PDB but it is unclear whether they are associated with more severe disease or the development of complications. Here we investigated the relationship between SQSTM1 mutations and complications of PDB in 676 participants of the PRISM study—a large scale clinical trial of PDB management based in the UK. We screened for SQSTM1 mutations by DNA sequencing and analysed the relationship between presence of SQSTM1 mutations, disease severity and complications. Mutations were identified in 72/676 (10.6%) patients overall but the prevalence of mutations was much higher (45.8%) in 103 patients who had a positive family history of PDB. Mutations identified were P392L (72.2%); G425R (8.3%); I424T (6.9%); M404V (6.9%); E396X (1.3%); Q400X (1.3%); M404T (1.3%) and a G425R/I424T compound heterozygote (1.3%). Two of these mutations are novel (Q400X and I424T). Patients with SQSTM1 mutations had an earlier age at first diagnosis than in patients without SQSTM1 mutations (59.0 ± 11.7 vs. 65.1 ± 10.4 years, $p < 0.0001$) and the number of affected bones was significantly greater (7.88 ± 3.4 vs 5.93 ± 2.5, $p < 0.001$). Several complications were more common in SQSTM1 mutation carriers including bone deformity (44% vs 36%); previous fractures (15.2% vs 9.3%); previous orthopaedic surgery (26% vs 15%); and hearing aid use for deafness (8.3% vs 7.1%). We combined information from disease extent with the presence of complications to generate a disease severity score. This showed that SQSTM1 carriers had disease severity which was 30% worse than non carriers (7.9 ± 3.4 vs 5.9 ± 2.5, $p < 0.00001$). We conclude that SQSTM1 mutations are an important cause of PDB in patients attending secondary referral centres in the UK, especially in those with a positive family history. Disease severity is increased and complications are more common in SQSTM1 carriers indicating the need for robust clinical trials to evaluate the effects of early intervention in these patients.

Conflict of Interest: The PRISM study was supported in part by a grant from Proctor & Gamble and Sanofi Aventis

OC07

NOVEL LOCI INFLUENCING MENOPAUSAL AGE AND RISK FOR SURGICAL MENOPAUSE ARE IDENTIFIED BY A GENOME WIDE ASSOCIATION STUDY

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Early menopause is a risk factor for osteoporosis. Age at menopause has a strong genetic component with heritability estimates of about 60% for both natural and surgical menopause. While few candidate gene polymorphisms have been found associated with menopausal age and/or susceptibility to surgical menopause, the genetic risk factors are mostly unknown. Understanding of human sequence variation and recent advances in genotyping technology allow testing millions of common genomic variants in large populations. Genome Wide Association (GWA), a hypothesis-free design, has been successful identifying genetic risk factors for complex traits and diseases. We searched for genetic factors influencing natural and surgical menopause using GWA data from two large population-based cohorts. A sample of 1338 women of the Rotterdam study genotyped with the Illumina 550 K SNP array was analysed for association with age at natural menopause. Risk for surgical menopause was analyzed dichotomously with 347 women with surgical menopause as cases and 1338 women with naturally occurring menopause as controls. PLINK software was used for QC and association testing. Top hits (p-value $<10^{-4}$) in both analyses were tested for in-silico replication in the Twins UK study (Illumina 300 K SNP array); 673 women were studied for age at natural menopause and 1003 for surgical menopause (338 cases and 665 controls). Top hits were 7×10^{-7} (age at natural menopause) and 6×10^{-8} (surgical menopause) and did not replicate. For age at natural menopause 8 of 37 top SNPs tested in Twins UK study were replicated: 4 SNPs mapping to a locus on 19q13.4 ($p \leq 10^{-3}$, combined p-value $\leq 4 \times 10^{-6}$), 2 SNPs on 5q11.2 and 2 SNPs on 8q22 and 16q23 all with $p \leq 0.05$ (combined p-value $\leq 4 \times 10^{-5}$). Effect sizes ranged between −0.69 years to +0.96 years per allele copy, for both studies. For risk at surgical menopause, 3 out of 27 top SNPs were replicated in Twins UK study: 2 SNPs mapping to the 2q14−21 locus (OR:1.4/1.5) and 1 to Xp22.2 (OR:1.8) all with $p < 0.05$. The SNPs on 5q11.2 (r^2 0.78) showed significant association with femoral neck BMD ($p = 0.001$); at least one SNP effect seems to be partly explained by age at menopause. In conclusion, 6 loci associated with age at natural menopause and surgical menopause were replicated across two large population-based cohorts. One locus associated strongly with BMD. Further replication and scrutiny of annotated genes in the identified regions is underway.

Conflict of Interest: None declared

OC08

DOMINANT GENDER-NON-SPECIFIC DIRECT REGULATION OF OSTEOBLASTS AND OSTEOCLASTS BY THE PITUITARY HORMONE OXYTOCIN

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Oxytocin is an hypothalamic hormone exerting a wide spectrum of central and peripheral effects. The multiple hormonal functions of Oxytocin are mediated by Oxytocin receptors expressed in brain and peripheral organs. As we report, Oxytocin receptor is expressed in osteoblasts and also in osteoclast and their precursors. Moreover, we have previously shown OTRs up-regulation in osteoblast and osteoclasts by estradiol-pretreatment, and increased bone cell activity in osteoblast and osteoclast in response of a subsequent oxytocin treatment. In order to study the role of oxytocin in bone cells, and in particular in osteoblasts, we took advantage of an oxytocin deficient in vivo model and studied the cellular bases of oxytocin involvement in osteoblast differentiation and function.

Bone marrow stromal and bone chip cells from Oxytocin−/− mice cultivated in presence of DEXA, ascorbic acid and β-glycerophosphate for 4 weeks exhibited a decrease in nodule formation in comparison with oxytocin +/+ cells, suggesting a defect in osteoblast differentiation. Defective mineralization does not correlate with a decrease in cell proliferation or viability, by contrast oxytocin−/− cells proliferate faster than wild type cells, corroborating the idea of a defect in osteoblast differentiation. Quantitative analyses of osteoblast markers by Real Time PCR showed a decreased expression of Osterix, alkaline phosphatase, Osteopontin and Osteocalcin, correlating with a defective mineralization and osteoblast differentiation. By contrast, expression of an early marker of osteoblast differentiation, Runx2, was higher in Oxytocin −/− cells, suggesting that lack of oxytocin causes a delay in the differentiation program, resulting in more proliferative immature oxytocin−/− osteoblasts. The mechanism of oxytocin involvement in osteoblast differentiation remains to be established, but a novel family of transcription factors regulator of Runx2 activity and BMP2 signaling, Schnurri-1, 2 and 3, could be responsible, since we found a decreased expression of all the three in oxytocin −/− cells. These in vitro data, correlate with in vivo skeletal abnormalities, indeed Oxytocin−/− mice show decreased mineral apposition rate (MAR) and reduced trabecular bone volume (TV/TB).

Conflict of Interest: None declared

OC09

TRIGLYCERIDES, BMD AND PROSPECTIVE FRACTURES IN WOMEN

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Background: Triglycerides in the form of chylomicrons and chylomicron remnants are important carriers of lipoproteins, fatty acids and lipophilic vitamins. These are in turn gaining an increasing attention with respect to anabolic effects on bone formation. We investigated the importance of triglycerides for the future fracture risk and death in a large cohort of women.

Methods: The cohort consisted of 12819 women at with a mean age of 46.8 ± 9.3 years (range 28–64) at baseline. These women attended the Västerbotten Intervention Programme (VIP) which is a health investigation that started in Västebotten County in 1985. At baseline, triglycerides were measured in serum (s-Tg) in a fasting state. Information was gathered about lifestyle factors such as smoking and physical activity. Blood pressure was measured and a 75 gram oral glucose tolerance test was performed. Bone mineral density (BMD) of the femoral neck was measured in a sub cohort of 2081 women, but not at the same time as the VIP examination was performed.

Results: During a mean follow up time of 3056 days (range 3–6264 days) a total number of 1050 validated low energy fractures were recorded in the cohort. In a survival analysis including body mass index (BMI kg/m2), age, smoking habits, physical activity, hypertension, and diabetes, levels of Tg decreased the risk of a future fracture (hazard ratio (HR) = 0.90, p = 0.036). This relationship was stronger when comparing those 1234 women that had hypertriglyceridemia, i.e. levels of Tg of at least 2, with the rest of the cohort (HR = 0.73, p = 0.005). BMD of the femoral neck was associated with s-Tg after adjustment for age (beta = 0.06, p = 0.005). In the total cohort 288 women died during follow-up. S-Tg was an independent risk factor for death in this cohort (HR = 1.19, p < 0.0001).

Conclusion: The novel results of the present study show that high levels of triglycerides are independently associated with a decreased risk of fractures, higher BMD, and an increased risk of death in a large cohort of women.

Conflict of Interest: None declared

OC10

RELATIONSHIP BETWEEN OSTEOCALCIN AND BLOOD GLUCOSE LEVEL IN MEN AND WOMEN—THE UFO STUDY

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A recent experimental study suggested that osteocalcin, a molecule secreted by osteoblasts, is involved in the regulation of glucose metabolism by improving eta-cell proliferation, insulin secretion and insulin sensitivity.

Our aim was to investigate whether serum levels of osteocalcin are related to blood-glucose in older men and women, participating in Umeå Fracture and Osteoporosis study (UFO).

The UFO study is a nested case-control study investigating associations between bone markers, lifestyle and osteoporotic fractures and is based on the Northern Sweden Health and Disease Study (NSHDS), consisting of blood samples and lifestyle data from around 100.000 subjects attending an intervention programme in the county of Västerbotten (VIP). The NSHDS cohort was merged with a prospective fracture database, capturing all osteoporotic fractures occurring in the county during 1993–2004. We identified 158 subjects (124 women, 34 men) as having donated blood before they sustained a hip fracture. Each case was compared with two controls identified from the same cohort and matched for age, gender and month of sample collection; yielding a total cohort of 474 subjects with a mean age at the time for sample collection of 60.1 years (range 49.3–74.3). Osteocalcin was measured in serum at baseline and a glucose tolerance test was performed, measuring glucose in a fasting state (FPG) and 2 h after a glucose load (2 hPG).

In this cohort we found no association between future fracture risk and FPG, 2 hPG, or osteocalcin measured at baseline. However, osteocalcin was negatively related to FPG (beta = −0.19, P = 0.006). The relationship remained significant after adjustments for age, BMI and current physical activity (beta = −0.17, P = 0.03). In our population, 34.8% had impaired fasting glucose (FPG ≥ 5.6 mmol/l) and 9.4% had impaired glucose tolerance (IGT) (2 hPG ≥ 8.9 mmol/l). The frequency of impaired FPG was 16.4% in the quintile with the highest osteocalcin levels (> 35 mug/l) compared to 55.5% in the quintile with the lowest levels (<21 mug/l) (P = 0.001 for comparison). No significant difference was found in the frequency of IGT.

In summary, we found no association between osteocalcin, blood glucose and future fracture risk. However, this study shows that high osteocalcin levels are associated with lower fasting glucose levels in older subjects, supporting the results from an experimental study that osteocalcin seems to be involved in glucose homeostasis.

Conflict of Interest: None declared

OC11

DENOSUMAB INCREASES TOTAL, CORTICAL, AND TRABECULAR BMD OF THE FOREARM AS MEASURED BY QCT IN POSTMENOPAUSAL WOMEN WITH LOW BMD

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Denosumab, a fully human mAb against RANKL, decreases bone turnover and increases areal bone mineral density (aBMD) by dual X-ray absorptiometry (DXA) at mixed and primarily cortical skeletal sites in postmenopausal women with low BMD. Here we describe the effect of denosumab on BMD in the total, cortical, and trabecular bone compartments of the distal region of the forearm as evaluated by quantitative computed tomography (QCT) using standard whole body spiral CT scanners in a phase 3 study. This 2-year randomized, double-blind trial was conducted in 332 postmenopausal women with DXA T-scores between −1.0 and −2.5. Subjects were randomized (1:1) to receive subcutaneous injections of denosumab 60 mg every 6 months or placebo. Randomization was stratified by time since menopause (≤ 5 years and > 5 years). The left forearm was scanned at baseline and at months 1, 6, 12, and 24 by QCT and DXA. Values are reported as least squares mean [95% CI]. As observed previously, denosumab administration increased aBMD at the 1/3 radius; aBMD was increased over placebo by 3.5 [2.8, 4.3]% (P < 0.0001) at 24 months. In addition, QCT measurement showed that denosumab significantly increased forearm BMD by 2.6 [1.5, 3.8]%, relative to placebo at the total region. Significant increases in the cortical (1.7 [1.1, 2.3]%) and trabecular (9.4 [1.1, 17.6]%) compartments were also seen. These positive changes in BMD were associated with significant improvements in bone geometry and derived indices of bone strength, including cortical thickness and total polar moment of inertia (P < 0.001). Similar trends were achieved in both time-since-menopause strata. In conclusion, denosumab significantly increased aBMD and both cortical and trabecular BMD at the distal radius and improved bone geometry and strength parameters. These data confirm and expand on the observation that DXA BMD at the distal radius improved with denosumab administration, a potentially differentiating outcome from existing therapies for osteoporosis.

Conflict of Interest: H Genant: Synarc, Shareholder; Amgen, Ad Board
K Engelke: Synarc Employee
M Omizo: Amgen, Res Grants and Ad Boards
J Brown: Amgen, Research Support and Consultant
H Bone: Amgen, Investigator and Consultant
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OC12

THIAZOLIDINEDIONE USE AND OSTEOPOROTIC FRACTURE RISK

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Background: Thiazolidinediones are increasingly used in patients with type 2 diabetes. Recent studies suggest that thiazolidinediones may adversely affect the skeleton with decreased bone formation and accelerated bone loss; however, data on whether thiazolidinedione use is associated with an altered fracture risk are limited. Based on the UK General Practice Research Database (GPRD) we examined the relationship between thiazolidinedione use and the risk of osteoporotic fracture.

Methods: Nested case-control analysis using the UK General Practice Research Database (GPRD). The study included 1020 case patients aged 50 to 89 years with an incident fracture diagnosis between 1994 and 2005, and 3728 controls, matched to cases on age, sex, calendar time, and general practice attended. The Odds ratios (OR) of having a fracture in association with use of rosiglitazone, pioglitazone, other oral antidiabetics or insulin were calculated.

Results: During the observation period, 1020 incident low-trauma fractures occurred. After adjustment for age, body mass index, diabetic complications, other comorbidities and co-medication (including other antidiabetic drugs), the OR for long-term (> 12 months) thiazolidinedione use was 2.43 (95% CI 1.49–3.95). Both rosiglitazone (OR 2.38, 95% CI 1.39–4.09) and pioglitazone (OR 2.59, 95% CI 0.96–7.01) were associated with an increased fracture risk, predominantly at the hip and wrist. The association was independent of the patients' age and gender and tended to increase with higher thiazolidinedione doses used. No materially altered fracture risk was found in association with use of other oral antidiabetic drugs.

Conclusion: The current case-control analysis provides further evidence for an increased fracture risk, particularly of the hip and wrist, associated with long-term use of thiazolidinediones.

Conflict of Interest: None declared

OC13

FOR HOW MANY YEARS CAN FRACTURE BE PREDICTED BY BONE TURNOVER MARKERS: A 7.5-YEAR FOLLOW-UP STUDY OF 1040 ELDERLY WOMEN

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Background: High levels of bone turnover markers (BTMs) are associated with increased risk for fracture. The information on how many years the prediction will last is more limited and the aim of this study was to prospectively evaluate the influence of follow-up time on the prediction of fractures by BTMs in the elderly. Methods: Fracture prediction was studied in a population-based random sample of 1040, 75-year-old women (the Malmö OPRA study). Six BTMs (S-TRACP5b, S-CTX-I, S-OC[1–49], S-TotalOC, S-bone ALP, urinary osteocalcin (U-OC)) were measured at baseline. Prospective fractures were recorded and verified 7.5 years after baseline. The ability of BTMs to predict fracture was estimated by Cox proportional Hazard ratios (HRs) and analyzed for three different lengths of follow-up, namely 2.5, 5 and 7.5 years. Results: During 7.5 years of follow-up, 314 women (30%) sustained at least one fracture of any type. Both formation and resorption markers were able to predict fractures sustained during the first 2.5 years from baseline. HRs (per SD) were from 1.13 (95% CI 0.95–1.36, S-boneALP) to 1.37 (1.10–1.70, S-CTX), and significant for all markers, except S-boneALP and U-OC. HRs decreased when the follow-up time was extended, and for 7.5-year follow-up were significant only for S-CTX and S-TRACP5b. BTMs were not able to predict only those fractures sustained between 2.5 and 5 or between 5 and 7.5 years. We also separately analyzed clinical vertebral fractures (87 women). All BTMs were able to predict vertebral fractures sustained within 2.5 years from baseline, with HRs from 1.44 (1.00–2.08, S-TotalOC) to 1.69 (1.08–2.63, S-CTX). In contrast to any fractures, HRs for vertebral fractures were statistically significant for most BTMs also when the follow-up was extended to 5 or 7.5 years. For S-CTX, HR for 5 and 7.5 year follow-ups were 1.61 (1.17–2.23) and 1.57 (1.20–2.06), respectively. Furthermore, most BTMs were predictive also for those vertebral fractures sustained only between 2.5 and 5 years (for S-CTX HR = 1.91). Conclusions: Increased levels of BTMs, particularly resorption markers, are associated with increased probability of fracture, particularly of vertebral fracture. Association to fracture risk is most pronounced for the fractures sustained within a few years after BTM measurement but remains significant at least for vertebral fractures sustained up to five years.

Conflict of Interest: None declared

OC14

PARENTAL HEIGHT AND CHILDHOOD MILK INTAKE AT 4 YEARS ARE ASSOCIATED WITH CATCH UP BONE MINERAL ACCRUAL IN EARLY CHILDHOOD

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Peak bone mass is a major determinant of osteoporosis risk in later life and is strongly influenced by patterns of post-natal growth. These patterns, in turn may be influenced by skeletal mineralization at birth and subsequent catch up or down. In this study we explored the parental and childhood determinants of skeletal growth relative to peers in 4-year old children.

Children were recruited from offspring in the Southampton Women's Survey (SWS), a longitudinal study of women aged 20–34 years, who were characterised in detail before and during pregnancy. The children underwent assessment of diet, lifestyle and health by questionnaire, anthropometry and DXA measurement of whole body bone mass (Hologic Discovery using paediatric software). 254 children (130 boys) had also undergone DXA assessment within 16 days of birth. Within group Z-scores for bone area (BA), bone mineral content (BMC), areal bone mineral density (aBMD) and estimated volumetric BMD (vBMD) were generated at both timepoints and change defined by 4 year Z-score minus birth Z-score, adjusted for the baseline measurement. Catch up or down was defined as a change of +/− 0.67 SD or more from birth to 4 years. Correlation and logistic regression methods were used.

There were strongly statistically significant relationships between indices of bone mass at birth and at 4 years old except for vBMD

(BA: $r = 0.32$, $p < 0.0001$; BMC: $r = 0.38$, $p < 0.0001$; aBMD: $r = 0.15$, $p = 0.002$; vBMD: $r = -0.04$, $p = 0.546$). After adjusting for neonatal BMC, 52 (20.5%) children showed catch up and 59 (23.2%) showed catch down for BMC. The predictors of increased chance of catch up vs down for BA and BMC included maternal height (BMC: OR = 1.07; 95%CI:1.00–1.14, $p = 0.049$), paternal height (BMC: OR = 1.16; 95%CI:1.05–1.28, $p = 0.004$) and childhood milk intake at 4 years (BMC: OR = 7.6; 95%CI:1.4–39.9, $p = 0.02$). The relationships with change in aBMD were weaker and there were no significant predictors of change in vBMD. All associations remained robust after adjusting for gestational age.

Parental height and childhood milk intake predicted chance of alteration of the skeletal growth trajectory in early childhood, after adjusting for birth size. These data are consistent with an interaction between genetic and environmental factors in determining the trajectory of skeletal mineral accrual in early childhood.

Conflict of Interest: None declared

OC15

VERTEBRAL FRACTURES ARE ASSOCIATED WITH INCREASED CORTICAL POROSITY IN MALES WITH IDIOPATHIC OSTEOPOROSIS

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Introduction: In men, vertebral fractures are poorly associated with bone density and both cortical and trabecular micro architectural changes could contribute to bone fragility. Cortical bone is a major determinant of bone strength in males. Bone histomorphometry gives the opportunity to investigate not only cortical thickness but also cortical porosity that was reported to be an important contributor to bone biomechanical properties. We therefore conducted a transversal study to investigate cortical structure in men with or without vertebral fractures (VFX).

Patients and methods: We selected 93 bone biopsies of male patients with idiopathic osteoporosis (defined as a T-score <-2.5), 40 to 70 years old. Patients were classified into two groups according to the presence ($n = 46$) or absence ($n = 47$) of prevalent VFX. We measured microarchitectural indices in trabecular and cortical bone by histomorphometry at the iliac crest.

Results: Patients with VFX had lower trabecular bone volume (BV/TV: 12.4 ± 3.8 versus $14.7 \pm 3.1\%$ ($m \pm SD$), $p < 0.01$) and higher trabecular separation (TbSp: 871 ± 279 versus 719 ± 151 μm, $p < 0.01$) and marrow star volume (MaSV: 1.617 ± 1.257 versus 0.945 ± 0.466 mm^3, $p < 0.01$). Cortical thickness was identical in patients with or without VFX, but cortical porosity was higher in patients with VFX (6.5 ± 2.6 versus $5.0 \pm 2.0\%$, $p < 0.01$) because of higher mean area of each Haversian canals (8291 ± 4135 versus 5438 ± 2809 μm^2, $p < 0.01$). There was no correlation between any trabecular and cortical microarchitectural parameters. Using a logistic regression model, we evaluated the VFX as a function of the cortical porosity and MaSV, adjusted on age. The odds ratio of VFX prevalence was 4.07 (95% CI 1.25–13.3, $p < 0.02$) for the 3rd tertile of cortical porosity and 3.89 (95% CI 1.19–12.7, $p < 0.02$) for the 3rd tertile of MaSV.

Conclusion: Our data show that both trabecular and cortical bone structures contribute independently to vertebral fractures in men with idiopathic osteoporosis. By contrast to data reported in women, cortical porosity but not cortical width is associated with vertebral fractures in men. This suggests that cortical deficit is different in men and women with fragility fractures.

Conflict of Interest: None declared

OC16

REGULATION OF T CELL FUNCTION BY OSTEOCLASTS

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The bone marrow (BM) is a unique microenvironment where a tight network of stromal cells, osteoblasts and osteoclasts (OCs) is in close proximity with immune cells. Among these, T cells have been recognized as key players in the maintenance of bone homeostasis and in models of pathological bone loss such as rheumatoid arthritis or post-menopausal osteoporosis. Although subsets of antigen-experienced, memory T cells are known to nest to the bone marrow, whether bone cells can retain and locally regulate T cells is still unclear. As OCs arise from antigen-presenting (APC) circulating progenitors, we asked whether mature OCs can retain and regulate T cells. CD11b+ cells were isolated from human PBMCs and OCs were obtained in vitro upon stimulation with M-CSF and RANKL. The question of whether OCs are immunocompetent cells was then approached through gene expression profiling by Real Time PCR, immunohystochemical staining and functional assays. Mature osteoclasts were compared to immature CD11b+ monocytes and Mesenchymal Stromal Cells (MSCs) for the expression of MHC-II (chain HLA-DR) molecules and key costimulatory molecules involved in T cell regulation. MHC-II was found to be expressed at high levels in CD11b+ cells; interestingly, after OC maturation MHC-II expression is only slightly down regulated but still sustained in TRAP+, multinucleated OCs. Remarkably, mature OCs showed a strong MHC-II staining by immunohystochemistry, a unique property of APC cells. Furthermore, immune co-receptors CD80 ($p < 0.001$), CD40 ($p < 0.005$) and PDL-1 ($p < 0.001$) were all significantly upregulated at the mRNA level upon OC differentiation. MSCs used as a control showed very low or undetectable levels of these markers. In functional adhesion assays, OCs showed a 2.5-fold ($p < 0.01$) increased ability to retain T cell as compared to MSCs. Multiplex analysis of T cell attracting chemokines in cell culture supernatants showed significantly higher levels of chemokines IP-10, MIG and MIP1a in OC supernatants compared to MSCs. Also, OCs blunted T cell proliferation in antigen-independent, polyclonal T cell stimulation by PHA or anti-CD3 in co-culture experiment. Thus, OCs are fully immunocompetent cells with the ability of retain and regulate T cell response in the local microenvironment.

Conflict of Interest: None declared

OC17

HIGH BONE MASS AND DECREASED OSTEOCLASTOGENESIS IN MICE NULL FOR INTERLEUKIN-15 RECEPTOR

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T lymphocytes play a major role in inflammatory bone resorption. Mouse models of T cell deficiency, such as nude mice, may be protected against estrogen-deficient bone loss, but in absence of a

high bone mass phenotype in these mice, the role of T cells in the physiological regulation of bone remodelling remains uncertain. Here we evaluate the influence of IL-15 signaling, a master T-cell growth factor, on the skeleton of intact and ovariectomized (OVX) mice.

The bone phenotype of mice null for the interleukin-15 receptor alpha subunit (IL-15Ralpha−/−) and their wild-type (WT) littermates was evaluated by X-ray absorptiometry, ex vivo micro-computed tomography and histomorphometry. Serum levels of the osteoclastic marker TRACP5b were measured, M-CSF/RANKL-induced osteoclastogenesis and Pit formation were evaluated from spleen and bone marrow cultures. Functions of T cells and dendritic cells (DC) were also investigated ex vivo and in vitro.

Compared to WT, BMD was significantly higher in adult IL-15Ralpha−/− (Spine, +16%: $p < 0.01$; Femur, +6%: $p < 0.05$). In OVX mice, a significant spine BMD loss was observed in WT ($p < 0.001$), but not IL-15Ralpha−/− mice, so that BMD remained actually higher in OVX IL-15Ralpha−/− than intact WT ($p = 0.006$). BV/TV, trabecular number, thickness and connectivity in vertebrae and/or distal femur, and femur cortical bone volume and thickness were all significantly higher in IL-15Ralpha −/− than WT mice ($p < 0.05$ by 2F-ANOVA in both intact and OVX groups). The number and surface of osteoclasts on trabecular bone surfaces was 40% lower, and serum TRACP5b 60% lower in IL-15Ralpha−/− compared to WT mice (both $p < 0.05$). The percentage of osteoclast precursors (c-kit+) and the No of TRAP+ cells from spleen, as well as the activity of bone marrow-derived osteoclasts on dentine slices were all significantly decreased in IL-15Ralpha−/−. T cell proliferation, production of IFN-gamma and RANKL, and antigen presentation by dendritic cells were deeply altered in IL-15Ralpha−/− mice.

In conclusions, impaired T-cell and osteoclastogenic functions in IL-15Ralpha−/− mice lead to a high bone mass phenotype that is predominant on loss of estrogen. Antagonizing the IL-15 pathway may represent a novel therapeutic approach to improve bone mass in primary and secondary osteoporosis.

Conflict of Interest: None declared

OC18

CANNABINOID RECEPTOR ANTAGONISTS INHIBIT OSTEOCLAST FORMATION IN VITRO AND OVARIECTOMY-INDUCED BONE LOSS IN VIVO THROUGH THE CB1 AND CB2 RECEPTORS

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We have recently shown that the Cannabinoid receptor 1 (CB1) antagonists are potent inhibitors of osteoclast formation and bone resorption *in vitro* and *in vivo*. However others have shown that the CB2 selective agonist HU308 inhibits osteoclast differentiation, stimulates osteoblast differentiation and partially protects against ovariectomy-induced bone loss in mice. Here we investigated the effects of pharmacological activation and blockade of cannabinoid receptors on osteoclast (OCL) formation and bone resorption *in vitro* and *in vivo*. The CB2-selective antagonist AM630 and the CB1-selective antagonist AM251 significantly inhibited OCL formation, actin ring formation and bone resorption in OB-bone marrow (BM) mouse co-culture. Both AM630 and AM251 inhibited OCL formation in RANKL-stimulated BM cultures indicating a direct effect on OCL and their precursors (IC50 0.22 μM for AM630 and 0.64 μM for AM251). Conversely, the CB2 selective agonists JWH133 and HU308 stimulated OCL formation (EC50 0.01 μM for both JWH133 and HU308) and partially reversed the inhibitory effects of AM630 on OCL formation. Further studies showed that OCL derived from CB2 knockout mice were resistant to the inhibitory effects of the CB2 selective antagonist AM630 at concentrations lower than 100 nM, therefore indicating a CB2 mediated effect at this concentration range. However, the CB2 selective antagonist AM630 was equally effective on OCL cultures generated from both wild type and CB2 knockout cultures at concentrations from 300 nM and above, therefore indicating a CB2 independent effect at this concentration range. Neither AM630 nor AM251 affected OB viability, alkaline phosphatase activity, collagen production, osteocalcin production and bone nodule formation. Ovariectomy induced bone loss was prevented in wild type mice by AM630 at doses of 0.1 mg/kg and 1.0 mg/kg and histomorphometry showed that this was mediated by inhibition of osteoclastic bone resorption with no change in bone formation. We also studied the effects of AM630 in CB2 knockout mice. These mice were resistant to the protective effects of AM630 on ovariectomy induced bone loss at 0.1 mg/kg, but were protected at the higher dose of 1 mg/kg, indicating a non-CB2 mediated effect. We conclude that the CB1 and CB2 receptors regulate osteoclast formation *in vitro* and bone loss *in vivo*. Our studies demonstrate that CB2 and CB1 receptor antagonists are potential anti-resorptive drugs that could be used in the treatment of osteoporosis and other bone diseases associated with increased osteoclast activity.

Conflict of Interest: None declared

OC19

TRANSCRIPTION FACTOR CCAAT/ENHANCER BINDING PROTEIN (C/EBP) BETA ISOFORM RATIO REGULATES OSTEOCLASTOGENESIS AND BONE HOMEOSTASIS

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The transcription factor CCAAT/enhancer binding protein beta (C/EBPbeta) regulates proliferation and differentiation in many mammalian cell types. Data obtained from transgenic mice and cell culture experiments indicated that C/EBPbeta might also play a role in bone cells, however, its precise function remained still unclear. The C/EBPbeta transcript gives rise to short (LIP) and long (LAP*, LAP) protein isoforms due to alternative translation initiation controlled by the mTOR pathway. We found that during ex vivo cell cultures of primary osteoblasts C/EBPbeta protein isoform levels increased, whereas they decreased during differentiation of bone marrow cells into osteoclasts, suggesting functions of C/EBPbeta in both bone cell types. A genetic approach was used to address the function of C/EBPbeta and its isoforms in bone. Histomorphometric analyses of tibiae of C/EBPbeta mutant mice showed that distinct C/EBPbeta isoforms differentially affect osteoclastogenesis and osteogenesis in vivo. Osteoblast function was reduced in C/EBPbeta null mice, but enhanced in mice that produce only the LIP isoform from their endogenous C/EBPbeta locus (LIP knock-in). Mice lacking all C/EBPbeta isoforms or LIP knock-in mice, both exhibited an increased osteoclastogenesis. Ex vivo bone marrow cell cultures stimulated with M-CSF and RANK-L confirmed the formation of larger and more active osteoclasts from both mutant strains. This suggested that lack of the long C/EBPbeta isoform(s) caused the

enhanced osteoclastogenesis. Indeed, ectopic expression of the long isoform LAP in the monocytic cell line RAW264.7 strongly inhibited osteoclastogenesis upon RANK-L stimulation, whereas LIP robustly stimulated the formation of very large multinucleated osteoclasts. Rapamycin, an inhibitor of mTOR signaling that increases the LAP to LIP ratio, blocked osteoclast differentiation from wild type osteoclast precursors in ex vivo cell cultures, but not from LIP knock-in or from C/EBPbeta null osteoclast precursors. This showed that C/EBPbeta mediates the inhibitory effect of rapamycin on osteoclastogenesis. In summary, these data identify the LAP isoform of C/EBPbeta as a novel inhibitor of osteoclast differentiation and demonstrate that the C/EBPbeta isoform ratio regulates osteoclastogenesis and bone homeostasis. Our results imply the mTOR pathway and the pharmacological adjustment of C/EBPbeta isoform ratio as an approach in the treatment of various bone diseases.

Conflict of Interest: None declared

OC20

SMALL MOLECULE INHIBITORS OF IKK-DEPENDENT SIGNALLING INHIBIT OSTEOCLAST FORMATION IN VITRO AND OVARIECTOMY-INDUCED BONE LOSS IN VIVO

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The activation of NFκB signalling pathway is vital for osteoclast formation and bone resorption. Inhibitors of IκB kinase (IKK) exhibit anti-inflammatory properties and have been shown to inhibit the development of inflammatory arthritis in mice. However, the effects of these compounds on bone metabolism are still unknown. Here we studied the effect of the inhibitors of IKK, Celastrol (IKKα/β and TAK-1 inhibitor), BMS345541 (IKKα/β inhibitor), Parthenolide and Wadelolactone (selective IKKβ inhibitors) on osteoclast (OC) formation in vitro and ovariectomy-induced bone loss in vivo. All compounds tested inhibited IL1β-stimulated OC formation in the mouse bone marrow osteoblast co-cultures and IL1β-induced activation of IκB in osteoblasts. The order of potency in inhibiting OC formation and IL1β-induced IκB activation was: Celastrol > BMS345541 > Parthenolide > Wadelolactone. All four compounds also inhibited RANK-L-induced OC formation (IC50 0.03 μM for Celastrol; 0.2 μM for BMS345541; 2 μM for Parthenolide and 10 μM for Wadelolactone), indicting a direct inhibitory effect on OC and their precursors. Pretreatment of mature OC or their precursors with Celastrol (0.5 μM), BMS345541 (2 μM) and Parthenolide (10 μM) for 1 hour prior to stimulation with RANK-L (100 ng/ml) completely abolished the phosphorylation of IκB and prevented NFκB nuclear translocation. We also studied the effects of these compounds on the recruitment of TAK-1 to the RANK receptor, one of the earliest events in this cascade. Celastrol, but not Parthenolide or BMS345541 prevented the recruitment and binding of TAK-1 to RANK. Celastrol (0.5 μM), BMS345541 (2 μM) and Parthenolide (10 μM) also inhibited the phosphorylation of MEK1/2 and ERK1/2 MAP kinase when activated by RANK-L, IL1β and TNFα but not by M-CSF and parathyroid hormone, two pathways which do not involve IKK activation. Both Celastrol (0.5 mg/kg/day) and Parthenolide (5 mg/kg/day) prevented the loss of trabecular bone ($p < 0.05$) following ovariectomy and preserved trabecular number ($p < 0.01$) and thickness ($p < 0.05$) in mice. In conclusion, Celastrol and Parthenolide are potent inhibitors of osteoclast formation and bone resorption that are effective both in vitro and in vivo. This identifies IKK inhibitors as a novel class of anti-resorptive agents which may be of clinical value not only in arthritis but also in the prevention and treatment of diseases characterized by increased osteoclastic bone resorption such as osteoporosis and Paget's disease of bone.

Conflict of Interest: Aymen Idris is a recipient of ECTS/Amgen Bone Biology Fellowship.

OC21

DIFFERENTIAL EXPRESSION OF PERIOSTIN AND TISSUE PLASMINOGEN ACTIVATOR BY MORPHOLOGICALLY DIVERSE HYPERTROPHIC CHONDROCYTE POPULATIONS

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During endochondral ossification, hypertrophic chondrocytes exist in two forms detectable ultrastructurally, 'dark' and 'light' cells. We have recently observed that these cells undergo distinct cell type-specific forms of non-apoptotic physiological death (1). The aims of the current study were to develop a culture system using rat chondrocytes for the study of molecular differences between dark and light cells, and to undertake gene expression profiling of the two populations. Chondrocytes were isolated from femoral epiphyseal cartilage from neonatal rats, and cultured as pellets in the presence of triiodothyronine (T3) in 0.1% FCS. After 14 days, the pellets were examined by light and electron microscopy. The proportion of hypertrophic light chondrocytes was twice that of hypertrophic dark chondrocytes. Hypertrophic light and dark chondrocytes from the T3-treated pellets could be purified by gradient centrifugation. The Affymetrix GeneChip® rat genome array was used to identify differences in gene expression between purified hypertrophic light and dark chondrocytes isolated from T3-treated pellets. A number of genes of interest were selected for confirmation of differential expression by quantitative PCR. These include periostin, osteoglycin and tissue plasminogen activator (TPA), which were shown to be 164-fold, 5-fold and 4-fold (respectively) more highly expressed in dark cells than in light cells. Immunohistochemical studies of rat growth cartilage during postnatal growth confirmed that periostin is selectively expressed by dark (as opposed to light) hypertrophic chondrocytes. Periostin was found to be uniformly expressed throughout the zone of prehypertrophic chondrocytes, but down-regulated in light chondrocytes with hypertrophy. In contrast, TPA staining was undetectable in the pre-hypertrophic zone, and detectable in both light and dark chondrocytes following hypertrophy. Thus, periostin appears to be a marker of dark hypertrophic chondrocytes. The identification in this study of multiple genes that are differentially expressed between light and dark hypertrophic chondrocytes is likely to assist in the elucidation of functional differences between these morphologically distinct populations.

1. Ahmed YA et al. (2007) Osteoarthr Cartilage 15:575–586.

Conflict of Interest: None declared

OC22

HOXA2 MISEXPRESSION IN CHONDROCYTES IMPAIRS THE CELL PROLIFERATION-TO-DIFFERENTIATION CHONDROGENIC SWITCH

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Background: Chondrogenesis is one major differentiation step in skeleton formation, as endochondral ossification gives rise to the bones of spine, limbs and skull basis. Molecular mechanisms leading to adequate ossification remain unclear. Several molecules known to play important roles in chondrocyte proliferation or hypertrophy have overlapping actions and interact with each other. Hox genes were found to be among the most important genes governing embryonic patterning. Hoxa2 is known to induce second branchial arch derivatives patterning, including the hindbrain, the trigeminal nerve and the corresponding skeleton parts. However, little is known about its action during the differentiation processes of those structures. While the Hoxa2 gene is expressed during proliferation of several mesenchymal condensations, it must fade out to allow chondrogenic differentiation. Accordingly, we recently demonstrated that the induced persistent expression of Hoxa2 in mesenchymal cells, normally committed to engage the chondrogenic programme, impedes cartilage development. Others showed that Hoxa2 expression rises during fracture healing, revealing that its action is not limited to the second arch derivatives. The present study investigated expression of signal transducers and transcription factors known to control endochondral ossification in order to determine at what level Hoxa2 interferes with chondrogenic differentiation.

Methods: We used transgenic mice allowing Cre-mediated conditional misexpression of Hoxa2 and induced this gene in Collagen 2 alpha 1 expressing cells (Col2a1-Cre transgenic line) committed to enter chondrogenesis. E13.5 embryos showing induced Hoxa2 expression were collected and simple Col2a1-Cre transgenics were used as controls. Ihh, BAPX1, Wnt5a, Sox9, Sox5, Sox6, RunX2, FGFR3, PTHrP, BMP4 and BMP7 were detected in axial skeleton by immunohistochemistry of sagittal paraffin sections as well as Western blotting of spine samples.

Results: Both immunohistochemistry and Western blotting highlighted a decrease of Ihh, BAPX1, Wnt5a, Sox5, Sox6, FGFR3, PTHrP and BMP7 expression in induced transgenic mice as compared with controls, whereas that of the other markers remained unchanged.

Conclusions: Our data provide evidence that Hoxa2 has a repressive effect on distinct key regulators of chondrogenesis. These results confirm the hypothesis that during skeletal patterning Hoxa2 is required to keep differentiation under control, while its persistent expression in mesenchymal condensation inhibits or delay chondrogenic differentiation.

Conflict of Interest: None declared

OC23

THE MATRIX PROLINE/ARGININE-RICH END LEUCINE-RICH REPEAT PROTEIN (PRELP) INHIBITS OSTEOCLASTOGENESIS INACTIVATING NF-KAPPAB SIGNAL

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PRELP is a matrix protein that binds heparin and heparan sulphate chains of e.g. membrane perlecan and surrounding tissue type I/II collagens. Although ubiquitous, it is highly expressed in cartilage and developing bone. We show that the heparin-binding domain of PRELP (hbdPRELP) irreversibly inhibited mouse bone marrow osteoclast formation and bone resorption, both in the presence and in the absence of osteoblasts, with an effect on late pre-fusion osteoclast precursors and impairment of osteoclast adhesion. Heparinase failed to rescue osteoclast formation in the presence of hbdPRELP, while its effect was blunted by chondroitinase treatment, suggesting the involvement of chondroitin sulphate cell surface proteoglycans. Tagged hbdPRELP co-localised with cell surphace chondroitin sulphates, was internalised in endosomes and transferred to the nucleus with a mechanism dependent on cell activity and abolished by choindroitinase. Internalisation also required annexin II, which co-localised with tagged hbdPRELP and surface chondroitin sulphates, and formed a complex with tagged hbdPRELP revealed by immunoprecipitation. hbdPRELP internalisation was impaired by an annexin II neutralising antibody, but not by an anti-CD44 antibody or by an irrilevant IgG. hbdPRELP recognised nuclear components and formed a complex with p65 NF-kappaB subunit. Nuclear localisation of p65 NF-kappaB was not affected by hbdPRELP, but its transcriptional activity was reduced by 50%, resulting in transcriptional inhibition of the downstream osteoclast-specific genes cathepsin K, calcitonin receptor, metalloproteinase 9, RANK, TRAcP, DC-STAMP and CD44, these latter implicated in osteoclast precursor fusion. Consistently, in hbdPRELP-treated pre-fusion osteoclasts the transcriptional factor NFATc1 was totally cytoplasmic with no nuclear localisation. hbdPRELP did not prevent MAPK phosphorylation, nor did it induce apoptosis, as shown by nuclear staining with DAPI and by the lack of modulation of caspase 3 and Bcl-2/Bax protein expression. Finally, hbdPRELP effect appeared osteoclast-specific, as it failed to affect mouse calvarial osteoblast differentiation, function and expression of osteoclastogenic cytokines as well as tumour cell growth, motility and invasion. We conclude that the heparin binding domain of PRELP is a novel direct negative regulator of osteoclast formation, that reduces NF-kappaB signalling in late stage pre-fusion committed osteoclast precursors.

Conflict of Interest: None declared

OC24

1,25-DIHYDROXYVITAMIN D3 MODULATES DEXAMETHASONE EFFECTS BY CHANGING TH1/TH17 AND TH2 CYTOKINE PRODUCTION BY PERIPHERAL BLOOD MONONUCLEAR CELLS IN EARLY RHEUMATOID ARTHRITIS PATIENTS

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Background: Corticosteroids are commonly used in the treatment of rheumatoid arthritis (RA), but also induce systemic bone loss. 1,25-dihydroxyvitamin D3 (1,25-(OH)2D3) stimulates bone formation and gives a shift from a pro-inflammatory into an anti-inflammatory immune response. It is unknown whether 1,25-(OH)2D3 may reduce corticosteroid induced bone loss by modulation of the cytokine balance in RA patients.

Aim: To study the effects of 1,25-(OH)2D3 and dexamethasone on the production of pro-inflammatory/pro-destructive Th1/Th17 cytokines IFNgamma, TNFalpha, IL-17A and the anti-inflammatory/anti-destructive Th2 cytokine IL-4.

Methods: PBMC from 18 healthy controls and 18 untreated early RA patients were stimulated for 72 hours with antiCD3/antiCD28 in the absence and presence of various concentrations of 1,25-OH2D3, dexamethasone and 1,25-(OH)2D3/dexamethasone combined. IL-17A, TNFalpha, IFNgamma and IL-4 were measured in the supernatants by a specific ELISA.

Results: TNFalpha levels in supernatants of unstimulated PBMC and IL-17A levels in supernatants of stimulated PBMC were higher in early RA patients versus healthy controls. In both groups 1,25-(OH)2D3 and dexamethasone significantly inhibited IL-17A and IFNgamma production. Dexamethasone also significantly inhibited

the levels of TNFalpha. Interestingly, the combination of 1,25-(OH)2D3 and dexamethasone almost completely inhibited IL-17A and IFNgamma production. Furthermore, 1,25-(OH)2D3 induced a three-fold increase in IL-4 and completely restored the IL-4 production that was inhibited by dexamethasone to levels as in supernatants of antiCD3/antiCD28 stimulated PBMC. 1,25-(OH)2D3 decreased TNFalpha/IL-4, IL-17A/IL-4 and IFNgamma/IL-4 ratios, while dexamethasone increased TNFalpha/IL-4 and IFNgamma/IL-4 ratios. The unfavorable effect of dexamethasone on TNFalpha/IL-4 and IFNgamma/IL-4 ratios could be overcome by 1,25-(OH)2D3.

Conclusion: These data show a beneficial effect of 1,25-(OH)2D3 on the Th2 cytokine IL-4 and inhibition of the Th17 cytokine IL-17A. In addition, 1,25-(OH)2D3 has an additional value on the inhibition of IL-17A by dexamethasone and restored the IL-4 production and the negative effect of dexamethasone on TNFalpha/IL-4 and IFNgamma/IL-4 ratios. These data suggest that 1,25-(OH)2D3 may contribute to a bone sparing effect in RA patients using corticosteroids by modulation of the cytokine balance.

Conflict of Interest: None declared

OC25

THE P38 MAP KINASE PATHWAY PLAYS AN ESSENTIAL ROLE IN MEDIATING TRABECULAR BONE LOSS INDUCED BY ESTROGEN DEFICIENCY

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Increased bone remodelling with excess bone resorption over formation is the principal mechanism of bone loss with estrogen deficiency and increased bone fractures. Different mechanisms have been described to explain the enhanced osteoclast activity with estrogen deprivation such as an increased cytokines production by stromal cells and alteration in osteoclasts apoptosis. The p38 pathway is known to be involved in controlling both cytokines effects on osteoclastogenesis and osteoclast activity. Thus, in this study we investigated the effect of a selective p38alpha inhibitor (p38INHIB), on the prevention of bone loss induced by estrogen deficiency in an adult ovariectomized (OVX) rat model.

Oral administration of 15 and 45 mg/kg BW of the p38INHIB for 8 weeks dose-dependently blunted the increase in urinary deoxypyridinolin excretion level, a marker of bone resorption, induced by OVX in adult rats ($p < 0.0001$). Interestingly, the p38INHIB did not reduce but significantly enhanced (2x, $p < 0.001$) the rise in osteocalcin level, a marker of bone formation, observed in OVX animals. Associated with this uncoupling effect on bone remodelling, the p38INHIB completely blunted vertebral bone loss associated with estrogen deficiency. A partial preventive effect was also observed in long bones (68%) with reduction of trabecular bone loss of the tibial metaphysis and enhancement of the cross sectional area of the tibial diaphysis ($p < 0.02$). Finally, prevention of trabecular bone loss and increased in cortical bone thickness were associated with improvement of the trabecular microarchitecture and the biomechanical resistance.

In conclusion, chronic administration of a selective p38alpha inhibitor blunted the increased bone turnover induced by estrogen deficiency and uncoupled bone resorption and bone formation. This effect resulted in a complete prevention of axial trabecular bone loss and alteration of bone microarchitecture induced by OVX. These data suggest that p38 is a critical pathway for regulation of bone resorption in response to estrogen deficiency. Selective inhibitors of this pathway are molecules of potential interest for prevention of bone loss in postmenopausal osteoporosis.

Conflict of Interest: J. Caverzasio, SCIOS Inc, Grant Research Support

OC26

COOPERATIVITY BETWEEN THE C-FOS PROTO-ONCOGENE AND FGF RECEPTOR SIGNALLING IN THE PATHOGENESIS OF BONE AND CARTILAGE TUMOURS

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The c-Fos proto-oncogene and AP-1 transcription factor is involved in the regulation of osteoblast and chondrocyte proliferation and differentiation. Inappropriate c-Fos expression deregulates these processes. Specifically, c-Fos transgenic mice develop bone and cartilage tumours and ectopic expression of c-Fos impairs the ability of osteoblasts and chondrocytes to differentiate. To understand the molecular mechanism of these effects, we screened for genes whose expression is modulated by exogenous c-Fos expression during in vitro chondrocyte differentiation. One gene identified was the Fibroblast Growth Factor Receptor 1 (FGFR1). Induction of exogenous c-Fos expression in chondrocytes and osteoblasts resulted in upregulation of FGFR1 RNA and protein expression and enhanced FGF-2-mediated MAPK signalling in a cell-specific manner, with ERK and p38 signalling specifically enhanced in chondrocytes and osteoblasts, respectively. Consistent with a role for c-Fos modulation of FGF signalling, the FGFR inhibitor, SU5402, rescued the differentiation of chondrocytes ectopically expressing c-Fos.

The inability to undergo terminal differentiation is a critical feature of transformed cells. We therefore investigated if modulation of FGF signalling by c-Fos is involved in its ability to induce transformation and tumour formation. High expression of FGFR1 protein was seen in c-Fos-induced bone and cartilage tumours and c-Fos and FGF-2 cooperate to induce anchorage-independent growth of chondrocytes, with ectopic expression of c-Fos enhancing FGF-2-induced colony formation by two-fold. Furthermore, SU5402 reduced anchorage-independent growth of c-Fos-transformed cells by over 50%. Inhibition of FGFR signalling also interfered with the transformed state of MG63 human osteosarcoma cells, with a 75% inhibition in colony growth in soft agar. That c-Fos and FGF signalling are important in the pathogenesis of human osteosarcomas was suggested by Tissue Microarray Analysis, where over 60% of biopsies expressed high levels of FGFR1, and $\sim$80% of biopsies expressed both c-Fos and FGFR1.

These results identify FGF signalling as a novel target for c-Fos and suggest that modulation of this pathway is involved in c-Fos-induced tumour formation. Moreover, these data suggest a novel role for FGFR1 signalling in the pathogenesis of primary human skeletal malignancies such as osteosarcomas and chondrosarcomas and may present a new therapeutic target for their treatment.

Conflict of Interest: None declared

OC27

KNOCK-IN OF THE P392L MUTATION OF SQSTM1 CAUSES A PHENOTYPE SIMILAR TO PAGET'S DISEASE IN MICE

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Introduction: Paget's disease of bone (PDB) is a common disease with a strong genetic component characterized by focal increases in

bone turnover. Mutations affecting the ubiquitin associated (UBA) domain of the SQSTM1 gene predispose to PDB, but it has been argued that these alone are insufficient to cause the disease and that an additional environmental trigger is required. Here we report upon the generation and characterisation of an animal model for PDB which carries the P392L mutation, which is the most common SQSTM1 mutation causing PDB in humans.

Methods: Mice with a 'knock-in' of the P392L mutation of SQSTM1 gene were generated using gene targeting and bred onto a mixed C57/Bl6-129 background. Using μCT analysis we conducted skeletal phenotyping of 8 and 12 month old mice.

Results: Mice with the P392L mutation developed lytic lesions in the lower limbs with a penetrance of 100% by 12 months in homozygotes ($n = 8$) and 75% in heterozygotes ($n = 4$) as compared to 0% in wild type controls ($n = 5$) ($p < 0.001$ between genotypes). The most severe lesions were observed in homozygous animals. At 8 months lesions were also present, but penetrance was reduced to 70% ($n = 10$) in the homozygotes and 25% ($n = 4$) in the heterozygotes as compared to 0% in wild type controls ($n = 8$) ($p = 0.008$). The lesions were less severe at this age with only 1 severely affected homozygous animal. At both time points lesions were predominantly localised to the distal femur and proximal tibia. Animals with severe lesions usually had only one limb affected. Studies in vitro showed a significant increase in RANKL-induced osteoclast formation in P392L homozygous mutants as compared with heterozygotes and wild type.

Conclusions: Mice carrying the P392L mutation of the SQSTM1 gene exhibit several phenotypic features of human PDB including focal osteolytic lesions, increased lesion severity with age and increased osteoclast formation in vitro. We conclude that the most common P392L mutation of SQSTM1 is sufficient to cause a PDB-like disorder in mice confirming that this mutation can cause PDB in the absence of any additional trigger. The mutant mice represent an animal model of PDB and will be a valuable resource to investigate disease mechanisms and to explore new treatment strategies.

Conflict of Interest: None declared

OC28

DIETARY CALCIUM DEFICIENCY INCREASES OSTEOLYSIS AND MODIFIES GENE EXPRESSION PROFILE IN AN ANIMAL MODEL OF MULTIPLE MYELOMA

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It is not clear why indolent Multiple Myeloma (MM) in patients could turn into an aggressive MM with a poor prognostic. Bone microenvironment has a key role in the development of tumor growth and osteolysis in MM. In a mouse model of MM, it was shown that a pre-existing high bone turnover due to ovariectomy dramatically increased MM cells growth with a faster development of osteolysis (Bone 2001, 33, 283–292). The altered microenvironment was able to select a more aggressive cell line (5THL) (BBRC, 328, 679–687). Here, we investigate the effect of a dietary calcium deficiency on tumor growth, osteolysis and gene expression in the 5THL murine model of MM. Two groups of mice were injected intravenously with 1.5 106 5THL cells and commenced a diet with a normal (0.8% Ca+MM+) or low calcium content (0.05%, Ca-MM+). Two other groups were not injected with MM cells but received either a normal or low calcium diet (control Ca+, control Ca−). Tumor growth was controlled by measurement of the paraprotein, osteolysis was assessed by X-ray and microCT, gene expression of the Wnt pathway and RANKL was monitored in the bone marrow by qPCR at 6, 8 and 10 weeks. In control Ca−, serum PTH was slightly increased after 10 days and microCT showed trabecular bone loss on the tibia and a significant decrease of cortical thickness (−28%, $p < 0.0001$). In the MM+ groups, the paraproteinemia was detected at 6 weeks in both groups. At 10 weeks, paraprotein level was significantly higher in the Ca-MM+ (17.3% vs 10.3%, $p < 0.05$). 4 out of 9 mice developed paraplegia at 8/9 weeks whereas no paraplegia was observed after 10 weeks in the Ca+MM+. Numerous bone lacunae in the long bones were observed in both groups with a higher decrease in cortical thickness in Ca-MM+. MicroCT performed on the vertebras L4 to L1 showed higher bone destruction In Ca-MM+. 3D measurements showed a significant decrease of BV/TV (%) (Ca+MM+: 16.8 ± 4.8, Ca-MM+: 7.3 ± 2.2, $P < 0.001$). qPCR revealed no difference in the expression of RANKL whereas differences were obtained in genes involved in the Wnt pathway. Among them, LRP5 and LRP6 were significantly 2.9 and 2.7 lower expressed at 6 weeks in Ca-MM+. In conclusion, a low calcium diet induced a modification in the expression profile of the MM with higher bone destruction both in the long bones and vertebrae. qPCR revealed an earlier decrease in bone formation level rather than a higher bone resorption level.

Conflict of Interest: None declared

OC29

GENDER-SPECIFIC CONTROL OF PEAK BONE MASS BY THE WNT PATHWAY: ANDROGEN SIGNALING PROTECTS AGAINST LEF1 HAPLOINSUFFICIENCY-INDUCED BONE LOSS

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Wnt signaling, which is critical for osteoblast function and bone mass control, is implicated here in the increased bone mass accrual in young males versus females. We investigated the role of *Lef1*, one of the four transcription factors that transmit Wnt signaling to the genome, in bone mass control *in vivo*. Because *Lef1*-deficient mice die perinatally, we analyzed peak bone mass accrual in *Lef1* heterozygous mice. Micro-computed tomographic analysis of femora and lumbar vertebral bodies revealed decreased bone mass accrual in female $Lef1^{+/-}$ mice. This was attributable to decreased bone formation demonstrated by vital calcein labeling of newly formed bone. Remarkably, male $Lef1^{+/-}$ mice displayed normal bone mass accrual. This is attributable to crosstalk between androgen and Wnt signaling, as suggested by increased *Lef1* expression in osteoblast cultures treated with dihydrotestosterone. Indeed, similar to female $Lef1^{+/-}$, male $Lef1^{+/-}$ mice lacking functional androgen receptor ($Lef1^{+/-}$; AR^{tfm}) also displayed a low bone mass (LBM) phenotype, suggesting that androgen signaling protects against *Lef1* haploinsufficiency-induced LBM. To further test whether the female skeleton is more sensitive to changes in Wnt signaling compared to males, we also analyzed femora of mice haploinsufficient for *GSK3β*, a negative regulator of the Wnt signaling pathway. Indeed, females were substantially more sensitive than males to *GSK3β* haploinsufficiency, displaying, in this case, a high bone mass phenotype. Given the

ubiquity and importance of the Wnt pathway in human health and disease, the crosstalk between androgen and Wnt signaling may contribute to the sexual dimorphism observed not only in the skeleton, but also in many other organ systems.

Conflict of Interest: None declared

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OC30

ROLE OF THE TRANSCRIPTION FACTOR RUNX2 AS A MEDIATOR OF THE ANTI-APOPTOTIC EFFECTS OF PARATHYROID HORMONE (PTH)-RELATED PROTEIN (PTHrP) IN THE DAMAGED KIDNEY

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The N-terminal fragment of both PTH and PTHrP has anti-apoptotic effects in osteoblasts by a mechanism which involves Runx2. This transcription factor is also present in mammary epithelial cells where it might modulate cell survival. We studied here the putative implication of Runx2 in the previously reported anti-apoptotic effects of PTHrP in the damaged kidney. We used unilateral ureteral obstruction (UUO) as a model of renal injury in transgenic mice overexpressing PTHrP in the proximal tubule and their control littermates in vivo, and mouse tubuloepithelial MCT cells in vitro. Changes in mRNA and protein expression were assessed by real-time PCR and Western blot, respectively. Apoptosis was evaluated by TUNEL in vivo, and by propidium iodide staining and trypan blue exclusion, in vitro. We found a higher PTHrP (both mRNA and protein) expression associated with higher Runx2 mRNA levels in the obstructed kidney than in the sham-operated kidney at day 4. Moreover, this increase in Runx2 was significantly higher in PTHrP-overexpressing mice, associated with a decrease in apoptotic tubulointerstitial cells. Administration of either the PTH/PTHrP type 1 receptor antagonist, PTHrP (7–34) (35 mug/day), or the angiotensin II receptor inhibitor losartan (10 mg/Kg per day), that normalizes PTHrP overexpression after UUO, significantly decreased Runx2 overexpression following UUO. In vitro, PTHrP (1–36) and also the C-terminal PTHrP (107–139) fragment, at 10–100 nM, maximally stimulated (1.5–2.0-fold over basal; $p < 0.05$) Runx2 protein expression in mouse tubuloepithelial MCT cells at 24 h. Moreover, both PTHrP fragments, within this dose range, induced Runx2 internalization into the cell nucleus after 6 h (by confocal microscopy) in these cells. PTHrP (1–36) and PTHrP (107–139) increased the expression of the pro-survival proteins Bcl-2 and Bcl-XL, and decreased that of the pro-apoptotic protein Bax, related to a decreased folic acid-induced apoptosis in these cells. Transfection with a Runx2 dominant negative construct significantly inhibited, but not abolished, the anti-apoptotic effect of both PTHrP fragments in these cells; an effect associated with a decrease in Bcl-2 protein expression.

Conclusions: These findings suggest for the first time that both the N- and C-terminal fragments of PTHrP can promote renal epithelial cells survival through a mechanism dependent in part on Runx2.

Conflict of Interest: None declared

OC31

LOSS OF FUNCTION ALPHA10BETA1 MICE EXHIBIT REDUCED BONE VOLUME

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The $\alpha 10\beta 1$ integrin is a major collagen-binding integrin known to be expressed by articular and growth plate chondrocytes during mouse skeletal development and in adult cartilage. Mice deficient in $\alpha 10$ integrin-subunit ($\alpha 10^{-/-}$) have previously been described to display no more than a mild phenotype, with a slight, time progressive shortening of the long bones (7–10%), associated with mild growth plate abnormalities. Due to the known interaction between longitudinal growth and bone mass and architecture, we have hypothesised that loss of $\alpha 10\beta 1$ function will also impact on bone structure and its capacity to adapt to mechanical load-bearing stimuli. Accordingly, tibial bone architecture of male and female $\alpha 10^{-/-}$ mice at three ages (10, 24 and 72 weeks representing young, adult and aged mice, respectively) was assessed via μCT and analysed using two-way ANOVA. Initial screening indicated that there was no significant interaction between age and genotype for any cortical or trabecular bone parameters measured, suggesting that $\alpha 10\beta 1$ does not independently impact on ageing-related changes in bone architecture. Despite this, dramatic modifications were apparent in the bones of $\alpha 10^{-/-}$ mice. Although no change in total tissue area, periosteal perimeter, or endosteal perimeter was detected at the cortical midshaft, there was a marked reduction in bone area in both male ($p < 0.01$) and female ($p < 0.05$) $\alpha 10^{-/-}$ mice characterised by significant cortical thinning (male $p < 0.01$, female $p < 0.05$) that was more pronounced in males. Due to the known age-related decline in trabecular bone volume, trabecular architecture could only be assessed in young and adult mice. Mice deficient in $\alpha 10\beta 1$ were found to exhibit a marked and statistically significant reduction in trabecular BV/TV in both genders (male $p < 0.001$, female $p < 0.01$), with decreased trabecular connectivity (TPf, male $p < 0.001$, female $p < 0.01$). Intriguingly, this reduction in BV/TV was characterised in male mice by a reduction in trabecular number ($p < 0.001$) but in females by a reduction in trabecular thickness ($p < 0.05$). Male, but not female $\alpha 10^{-/-}$ mice also exhibited an increased SMI ($p < 0.001$). Our findings are the first to describe a vital role for the $\alpha 10\beta 1$ integrin in both cortical and trabecular bone architecture, and highlight the possibility that cell:matrix interactions via the $\alpha 10\beta 1$ integrin plays a significant role in the regulation of bone mass.

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A. Pitsillides, RVC, Grant Research Support

OC32

TGF2 BETA PREVENTS OSTEOBLAST APOPTOSIS INDUCED BY SKELETAL UNLOADING BY UPREGULATING ALPHA5/BETA1/PI3K/AKT SIGNALING IN VIVO

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Bone loss in immobilized patients is a growing problem in modern countries in which the aging population is increasing. Immobilized

patients show reduced bone formation and increased bone resorption, resulting in rapid bone loss. The cellular mechanisms involved in this deleterious skeletal effect remain poorly understood. In rats, skeletal unloading decreases bone mass mainly as a result of decreased bone formation. We previously showed that the reduced bone formation induced by hind limb suspension in rats results from decreased recruitment of osteoblast precursors and osteoblast function associated with reduced TGF beta expression in unloaded bone. Recently, we showed that hind limb suspension in rats alters alpha5/beta1 integrin expression in metaphyseal bone, resulting in reduced PI3K signaling and Bcl-2 expression and subsequent osteoblast apoptosis. In this study, we investigated whether treatment with TGF beta2 may prevent apoptotic signals in metaphyseal bone cells in unloaded rats. Histomorphometric analysis showed that hind limb suspension decreased metaphyseal bone volume in tibia at 2–7 days. We showed that bone loss was associated with decreased alpha5/beta1 integrin protein levels and decreased phosphorylated phosphatidyl-inositol-3 kinase (p-PI3K/PI3K) and p-Akt/Akt levels compared to loaded bones. Continuous administration of TGF beta2 using osmotic minipumps prevented the metaphyseal bone loss in tibia at 2–7 days of skeletal unloading. TGF beta2 also prevented the decrease in alpha5/beta1 integrin protein levels and the altered p-PI3K/PI3K and p-Akt/Akt induced by skeletal unloading. In contrast, p-FAK and p-ERK p42/44 levels were not significantly altered in unloaded bone. The reduced PI3K and Akt activity in unloaded long bone was associated with decreased levels of the survival protein Bcl-2 with unaltered Bax levels, causing increased Bax/Bcl-2 levels. Treatment with TGF beta2 prevented the increased Bax/Bcl-2 levels at 4 and 7 days of suspension, resulting in prevention of decreased p-Bad, a target of Akt. Overall, the results indicate that TGF beta2 protects osteoblast apoptosis induced by mechanical unloading by upregulating alpha5/beta1/PI3K/Akt signaling in vivo. This suggests that defective TGF beta signaling in unloaded bone is involved in osteoblast apoptosis and bone loss induced by hypokinesia.

Conflict of Interest: None declared

OC33

ZFP521, AN ANTAGONIST TO RUNX2 AND EBF1 TRANSCRIPTIONAL ACTIVITY, FAVORS BONE FORMATION IN VIVO AND INHIBITS MINERALIZATION IN VITRO

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We recently identified a novel zinc-finger protein 521 (Zfp521) that is expressed in prehypertrophic chondrocytes and in osteoblasts. Overexpression of Zfp521 in primary osteoblasts inhibited osteoblast differentiation but in vivo overexpression of Zfp521 lead to a dramatic increase in osteoblast numbers, bone formation rates and bone mass with no changes in bone resorption (Wu et al., JBMR 20:Supll 1, 2005). We also showed that Zfp521 interacts with Runx2 and antagonizes its transcriptional activity. As Zfp521 also regulates early B-cell factor 1 (Ebf1) activity during B-cell differentiation and Ebf1$^{-/-}$ mice exhibit high bone mass (Hesslein et al., JBMR 20:Supll 1, 2005), we determined whether Ebf1 was a target of Zfp521 in osteoblasts. Retroviral overexpression of Ebf1 in MC3T3-E1 cells and in primary osteoblasts enhanced, whereas overexpression of Zfp521 repressed, osteoblast differentiation. Zfp521 physically interacted with Ebf1 and repressed its transcriptional activity. Ebf1 and Runx2 activated osteocalcin promoter in a reporter assay and Zfp521 was able to suppress both Runx2 and Ebf1 induced transcriptional activity. To better establish the physiological role of Zfp521 in osteoblasts we then analyzed Zfp521 knockout mice. Zfp521$^{-/-}$ mice were severely runted and exhibited a short life span. At 2 weeks of age, the tibias of Zfp521$^{-/-}$ mice were significantly shorter and the formation of secondary ossification center in the proximal tibia was delayed. The rate of trabecular bone formation was decreased in Zfp521$^{-/-}$ mice, contributing to decreased bone volume. The cortices were thinner with reduced endocortical bone formation. Surprisingly, ex vivo Zfp521$^{-/-}$ calvarial osteoblasts formed more mineralized bone nodules than control cells in culture. Moreover, there were more osteoblast precursor cells in the bone marrow of Zfp521$^{-/-}$ mice than in controls, as measured in a colony forming unit assay. In conclusion, the bone phenotype of Zfp521$^{-/-}$ mice is a mirror image of that of Zfp521 transgenic mice and of Ebf1$^{-/-}$ mice, which share some phenotypic characteristics. Inactivation of Zfp521 leads to enhanced osteoblast differentiation ex vivo putatively via increased transcriptional activity of Runx2 and Ebf1. However, in vivo Zfp521$^{-/-}$ mice exhibit decreased bone formation and bone mass. We conclude that Zfp521 is a novel regulator of osteoblast differentiation and bone formation.

Conflict of Interest: None declared

OC34

HISTAMINE PROMOTES OSTEOCLASTOGENESIS THROUGH DIFFERENTIAL EXPRESSION OF HISTAMINE RECEPTORS ON OSTEOCLAST AND OSTEOBLAST

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The bioactive amine histamine is involved in many regulatory functions through 4 receptors (H1R to H4R). Histamine might also play a role in bone metabolism. Our aim was to identify the role(s) and target(s) of histamine in bone resorption using in vivo and in vitro approaches.

We used a model of synchronized resorption and treated 90 Wistar rats with an H1R antagonist, an H2R antagonist or histamine after induction of the periosteal remodeling. The two anti-histamine treatments decreased ED1+ osteoclast precursors recruitment, with differential effects, whereas histamine treatment strongly increased their numbers. Osteoclast numbers and resorption surface were markedly decreased by the anti H2R (-42% $p < 0{,}001$), moderately by the anti H1R (-21%, $p < 0{,}03$) and highly increased with histamine ($+57\%$ $p < 0{,}001$).

In vitro osteoclastogenesis was analyzed using primary bone marrow cells (BMC) or spleen cultures derived from wild type (WT) or histidine decorboxylase (the histamine-synthesizing enzyme) knock out (HDC−/−) mice. Comparing WT to HDC−/− , BMC treated with vitamin D and ascorbic acid gave rise to reduced number of TRAP+ multi nucleated cells (-31%, $p < 0{,}001$) and resorption on calcium phosphate discs. By increasing concentrations of histamine, osteoclast formation was restored in HDC−/− and enhanced in WT. HDC−/− spleen cells treated with RANKL and M-CSF exhibited reduced osteoclast formation which was increased by histamine treatment, as in BMC. Histamine treatment increased markedly osteoclast differentiation when started early during spleen cultures. HR 1, 2, 3 and 4 mRNA expressions on these cells were analyzed by quantitative RT-PCR at different time points during osteoclastogenesis. Over the 10 days of culture, H1R expression was stable, H2R

was down-regulated, H4R up-regulated and H3R not detected. The early effects of histamine could be explained by this differential receptors expression during osteoclast differentiation and the preferential early expression of H2R on osteoclast precursors. Finally, in primary WT calvarial osteoblasts cultured with ascorbic acid and vitamin D, histamine treatment increased the RANKL/OPG ratio due to increased RANKL mRNA expression. We also found that H1R was up-regulated on osteoblast by vitamin D.

In conclusion, histamine acts on both osteoclasts and osteoblasts as a physiological regulator of bone remodeling, promoting osteoclastogenesis through different expression of histamine receptors on these cells.

Conflict of Interest: None declared

OC35

N-CADHERIN-LRP5 INTERACTION NEGATIVELY CONTROLS OSTEOBLAST PROLIFERATION BY ANTAGONIZING WNT CANONICAL AND NON-CANONICAL ERK MAPK AND PI3K/AKT SIGNALING PATHWAYS

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Wnt signaling is known to promote osteoblastogenesis by stimulating preosteoblast proliferation and differentiation and reducing osteoblast apoptosis. We recently demonstrated that N-cadherin interacts with the Wnt co-receptor LRP5 to negatively regulate the canonical beta catenin signaling pathway, osteoblast differentiation and bone formation. Here, we investigated the role of N-cadherin-LRP5 interaction in the control of osteoblast proliferation. Enforced expression of N-cadherin in murine MC3T3-E1 osteoblasts reduced cell proliferation, as shown by BrdU assay and cell number. Treatment with a N-cadherin neutralizing antibody that antagonized N-cadherin-LRP5 interaction corrected the altered cell proliferation in these cells, implying the N-cadherin-LRP5 interaction in the altered osteoblast proliferation. Treatment with Wnt3a condionned medium (CM, 15%) increased cell proliferation in both N-cadherin overexpressing cells and control cells, indicating that cell proliferation can also be induced by Wnt non-canonical pathways. The addition of ERK1/2 MAPK inhibitor (U0126) or PI3K inhibitor (wortmannin) reduced Wnt3a-induced cell proliferation in both control and N-cadherin overexpressing cells, indicating a role of ERK1/2 and PI3K in Wnt3a-induced osteoblast proliferation. Western blot analysis revealed decreased phosphorylated (p)-ERK1/2, p-PI3K and p-Akt levels in basal conditions and reduced phosphorylation in response to Wnt3a in N-cadherin overexpressing cells compared to control cells. Furthermore, treatment with N-cadherin neutralizing antibody increased p-ERK1/2 and p-PI3K levels in basal conditions and in response to Wnt3a, showing that N-cadherin-LRP5 interaction negatively controls ERK1/2 and PI3K/Akt pathways. Further transfection of these cells with DKK1 that inhibits canonical Wnt-signaling by binding to LRP5, abolished the effect of N-cadherin neutralizing antibody and reduced ERK1/2 and PI3K activation induced by Wnt3a, further indicating that LRP5 interaction with N-cadherin negatively controls ERK1/2 and PI3K in osteoblasts. Altogether, the results indicate that N-cadherin-LRP5 interaction reduces basal and Wnt3a-induced osteoblast proliferation through alteration of Wnt signaling and ERK1/2 and PI3K pathways. These data reveal a novel negative role of the cell-cell adhesion molecule N-cadherin in the control of osteoblast proliferation by antagonizing Wnt-canonical and non-canonical signaling pathways.

Conflict of Interest: None declared

OC36

EFFICACY OF DENOSUMAB ON BONE MINERAL DENSITY IN POSTMENOPAUSAL WOMEN WITH LOW BONE MASS AND PATIENTS WITH BREAST CANCER ON ADJUVANT AROMATASE INHIBITOR (AI) THERAPY

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Under conditions of estrogen deficiency, excess osteoclast activity driven by RANKL may lead to bone loss. Denosumab is an investigational fully human mAb against RANKL. The effects of denosumab were evaluated in 2 similarly designed double-blind, placebo-controlled phase 3 studies in distinct patient populations. In the respective clinical trials, postmenopausal (PM) women with low bone mass ($N = 332$) or women with low bone mass receiving adjuvant AI therapy for non-metastatic breast cancer ($N = 252$) were randomized (1:1) to receive subcutaneous denosumab 60 mg Q6M or placebo for 2 yrs. All patients were instructed to take daily supplements of calcium and vitamin D. Denosumab significantly increased DXA BMD at the lumbar spine, total hip, 1/3 radius, and total body relative to placebo at 24 months in both patient populations (Table 1). Denosumab also significantly ($P < 0.001$) reduced serum levels of CTX-I and P1NP in both studies. The overall incidence of adverse events (AE) was similar between the denosumab and placebo groups within each study. In the PM study there were 18 serious AEs (SAE) in the denosumab group and 9 in the placebo group; this difference was driven by hospitalized infections. In the AI study there were 19 vs 11 SAEs for denosumab and placebo, respectively, and no specific pattern of SAEs was identified. No deaths occurred in the PM study, and 2 deaths (1 denosumab, 1 placebo) occurred in the AI study, both due to progressive breast cancer. In postmenopausal women with low bone mass and in women with a history of breast cancer and low bone mass receiving adjuvant AI therapy, denosumab increased BMD at all sites that were studied, including primarily cortical bone sites. Further clinical studies in both patient populations are underway to assess the potential of denosumab therapy in these patients.

Table 1 Difference in % Change at Month 24 Relative to Placebo

	PM Study*	AI Study*
Lumbar Spine	7.0 (6.2, 7.8)	7.6 (6.6, 8.6)
Total Hip	4.8 (4.3, 5.3)	4.7 (4.0, 5.5)
1/3 Radius	3.8 (3.1, 4.6)	6.1 (5.0, 7.1)
Total Body (no head)	3.9 (3.2, 4.7)	4.2 (3.2, 5.2)

All values $P < 0.0001$ vs placebo; * LS mean (95% CI)

Conflict of Interest: HG Bone: Amgen, Investigator, Consultant; G Ellis: Amgen, Res Grants; CK Yuen: Amgen, Res Grants; D Kendler: Amgen, Ad Board, Res Grants; M Fan, H Wang, D Kim, Y Liu, R Dansey, J San Martin, Amgen Employees

OC37

A NOVEL LUNG CANCER SIGNATURE MEDIATES METASTATIC BONE COLONIZATION BY A DUAL OSTEOLYTIC AND METALLOPROTEOLYTIC MECHANISM

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Bone is a frequent target of lung cancer metastasis, which is associated with a dismal prognosis. We have developed a model in which to identify and characterize genes involved in bone metastasis of lung cancer. After intracardiac inoculation (i.c.) in nude mice, highly metastatic subpopulations (HMS) were selected with overt prometastatic activity. Transcriptomic analysis revealed a novel gene signature of differentially expressed genes in HMS. Four overexpressed genes in all HMS were further validated by real time qPCR. These genes encode signaling molecules (such as TCF4 and PRKD3) and cell anchorage related proteins (MCAM, SUSD5). To delineate their functional contribution we assessed metastatic area by X-ray image analysis and μCT scan of retrovirally transduced simple and triple gene combinations. I.c. of single overexpressors TCF4, PRKD3 or SUSD5 induced a significant increase in metastatic area as compared to mock cells. Furthermore, i.c. of triple overexpressors in nude mice synergistically induced a dramatic increase in prometastatic activity, with prominent osteolytic lesions and metastatic area ($p < 0.001$), whereas cell homing activity to bone was unaltered. Moreover, intratibial injection of triple overexpressors induced aggressive bone colonization leading to overt osteolytic lesions as compared to mock cells ($p < 0.001$). These results were correlated with high osteoclastogenic activity induced by conditioned medium of triple overexpressors and a marked enhancement of global metalloproteolytic activities in vitro. Interestingly, TCF4 and SUSD5 were strongly up-regulated in a coculture system mimicking in vivo tumor-stroma interactions, an effect that was further abrogated by an anti-TGF-β peptide. After i.c. of HMS, in vivo treatment with a specific anti-TGF-β peptide severely reduced tumor burden and osteolytic activity of tumor cells (osteoclast number) compared to an irrelevant peptide. More importantly, in vivo global inhibition of metalloproteolytic activities and simultaneous TGF-β blockade led to increased survival and a stunted abrogation of bone tumor burden and osteolytic metastasis ($p < 0.001$).

Thus, this metastatic gene signature mediates bone-matrix degradation by a dual mechanism of induction of TGF-β—dependent osteoclastogenic bone resorption and enhancement of stroma-dependent metalloproteolytic activities. Our findings suggest the cooperative contribution of host-derived and cell-autonomous effects directed by a small subset of genes in mediating aggressive osseous colonization.

Conflict of Interest: F. Lecanda, Digna Biotech, Grant

OC38

GENOME-WIDE ASSOCIATION STUDY IDENTIFIES KLOTHO AND OTHER NOVEL LOCI AS CONTRIBUTORS TO BMD VARIATION IN POSTMENOPAUSAL WOMEN

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Osteoporosis is a highly heritable disease, with estimates of genetic contribution to variance of BMD up to 80%. Genetic studies aiming to identify loci influencing BMD variation have had little success to date, largely due to inadequate power to detect the genes of small to moderate effect size likely to be involved. Adequately powered genome-wide association studies [GWAS] have successfully identified disease-associated genes in complex polygenic disorders. We performed a GWAS to search for BMD loci.

The GWAS discovery cohort was an extreme truncate selection of 69 high ($+1.5 <$ Z-score $< +4$) and 66 low ($-4 <$ Z-score < -1.5) cases, with BMD measured at total hip. All cases were white Caucasian women, aged > 50 years and > 5 years postmenopausal, from Australia and Britain. Genotyping for 317,000 SNPs was performed with the Illumina HumanHap300 array. No significant difference was seen between Australian and British cases (mean $chi^2 = 1.0067$) and no population stratification was observed using 'Structure'.

The top 1000 associated SNPs (p-values 1×10^{-6} to 3×10^{-3}) were tested for *in-silico* replication in an unselected sample of 1600 elderly women of the Rotterdam Study, a population-based GWAS typed with the Illumina HumanHap550 array, with BMD measured at lumbar spine (LS) and femoral neck (FN).

Regions on chromosomes 6p, 8q, 9q, and 14q achieved $p < 10^{-5}$, below which significance level approximately half of observed associations are robust. Replication at $p < 0.05$ at either LS or FN was seen for 96 SNPs (50 expected), with 21 SNPs achieving both $P < 0.005$ in the phase 1 study and $P < 0.01$ in the Rotterdam study. Some of these SNPs lie in strong candidate genes for osteoporosis. For example, association was observed in both datasets with SNPs in the 5' region of *klotho* (phase 1 study SNP1 $P = 6 \times 10^{-4}$ and SNP2 7×10^{-4}; Rotterdam study $P = 0.01$, 0.007 respectively). These SNPs contributed 7–8% of the variance of BMD in the phase 1 study and 0.3–0.4% in the Rotterdam study. Similar effects were identified in SNPs within *TGF eta* and *PDGF*. Further evaluation of these effects is ongoing.

In conclusion, several loci identified by the Anglo-Australian cohort have replicated in an independent Dutch cohort. Our findings suggest that genetic variation in *klotho* significantly contributes to population variation in BMD. Large well-powered studies and consortia will provide robust information about the genetic contributions to this highly heritable disease trait.

Conflict of Interest: None declared

OC39

MICRORNAS CONTROL BONE FORMATION IN VIVO AND OSTEOBLAST DIFFERENTIATION EX VIVO

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MicroRNAs (miRNAs) are small, single-stranded RNA molecules which regulate gene expression. The enzyme Dicer is essential in mammalian cells for the processing of precursor miRNA into 21–23 nucleotides long mature miRNA. Loss of Dicer function induces embryonic lethal at E7.5 and conditional excision of Dicer in forelimb mesoderm by Prx1-Cre results in cell death and developmental delay. Many of the greater than 5000 miRNAs present in the global miRNA databases putatively target key osteogenic factors. To better understand the requirement of miRNA for bone development, we generated a cre-inducible conditional Dicer knockout mouse by flanking the PAZ domain (responsible for recognition and binding to pre-miRNAs) with loxP sites. We crossed $Dicer^{cond/cond}$ mice with mice bearing a Cre transgene placed under transcriptional control of a collagen 2.3 promoter element. Thus, loss of Dicer function will be restricted to osteoblast lineage cells. Retarded growth and complete absence of bone formation was observed at E14.5 and E15.5 in embryos deleted for Dicer, and no homozygous embryos were recovered after E16.5, suggesting loss of Dicer in osteoblast cell results in embryonic loss after E15.5. Therefore, we performed ex vivo osteoblast differentiation of marrow stromal cells obtained from $Dicer^{cond/cond}$ mice and induced Dicer excision by Adeno-Cre infection. Removal of Dicer significantly decreased osteoblast maturation with complete absence of mineralization. Quantitative PCR for bone markers Collagen type I, Runx2, Alkaline Phosphatase and Osteocalcin confirmed the phenotype of the cell populations altered by excision of Dicer. This suggests a critical requirement for miRNA biogenesis in osteoblastogenesis, particularly in the late stage development of the osteoblast phenotype. To explore further, we have performed a miRNA profiling study in preosteoblasts MC3T3 at the stages of maturation. Functional studies of selected miRNAs expressed during mineralization in Dicercond/cond and MC3T3 cultures are ongoing to identify those miRNAs required for regulation of bone formation in vivo and osteoblast differentiation in vitro.

Conflict of Interest: None declared

OC40

EXPRESSION OF TRUNCATED ACTIVATOR PROTEIN-1 MEMBERS IN THE HYPOTHALAMUS INCREASES BONE FORMATION

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ΔFosB a naturally occurring C-terminally truncated form of the FosB transcription factor has been reported to have reduced AP-1 transcriptional activity. Mice overexpressing ΔFosB under the control of the enolase 2 promoter (ENO2), which drives expression in bone, fat and the brain, results in increased bone mass and decreased adipose mass (Sabatakos et al. Nat Med, 2000). This effect of ΔFosB on bone mass is in part the result of a cell-autonomous effect, since targeted expression of ΔFosB to osteoblast with the osteocalcin promoter (OG2) recapitulated the bone phenotype with no effect on adipose mass (Kveiborg et al., MCB, 2003). In situ hybridization revealed that ΔFosB is also overexpressed in the hypothalamus of ENO2-ΔFosB mice. Given the recent evidence of a hypothalamic relay in the regulation of bone mass, we hypothesized that ΔFosB could affect this brain circuitry and thereby contribute to the increase in bone mass observed in the ENO2-ΔFosB mice. Stereotaxic injections of adeno-associated viruses (AAV) encoding ΔFosB was targeted to the hypothalamus. Six weeks post-injection, the mice injected with ΔFosB exhibited an $> 3X$ increase in bone formation rates and higher bone mass. To determine if reduced AP-1 transcriptional activity was responsible for this increase in bone formation we also tested the effect of dominant negative JunD (DNJun) which lacks the transactivation domain, but still retains JunD dimerization and DNA binding abilities. Interestingly, DNJunD injected mice had an ever higher increase in bone formation rate (5X) and significant increase in bone mass. The changes in bone formation were observed in both long bones and calvaria, suggesting that they were not dependent upon mechanical loading. The observed changes in bone mass were also independent of possible alterations in pituitary hormones levels. To determine if ΔFosB affected the sensitivity of osteoblasts to sympathetic inputs, we stimulated primary osteoblasts from the ENO2-ΔFosB mice with isopreterenol (a beta-adrenergic agonist). Primary osteoblast from the ENO2-ΔFosB did not exhibit any differences in sensitivity compared to control cells upon beta-adrenergic activation. These results suggest that regulation of AP-1 activity in the hypothalamus can lead to profound increases in bone formation and bone mass.

Conflict of Interest: None declared

OC41

IMPACT OF BALLOON KYPHOPLASTY ON QUALITY OF LIFE AND RISK OF RECURRENT VERTEBRAL FRACTURES: A RANDOMIZED TRIAL IN PATIENTS WITH ACUTE VERTEBRAL COMPRESSION FRACTURES

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Objectives: Balloon kyphoplasty is a minimally invasive treatment for vertebral fractures that aims to correct deformity using balloon tamps and bone cement to stabilize the body. The aim of this randomized trial was to assess the effect of kyphoplasty on quality of life and recurrent fracture risk.

Methods: Patients with up to 3 non-traumatic acute vertebral fractures were enrolled within three months of diagnosis and randomly assigned to receive either kyphoplasty ($N = 149$) or usual nonsurgical care ($N = 151$). Measurements of quality of life, back pain and function, days of disability and spine radiographs were assessed through twelve months of follow-up.

Results: Compared with the control, participants assigned to kyphoplasty had 5.2 points (95%CI, 2.9 to 7.4; $p < 0.0001$) greater improvement in the physical component summary of the SF-36 quality of life questionnaire at one month and 1.5 points (95%CI, -0.8 to 3.8; $p = 0.2$) more at twelve months. Kyphoplasty resulted in greater improvement in quality of life by the EuroQol questionnaire at one (0.18 points; 95%CI, 0.08 to 0.28; $p = 0.0003$) and twelve months (0.12 points; 95%CI, 0.01 to 0.22; $p = 0.025$) and improved disability by the Roland-Morris scale at one (4.0 points; 95%CI, 2.6 to 5.5; $p < 0.0001$) and twelve (2.6 points; 95%CI, 1.0 to 4.1; $p = 0.0012$) months. Kyphoplasty patients had less back pain on a

numeric rating scale at seven days (2.2 points; 95%CI, 1.6 to 2.8; $p < 0.0001$) and twelve months (0.9 points; 95%CI, 0.3 to 1.5; $p = 0.0034$) and reported fewer days of limited activity at one month (2.9 days per 2 weeks; 95%CI, 1.3 to 4.6; $p = 0.0004$) and twelve months (1.6; 95%CI, −0.1 to 3.3; $p = 0.068$). New radiographic vertebral fractures occurred in 41.8% of kyphoplasty subjects and 37.8% in the control (4% difference; 95%CI −7.5 to 15.6; $p = 0.5$). There was no significant difference between groups in the number of patients with adverse events.

Conclusiton: Compared to the control, balloon kyphoplasty improved quality of life, reduced back pain and disability and decreased pain medication and walking aid usage. Most of these improvements last at least 1 year without increasing adverse events including new vertebral fractures. (Clinicaltrials.gov number, NCT00211211).

Conflict of Interest: This study was supported by Kyphon (Medtronic).

OC42

THE TIMING OF DOSING OF ZOLEDRONIC ACID 5MG AFTER RECENT HIP FRACTURE AFFECTS ANTIFRACTURE EFFICACY AND REDUCTION OF MORTALITY: RESULTS FROM HORIZON-RECURRENT FRACTURE TRIAL

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The HORIZON Recurrent Fracture Trial showed that ZOL 5 mg reduced clinical, clinical vertebral, and non-vertebral fractures, and all-cause mortality in subjects with a recent hip fracture (Lyles et al NEJM;2007;357:1799–809). In the trial, 2127 men and women received their 1st dose of zoledronic acid (ZOL) 5 mg within 90 days after surgical repair of a hip fracture and once yearly thereafter. The primary end point was time to 1st new clinical fracture. Secondary end points included changes in total hip and femoral neck bone mineral density (BMD) in the non-fractured hip measured annually by DEXA, and clinical vertebral, non-vertebral, and hip fractures. New analysis used Cox and ANOVA proportional hazards regression, respectively, to model change in incidence of clinical fractures, death and BMD adjusting for the timing of 1st infusion. The median time of 1st infusion after hip fracture repair was approximately 6 weeks (46 days, range 1 to 123 days). Baseline characteristics of patients dosed <6 weeks and > 6 weeks were well matched, and mortality in the placebo groups was comparable. The risk reduction (RR) for clinical fractures was statistically significant regardless of when the 1st infusion was administered (≤6 weeks, >6 weeks) (37% RR vs 33% RR, both $P < .04$). The RR for vertebral fractures (67% RR vs 15% RR) and hip fractures (61% RR vs 36% RR increase) were significantly greater when the ZOL 5 mg dose occurred more than 6 weeks after hip fracture repair (both $P < .01$). The reduction in mortality was 46% in those dosed > 6 weeks ($P = .002$) and 10% in those dosed earlier ($P = .55$). Increases in total hip and femoral neck BMD relative to placebo at 24 months ranged from 3.43% to 8.40% and 2.47% to 5.14% respectively and were maximized when the dose was administered 4 to 12 weeks after hip fracture repair. In general, the data suggest the risks of vertebral and hip fractures and mortality can be optimized by administering the 1st infusion 4–12 weeks after hip fracture repair. ZOL 5 mg had no adverse effects on fracture healing, regardless of the timing of infusion. The most common AEs with ZOL were transient post-dose symptoms.

In conclusion, timing of ZOL 5 mg infusion in relation to fracture repair had a significant impact on the BMD response, antifracture efficacy, and mortality. This post-hoc analysis suggests that the timing of infusion of ZOL 5 mg may optimize antifracture efficacy and mortality in post-hip fracture patients.

Conflict of Interest: E Eriksen, Novartis, Shareholder, Employee

CC01

A NOVEL DISORDER OF TYPE I COLLAGEN CHARACTERISED BY HIGH BONE MASS, A MINERALIZATION DEFECT AND TENDON CALCIFICATION

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The most prevalent forms of osteogenesis imperfecta (OI) arise from mutations in the genes COL1A1 or COL1A2. These conditions are characterized by skeletal fragility, narrow long bones and low bone mass, but normal mineralization of bone.

We describe two siblings - a man aged 41 and his 39 year old sister - with a fracturing bone disease inherited from their late father. By the age of 15 the elder sibling had suffered more than thirty long bone fractures, and developed marked scoliosis. The younger sibling sustained nine major fractures by the age of 16. Their father had started to fracture in infancy and sustained more than fifty fractures. All three subjects had significant hearing loss; audiometry demonstrated a conductive component suggestive of cochlear ossification. Both father and son suffered Achilles tendon rupture that healed with extensive calcification. Stature was normal, the sclerae were white, and there was no dentinogenesis imperfecta.

The radiographs showed osteosclerosis of the axial skeleton. The long bones were not narrow: the z-score for the total width of the second metacarpal was > 2. The cortices of the long bones were thick with loss of tubulation. The femoral neck BMD z-score was +0.3 in the elder sibling and +6.2 in the younger. Bone turnover markers were increased. Transiliac bone biopsy specimens showed thick osteoid covering much of the bone surfaces (8–13 osteoid lamellae - normal ≤4).

Sequence analysis of the type I collagen genes identified a mutation (c.3652 G > A) in one allele of COL1A1 in both affected siblings. The mutation results in an Ala→Thr substitution at position 1218. This mutation disrupts the alanine-aspartic acid site where the C-terminus propeptide of type I procollagen is cleaved by BMP1. The mutation could slow formation of type I collagen fibrils in the extracellular matrix and impair mineralization—as it is in the regions that would be occupied by propeptide extension fibrils that mineral starts to form.

This novel bone disorder extends the phenotype of type I collagen disorders to include features (mineralization defect, high bone mass and tendon calcification) hitherto unrecognized in the OI spectrum.

Conflict of Interest: None declared

CC02

SEVERE HIGH TURNOVER OSTEOPOROSIS AND MULTIPLE FRACTURES IN A YOUNG MAN WITH AUTOIMMUNE DISEASE AND CIRCULATING ANTIBODIES TO OSTEOPROTEGERIN: A NOVEL SYNDROME

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A 40 year old Caucasian man presented with height loss and a low trauma fracture of the clavicle. He had severe osteoporosis on DEXA scanning with a T score of −6.6 in the spine and −2.9 at the hip. Further investigations showed he had high alkaline phosphatase (2610 u/l; normal 25–120) and primary hypothyroidism with low T4 value (<5 pmol/l), raised TSH (>65 mU/l) and positive thyroid autoantibodies. Serum level of parathyroid hormone was low (8 ng/l; normal 10–65) and 25 OH vitamin D was low/normal (35 nM; normal 25–150). He was given thyroxine replacement but the ALP rose further to 3595 u/l and he was found to have mild hypercalcaemia (2.8 mM) with undetectable levels of PTH. A radionuclide bone scan showed diffusely increased tracer uptake throughout the skeleton but no focal lesions. A transiliac bone biopsy showed high bone turnover with a dramatic increase in numbers of osteoclasts and osteoblasts. A mild mineralisation defect was noted (increased extent of osteoid seams of normal thickness). Further investigations revealed occult coeliac disease. Despite a gluten free diet and treatment with ergocalciferol 10,000 u/d and calcium, over the next 6 months he suffered further height loss and sustained a low trauma fracture of the left humerus. Repeat DEXA scanning showed that spine BMD values had fallen to a T-score of −7.7. In view of the high bone turnover he was given two infusions of Zoledronic acid 4 mg. This resulted in normalisation of ALP within 12 months of treatment (96 u/l) and a substantial increase in BMD to a T-score of −2.5 in the spine and −1.3 at the total hip.

Further studies were initiated to determine the cause of the increase in bone turnover. We screened for auto-antibodies to the TSH receptor in view of previous reports which suggested that TSH might suppress bone turnover, but no antibodies were found. We therefore screened for auto-antibodies to osteoprotegerin in view of its critical role in regulating bone turnover. We found that serum from the patient immunoprecipitated recombinant osteoprotegerin in vitro, whereas serum from 10 controls and 5 patients with primary hypothyroidism were negative. Preliminary studies have also shown that the patient's serum abrogates the inhibitory effect of recombinant osteoprotegerin on RANKL induced NFkB signaling in RAW cells in vitro. In conclusion this patient seems to have developed a novel syndrome of high turnover osteoporosis due to the development of autoantibodies to osteoprotegerin.

Conflict of Interest: None declared

CC03

EVIDENCE OF REDUCED BONE TURNOVER AND DISTURBED BONE MATRIX MINERALISATION IN A BOY WITH STICKLER SYNDROME

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Osteochondrodysplasias are a heterogeneous group of genetic skeletal dysplasias. Skeletal dysplasias are diagnosed and classified by their clinical features, radiographic findings, and their genetic pattern of inheritance. Patients with these diseases commonly develop cartilage matrix protein defects with adverse skeletal outcome. We report on a 12-years-old boy who manifested a constellation of clinical and radiographic features suggestive of Stickler syndrome (ophthalmoarthropaty). Early childhood development of severe myopia associated with a broad spectrum of skeletal manifestations has been emerged in connection with spondyloepiphyseal dysplasia and extensive ligamentous hyperlaxity. COL2A1 mutations have been reported in some families, including the original Stickler kindred (Williams et al., 1996). Our patient manifested genu valgum (knock knees), mild kyphosis and chronic osteomyelitis. An examination of a transiliac biopsy for histomorphometric indices and for parameters of bone matrix mineralization density distribution (BMDD) using quantitative backscattered electron imaging (qBEI) revealed: i) a severe osteopenia (e.g. BV/TV = −43%, TbTh. = −20% and Tb.N = −29%); ii) a distinct reduction bone turnover as reflected by indices like OS/BS = −48%, Ob.S/BS = −27% and Oc/BS = −47%. iii) a remarkable increase in osteoid thickness (O.Th = +124%) iv) a decrease in average and most frequent calcium content (CaMean = −10.5% and CaPeak = −8.5%, respectively). v) a strong increase in heterogeneity of mineralization (CaWith = +55.7%. vi) a dramatic increase in amount of bone less mineralized than 17.86 weight % calcium (CaLow = +341%). The results showed evidence of reduced bone turnover associated with disturbed bone matrix mineralisation most likely due to alterations in the organic matrix. To the best of our knowledge this is the first clinical report describe the clinico-radiographic features in combination with the bone biopsy findings in a boy with Stickler syndrome.

Conflict of Interest: None declared

CC04

CASE REPORT: A WOMAN WITH HYPOPHOSPHATASIA TREATED WITH TERIPARATIDE FOR NINE MONTHS; TOLERABILITY, SYMPTOMS AND LABORATORY FINDINGS

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Hypophosphatasia is a rare inborn error of bone metabolism caused by missense mutations of the tissue-nonspecific alkaline phosphatase (TNALP) gene with variable phenotypical presentations affecting the development and mineralization of the human skeleton. We present a 49-year old woman with early loss of decidual teeth. At the age of 10 years hematuria was interpreted as urinary tract infection and that was treated with antibiotics and followed by persistent proteinuria. At the age of 25 years she was diagnosed with hypertension. She developed makroproteinuria and renal biopsy was consistent with segmental glomerular sclerosis. Extremely low levels of serum total ALP was found during the evaluation of hypertension. A further assessment with HPLC revealed low activities of the bone and liver ALP isoforms normally present in the circulation.

She has recurrent pains in both feet since 1985 and X-rays have shown fractures of metatarsal bones II-V bilaterally. In 2000 she suffered pain in both hip regions and MRT showed bilateral fissures in both hips and she was treated conservatively. After a minor fall, the right hip fissure was dislocated and she was operated with bilateral hip prosthesis. In April 2006 she had a spontaneous painful fracture of the right proximal fibula with delayed healing.

Following a poster presentation by Whyte et al. (ASBMR 2006) who reported a successful treatment with PTH of a woman with similar problems, we initiated treatment with the PTH analogue teriparatide 20 microg/day sc. Our patient took her injections daily except for a short break (totally 2 weeks) for 9.5 months. During teriparatide therapy her ionized calcium increased from approximately 1.30 mmol/l to 1.40 mmol/l, serum phosphate was 1.8–2.3 mmol/l before and 1.6–2.0 during teriparatide treatment. The serum creatinine values remained around 180 micromol/l. Her total serum ALP levels were measurable at 2/12 assays. Serum PTH decreased from approximately 60 ng/l to 35 ng/l. She experienced a slight decrease of bone pain most noticeable in the lower extremities.

In conclusion: The treatment of this woman with osteomalacia due to hypophosphatasia with teriparatide 20 microg/d sc for 9.5 months did not cause any adverse effects and no symptomatic fissures or fractures occurred. The patient experienced less bone pain, however, the conventional total serum ALP levels did not improve in our patient.

Conflict of Interest: None declared

Su-OP01

A SET OF GENES EXPRESSED IN BREAST CANCER BONE METASTASES DISCLOSES PREDISPOSITION TO GENERATE METASTASES IN MULTIPLE ORGANS

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Bone is one of the preferential sites of distant metastasis in breast carcinoma (BrCa). While BrCa patients with bone-only metastases have frequently a better overall survival, the formation of metastases in other sites dramatically compromises the clinical outcome. We performed a microarray analysis with the Affymetrix platform on bone metastasis samples from breast cancer patients with bone-only metastases (BoMes) and patients who developed secondary tumours in bone and other sites (MuMes). We analysed the profiles obtained using an unsupervised hierarchical clustering and showed that the transcriptomes correlated with the clinical features, segregating the BoMes profiles from those of MuMes samples. In order to obtain a MuMes gene signature a statistical analysis was performed to identify the 2-fold regulated genes expressed in MuMes samples relative to BoMes samples. This analysis revealed 100 up-regulated and 18 down-regulated genes in MuMes vs. BoMes ($p < 0.05$). However, this gene signature was validated by real time RT-PCR analysis only for 66% of the selected genes evaluated in the same specimens previously analysed with the microarray. This latter result prompted us to perform another whole genome microarray analysis on new BoMes and MuMes samples using the Agilent platform. We then matched the Affymetrix signature with the most regulated genes in the Agilent profiles and found 15 genes (13 up-, 2 down-regulated) commonly modulated in MuMes vs. BoMes in both platforms, also validated by real time RT-PCR. These genes perhaps represent the most reliable gene set separating MuMes from BoMes. Mapping these genes using the GOTM (gene ontology tree machine) software, which allowed to identify the related biological processes and molecular functions, we observed up-regulation of many processes involved in oxygen transport, plus additional processes associated with fatty acid metabolism, gametogenesis and circulation. In conclusion, this study evidenced a group of genes that are likely to characterise breast cancer patients with bone only metastases from those who also developed multiple metastases. Our results provide a rationale for setting custom-made gene arrays for diagnostic and prognostic purposes.

Conflict of Interest: None declared

Su-OP02

NOVEL ASSOCIATION BETWEEN A POLYMORPHISM IN THE P2X7 RECEPTOR GENE AND LOSS OF LUMBAR SPINE BONE MINERAL DENSITY IN POSTMENOPAUSAL WOMEN

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The $P2X_7$ receptor ($P2X_7R$) is a unique pore-forming receptor we have demonstrated to be functionally expressed by osteoblasts and osteoclasts. A $P2X_7R$ knockout mouse model has reduced cortical bone density and reduced responses to mechanical loading, revealing the $P2X_7R$ as a mechanotransducer of bone remodelling. The gene for the $P2X_7R$ (*P2RX7*) is highly polymorphic; four SNPs (c.474G > A, c.1096C > G, c.1513A > C, and c.1729T > A) are known to cause amino acid changes and 50% reduced function in the heterozygous state, whilst the c.946C > A substitutes glutamine for arginine at the ATP binding site and reduces function by over 70% in the heterozygous state. Two polymorphisms have recently been shown to be associated with an increased 10-year fracture risk in older Danish postmenopausal women. The aim of this study was to survey a cohort of younger women for an association between functional *P2RX7* polymorphisms and key determinants of osteoporosis, specifically bone mineral density (BMD). We have genotyped 508 perimenopausal women from the Aberdeen Prospective Osteoporosis Screening Study (APOSS) for eight known *P2RX7* polymorphisms. Females were recruited randomly from among the general population in the North East of Scotland from 1990–94 when aged 45–54 y. Lumbar spine (LS) and femoral neck (FN) BMD was measured at baseline and at 6–7 year follow up. Blood samples were collected for DNA extraction at follow-up. *P2RX7* genotyping was performed by homogeneous mass extension. Genotype frequencies for all *P2RX7* variants were in Hardy-Weinberg equilibrium (p-values 0.2–1.0). We found association of c.946A with LS-BMD at both baseline ($p = 0.006$, $\beta = -0.12$) and follow-up ($p = 0.004$, $\beta = -0.13$). In a separate study of 125 Australian post-menopausal women (mean age 54 y) where BMD was assessed by both DXA and peripheral QCT (Stratec XCT 3000), the c.946A showed trends to lower LS and trochanter BMD ($p = 0.1$ and 0.05 respectively) and lower radial cortical area ($p = 0.07$). This is the first report detailing the association of the *P2RX7* c.946A, which is known to have deleterious functional effects *in vitro* and is expressed by both osteoblasts and osteoclast, with loss of LS BMD in younger postmenopausal women. Although the c.946 G > A polymorphisms is of low frequency (1–2%) in the Caucasian population, it has a major effect on lumbar spine BMD, thus confirming *P2X7R* as a novel bone anabolic therapeutic target, and early diagnostic tool for prevention of bone loss.

Conflict of Interest: None declared

Su-OP03

EFFECTS OF COL1A1 POLYMORPHISMS AND HAPLOTYPES ON PERIMENOPAUSAL BONE MASS AND POSTMENOPAUSAL BONE LOSS

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Collagen type I is an important component of bone. It is encoded by the COL1A1 gene. The fragile bones of patients with osteogenesis imperfecta are caused by COL1A1 mutations.Previous studies have found that -1997GT, -1663indelT and +1245GT (Sp1) polymorphisms in the COL1A1 gene are associated with low BMD and osteoporotic fracture risk. We wanted to investigate whether these effects were mediated through effects on peak bone mass or postmenopausal bone loss and could be prevented by treatment with estrogen.

The study population comprised 1739 perimenopausal women from the DOPS study. Genotyping was performed by Taqman-assays and sequence analysis. BMD was measured by DXA.

As the 3 polymorphisms are in strong LD, haplotypes were determined. The 3 most frequent haplotypes comprised 98 % of the women.

Women carrying the T allele of the -1997GT had lower BMD at all measured sites with a clear allele-dose effect: lumbar spine BMD 1.030 ± 0.137 g/cm^2 in GG genotype, 1.016 ± 0.147 g/cm^2 in GT genotype and 0.988 ± 0.124 g/cm^2 in TT genotype, $p < 0.05$; total hip BMD 0.921 ± 0.116 g/cm^2 GG, 0.904 ± 0.123 g/cm^2 GT and 0.887 ± 0.109 g/cm^2 TT, $p = 0.01$. The effect remained after 5 and 10 years, however the differences were no longer statistical significant, which might be explained by a loss of statistical power caused by dividing the original population into treated with HRT and not treated.

In haplotype 3 (T-inT-G) similar effects were found: Lumbar Spine BMD 1.014 ± 0.145 g/cm^2 in carriers of at least one haplotype 3 allele vs. 1.030 ± 0.137 g/cm^2 in non-carriers, $p < 0.05$. Total hip BMD 0.903 ± 0.122 g/cm^2 in carriers vs. 0.921 ± 0.117 g/cm^2 in non-carriers, $p < 0.01$. Again the effect was sustained throughout 10 years but did not reach statistical significance.

The -1997GT polymorphism and haplotype 3 also affect biochemical markers of bone turnover: P-osteocalcin 18.2 ± 14 ng/ml in carriers of the T allele vs.16.9 ± 6.3 ng/ml in non-carriers, $p = 0.014$. P-alkaline phosphatase 71.4 ± 31.2 U/l in women with at least one T-allele vs. 68.2 ± 25.2 U/l in non-carriers, $p < 0.05$.

No consistent effects of the the other polymorphisms and haplotypes were found on perimenopausal bone mass and postmenopausal bone loss. Furthermore, no interaction with HRT could be demonstrated.

In conclusion, the -1997GT polymorphism significantly affects perimenopausal BMD and it is possible that the effect is sustained throughout the first ten years after menopause.

Conflict of Interest: None declared

Su-OP04

FUNCTIONAL ROLE OF TNFSF11 GENE PROMOTER POLYMORPHISMS IN POSTMENOPAUSAL OSTEOPOROSIS

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RANKL is an important regulator of osteoclastogenesis. Increased osteoclastogenesis is the main cause of osteoporosis and *TNFSF11* gene, which encodes for RANKL protein, is therefore one of the candidate genes for the genetic susceptibility to osteoporosis. As nucleotide changes in the promoter could alter gene expression, the aim of our study was to investigate the association of 3 SNPs in *TNFSF11* gene promoter with BMD in postmenopausal women. The role of 2 most common haplotypes was also evaluated in functional studies of *TNFSF11* gene promoter.

404 postmenopausal women, 182 osteoporotic and 222 non-osteoporosis, were genotyped for TNFSF11 gene promoter SNPs $-290C > T$ (rs9525641), $-643C > T$ (rs9533156) and $-693G > C$ (rs9533155). Haplotypes were inferred using PHASE software. For PCR amplification, genomic DNA from 2 women with common haplotypes CCG and TTC, occurring in 44.3% and 49.3% of subjects, respectively, was used. Amplified fragments were cloned into pGL3-basic vector, which was co-transfected with pRL-TK plasmid into HEK293 cells. Dual Luciferase Reporter Assay was performed. To evaluate association of SNPs and 2 common haplotypes with BMD, ANCOVA, with age and BMI as covariates, was used. To compare luciferase activities between common haplotypes, t-test was used.

In postmenopausal osteoporosis, the association with lumbar spine BMD was found for the $-290C > T$, $-643C > T$ and $-693G > C$ (P values: < 0.001, 0.029 and 0.014, respectively) and haplotypes TTC and CCG (P values: 0.002 and 0.006, respectively). The association with femoral neck BMD was also shown in $-290C > T$ and $-693G > C$ SNPs, and TTC haplotype (P-values: 0.026, 0.037 and 0.045, respectively). In non-osteoporotic women, no association with BMD was found for any of the studied SNPs or haplotypes. Reporter gene analysis showed significantly higher luciferase activity in the CCG compared to TTC haplotype 48 hours post transfection. Furthermore, osteoporotic postmenopausal women with 2 CCG haplotype copies have significantly lower lumbar spine BMD than those with 2 TTC haplotype copies ($P = 0.018$). In CCG haplotype *TNFSF11* gene expression may higher, and could lead to increased osteoclast activity and bone resorption. To confirm these data, further analysis will be necessary.

Our results suggest that in postmenopausal osteoporosis *TNFSF11* gene promoter polymorphisms $-290C > T$, $-643C > T$ and $-693G > C$ have a functional role in the genetic regulation of BMD.

Conflict of Interest: None declared

Su-OP05

PROMOTER AND INTRON 1 POLYMORPHISMS OF COLIA1 PREDISPOSE TO OSTEOPOROSIS BY REGULATING TRANSCRIPTION AND ALTERING BINDING OF SP1, NMP4 AND OSTERIX

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The COLIA1 gene is an important candidate for susceptibility to osteoporosis. Previous studies have shown that haplotypes define by three single nucleotide polymorphisms (-1997G/T; -1663IndelT and +1245G/T)) in the 5′ flank of COLIA1 are associated with osteoporosis-related phenotypes in several populations. The mechanisms underlying these associations are unclear. Here we conducted functional studies on the effects of COLIA1 5′ haplotypes on gene expression; and the binding of transcription factors to DNA and chromatin surrounding the polymorphic sites. Reporter assays showed significant differences between the ability of different haplotypes to

drive transcription with 2-fold higher levels in the G-delT-T haplotype and 50% higher in the T-insT-G haplotype compared with the wide type G-insT-G. Further studies showed that these effects on transcription were regulated by the combination of all three SNP rather than by the individual polymorphisms. Gel shift assays showed the region surrounding the -1663IndelT polymorphism recognised a complex of nuclear proteins including Sp1, Nmp4 and Osterix and also showed that the -1663delT allele had significantly increased binding affinity for this complex ($p = 0.001$). The region surrounding the -1997 polymorphism bound purified Sp1 and the G allele had increased binding affinity ($p = 0.009$), and as previously reported, the +1245 site bound Sp1 with increased binding affinity for the T allele ($p < 0.001$). Further studies were performed using chromatin immunoprecipitation (ChIP) assays. This confirmed that the region flanking the -1663 site bound Sp1, Osterix and Nmp4, whereas the region flanking the -1997 site bound Sp1 and Nmp4. The region surrounding the +1245 site also bound Sp1. This study confirms that three common polymorphisms in the 5′ flank of COLIA1 interact to regulate transcription and bind several transcription factors that play a critical role in osteoblast function including Sp1, Nmp4 and Osterix. This suggests that the polymorphisms predispose to osteoporosis by altering binding affinity for several critical transcription factors and by altering expression of the COLIA1 gene.

Conflict of Interest: None declared

Su-OP06

BOTH N- AND C-TERMINAL FRAGMENTS OF PARATHYROID HORMONE-RELATED PROTEIN (PTHRP) REVERSE THE CATABOLIC EFFECTS OF 3-METHYLPREDNISOLONE ON BONE REGENERATION IN MICE

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Glucocorticoids (GCs) inhibit proliferation and differentiation of osteoblasts, leading to a decreased bone formation. PTHrP is an important modulator of bone formation, and its osteoblastic expression is decreased by GCs. We assessed the putative osteogenic effects of PTHrP (1–36) and PTHrP (107–139) during bone regeneration in GC-treated mice. We administered 3-methylprednisolone (10 mg/Kg, s.c., every other day) or vehicle to C576J/BL mice for 16 d. Some GC-treated mice were injected with either PTHrP peptide (100 μg/Kg, s.c., every other day). Bone marrow (BM) ablation was performed under anaesthesia in both tibiae at day 12 before sacrifice. BM of one tibia was cultured for up to 21 d. Cell colonies (crystal violet staining), alkaline phosphatase (ALP) activity, and bone nodules (alizarin red staining) were evaluated. Total RNA was isolated from the remaining hard tissue for mRNA analysis of osteoblast-related genes (real-time PCR). The other tibia was decalcified and included in paraffin for histological studies. MC3T3-E1 cells were grown in differentiation medium, with or without 1 μM dexamethasone (Dexa). Each PTHrP peptide (100 nM) was added to Dexa-treated cells for 24 h of each consecutive 48-h incubation cycle up to 3 cycles (80% confluence). GC decreased BM-derived cell proliferation and mineralization, with a drop in ALP+ staining and the number and size of bone nodules. These changes were reversed by PTHrP (1–36), and partially by PTHrP (107–139). A decrease (mean:−40%) in the expression of the following osteoblastic products: Runx2, osterix, osteocalcin, and osteoprotegerin, associated with a lower PTHrP expression, occurred in the GC mouse tibia. The alterations in these osteoblastic markers were partially recovered by either PTHrP peptide. RANKL mRNA levels also decreased (mean:−25%) by GC; while it remained unaltered with PTHrP treatments. GC mice showed a decreased osteoid surface (Masson staining) and osteoblast number (mean: −25%) in the metaphysis of the regenerating tibia and an increased number of adipocytes (2 fold vs control). These effects were reversed after treatment with either PTHrP peptide. In MC3T3-E1 cells, Dexa induced a decrease (−40%) in mineralization (alizarin red), reversed by either PTHrP peptide. Conclusions: GC decreases osteoblastic differentiation in the mouse regenerating tibia. Both N- and C-terminal PTHrP can restore the altered osteoblastic function and thus accelerate bone repair.

Conflict of Interest: None declared

Su-OP07

EFFECT OF VITAMIN-D3 AND CALCIUM ON FRACTURE RISK IN 65–71 YEAR OLD WOMEN IN A 3-YEAR RANDOMIZED CLINICAL TRIAL—PRELIMINARY RESULTS OF THE OSTPRE-FRACTURE PREVENTION STUDY

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Although Ca+Vitamin D supplementation has been shown to prevent fractures in the elderly, the effect of such a supplementation on fracture prevention in younger ambulatory postmenopausal women is still debatable.

The aim of this population level intervention was to determine whether daily Vitamin-D3 800 IU and Ca 1000 mg administered in two daily doses (Calcichew-D3 forte 500 mg/400IU, Leiras Nycomed Ltd. Finland) could decrease the incidence of fractures in postmenopausal women.

The women within the OSTPRE-cohort who were 65 years or older on 30.11.2002 ($n = 5407$) were asked regarding their willingness to participate in a randomized controlled trial with questions about medications, health disorders and health behaviour. Of the questionnaires that were adequately completed with written consent, 3432 subjects were randomized to have either Ca+Vit D supplementation ($n = 1718$) or no supplementation ($n = 1714$) for 3 years. Primary end points were number of falls and fractures. Fracture data was collected from the entire cohort by telephone interviews once a year and also from a subsample every 4 months. All self reported fractures were included in the analysis. Risk of fracture (HR) was calculated using Cox proportional hazards model. The study was approved by the local ethics committee.

Mean age of women was 67.4 (SD 1.8, range 65–71). At baseline ($n = 3432$) there were no statistically significant differences in age, weight, bone mineral density, HRT-use, hormonal status, age at menopause, smoking habits, alcohol use or functional capacity. A total of 290 fractures were reported including 144 osteoporotic fractures (vertebral $n = 26$, hip $n = 10$, distal forearm $n = 82$ and humerus $n = 26$). The incidence of distal forearm fractures was lower (1.86 %) in the intervention group compared to the control group (2.68 %) ($p = 0.107$, chi square test). In the Cox regression model ($n = 2946$) the hazard ratios (HR with 95% CI) in the intervention group compared to the control group were 0.84 (0.65–1.08) for all fractures, 0.74 (0.51–1.06) for osteoporotic fractures and 0.60 (0.37–0.98) for distal forearm fractures, respectively.

In conclusion, this population level intervention study suggests that daily Vitamin-D3 and Calcium supplementation has a trend towards fracture reduction in all fractures and particularly distal forearm fractures in postmenopausal women.
Conflict of Interest: OSTPRE-FPS received financial support from Academy of Finland and Leiras Nycomed

Su-OP08

NON-OSTEOPOROTIC WEDGE DEFORMITY AND OSTEOPOROTIC VERTEBRAL FRACTURE IMAGED BY X-RAY ABSORPTIOMETRY IN PRE- AND POSTMENOPAUSAL WOMEN (OPUS STUDY)

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Qualitative inspection of the endplate may improve the specificity for diagnosis of osteoporotic vertebral fracture (OVF), by excluding wedge-shaped vertebrae with short anterior height but no endplate fracture (wedge-SVH). It has been suggested that these may represent "gradual" fractures and if so, would be expected to be associated with low BMD. Our aims were to 1) test the hypothesis that most cases of wedge-SVH in postmenopausal women are long-standing and represent normal or developmental variation; 2) compare the characteristics of women with and without wedge-SVH or OVF.

We studied 1517 women ages 21 to 80 years from 3 European centres participating in the population-based OPUS study. Absorptiometric vertebral fracture assessment (VFA) and BMD DXA measurements at the lumbar spine (LS) and total hip (TH) were performed using Hologic densitometers. A single observer classified women as OVF (one or more endplate fractures with or without cortical fracture); wedge-SVH (one or more vertebrae with short anterior height, but no endplate fracture) or normal (no evidence of OVF or wedge-SVH). We compared the proportions of women with wedge-SVH in pre-and postmenopausal women (chi squared test) and mean age, height, weight and BMD T-scores in each group according to VFA classification (analysis of variance, or 2 sample t test).

The prevalence of wedge-SVH in pre- and postmenopausal women (Table 1) did not differ significantly ($p = 0.30$). In postmenopausal women, those with OVF were significantly older (by 3 yrs), shorter (by 1.9 and 2.3 cm) and had lower BMD T-scores by 0.47 and 0.60 (LS) and 0.64 and 0.61 (TH) compared to women classified normal or wedge-SVH respectively ($p < 0.01$). Premenopausal women classified as normal or wedge-SVH differed only by LS BMD T-score: this was significantly higher by 0.56 in women with wedge-SVH ($p < 0.01$).

Table 1 Classification by VFA

	Premenopausal, n	Postmenopausal, n
Normal	128 (56%)	612 (48%)
Wedge-SVH	96 (42%)	598 (46%)
OVF	4 (2%)	79 (6%)
Total	228 (100%)	1289 (100%)

Conclusion: vertebral wedge deformity without endplate fracture identified by VFA is equally prevalent in pre- and postmenopausal women and is not linked to low BMD.
Conflict of Interest: None declared

Su-OP09

EFFECT OF THYROID STATUS ON THE SKELETON IN POST-MENOPAUSAL WOMEN: THE OPUS STUDY

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OPUS is a population-based prospective cohort study of post-menopausal women from five European cities. We investigated whether TSH, fT4 and fT3 are associated with fracture risk, bone mineral density (BMD) at hip and spine, bone formation (procollagen type 1 N-terminal propeptide), bone resorption (N- and C-telopeptides of type 1 collagen, NTX, CTX), pulse rate, grip strength and balance. Vertebral fractures were determined at baseline and 6 years follow-up. Incident hip and other non-vertebral fractures were recorded and verified. Using strict exclusion criteria we defined a normal reference interval for thyroid function tests (TFTs)(thyroid disease-free group). Results: Over the 6 year follow-up period, 67 women had a new vertebral fracture, 11 women a hip fracture and 169 women a non-vertebral fracture. In the thyroid disease-free group ($n = 1754$) with adjustments for age and BMI, higher fT4 levels were associated with higher levels of NTX ($r = 0.064$, $P < 0.031$), lower BMD at the spine ($r = -0.063$, $P < 0.028$) and hip ($r = -0.057$, $P < 0.049$) and with incident hip fracture (mean fT4 [no fracture] 12.7 ± 1.9 vs fT4 [with fracture] 14.4 ± 2.3 pmol/L, $P = 0.028$). Hierarchical multiple regression, following adjustment for age and BMI, confirmed fT4 was associated with spine BMD ($\beta = -0.069$, $P = 0.032$). In the thyroid disease-free group, higher fT3 was associated with better balance ($r = 0.106$, $P < 0.001$; $F = 4.492$, $P = 0.023$) and increased grip strength ($r = 0.179$, $P < 0.001$; $F = 7.68$, $P < 0.001$). Hierarchical multiple regression confirmed these associations ($\beta = 0.094$, $P = 0.003$ for balance and $\beta = 0.187$, $P < 0.001$ for grip strength). Higher TSH was associated with lower NTX ($r = -0.134$, $P < 0.001$), P1NP ($r = -0.062$, $P = 0.031$), CTX ($r = -0.057$, $P = 0.05$) and poorer balance ($r = -0.083$, $P < 0.005$). Within the reference range, there was no association between fT3 or TSH and incident fractures. Conclusions: In post-menopausal women, the relationship between thyroid function and fracture risk is complex. Higher fT4 levels within the normal range are associated with reduced BMD. In contrast, low fT3 levels are associated with poorer balance and reduced muscle strength, both of which are potential risk factors for falls and fractures.
Conflict of Interest: None declared.

Su-OP10

INFLUENCE OF MENARCHEAL AGE ON DISTAL TIBIA MICROSTRUCTURE IN 20-YEAR OLD AND PREMENOPAUSAL MIDDLE-AGED WOMEN

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Late menarche is a risk factor for fragility fractures, probably by permanently affecting bone structural components so that pubertal-timing dependent alterations may persist from peak bone mass to menopause. We studied the influence of menarcheal age (MENA) on bone microstructure in healthy young adult (YOUNGAD, 20.4 ± 0.6 (±SD) years, n = 124) and premenopausal middle-aged (PREMENO, 45.8 ± 3.4 yrs, n = 120) women. Thirty-eight percent of the cohort were mother-daughter related. Volumetric bone density and microstructure were determined at distal tibia by non-invasive high resolution pQCT (XtremeCT, Scanco medical AG®, CH) including: total (Dtot), cortical (Dcort), and trabecular (Dtrab) volumetric bone density and fraction (BV/TV), trabecular number (TbN), thickness (TbTh) and spacing (TbSp), cortical thickness (CTh) and cross-sectional area (CSA). Areal bone mineral density (aBMD) was also measured by dual X-ray absorptiometry (DXA) at femoral neck (FN) and total hip (TH). Mother-daughters MENA were significantly correlated ($R = 0.39$, $n = 46$, $P < 0.01$). Median of MENA were 13.0 ± 1.2 and 13.1 ± 1.7 years in YOUNGAD and PREMENO, respectively. At distal tibia a significant inverse relationship was observed in YOUNGAD between MENA and Dtot ($R = -0.26$, $P = 0.004$), Dcort ($R = -0.22$, $P = 0.016$) and CTh ($R = -0.22$, $P = 0.015$). In PREMENO below the median of MENA as compared with above MENA, Dtot (295 vs. 322 mgHA/cm^3, $P = 0.009$), Dtrab (153 vs 167 mgHA/cm^3, $P = 0.022$), BV/TV (12.8 vs. 14.0 %, $P = 0.022$) and CTh (1138 vs 1232 μm, $P = 0.022$) were significantly lower, whereas there was a trend for greater CSA (669 vs. 630 mm^2, $P = 0.059$). Mean FN aBMD values were significantly lower in both YOUNGAD (838 vs. 878 mg/cm^2, $P = 0.042$) and PREMENO (785 vs. 825 mg/cm^2, $P = 0.042$) below median of MENA as compared with above. In conclusion, late menarcheal age is negatively associated with not only femoral neck aBMD, but also several microstructural elements of distal tibia. This influence of pubertal timing on both bone mass and microstructure, as observed at 20 years of age, i.e. at time of peak bone mass attainment is also expressed 25 years later at a time close to age of menopause. Alterations in both bone mineral mass and microstructural components could explain the increased risk of fragility fractures associated with later menarcheal age.

Conflict of Interest: None declared

Su-OP11

ACTIVATION OF THE CANONICAL WNT SIGNALING PATHWAY INDUCES CHONDROCYTE DEDIFFERENTIATION

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Proper function of both growth plate and articular cartilage requires a finely tuned balance between cartilage formation and resorption. Disruption of this balance causes either abnormalities in skeletal growth or degenerative cartilage diseases like osteoarthritis. The Wnt/β-catenin signaling pathway plays important roles in chondrocyte differentiation and in the maintenance of the chondrocytic phenotype through molecular mechanisms that are not yet fully understood.

To address this issue, the effect of increased canonical Wnt signaling on chondrocytes was studied during cartilage formation in conditional Col2a1-*Cre*;*Apc*$^{15lox/15lox}$ mouse embryos, which, due to *Apc* inactivation, exhibit high levels of transduced canonical Wnt signaling in chondrocytes. In addition, metatarsals of E18.5 wild-type mouse embryos were cultured *ex vivo* in the presence of a highly specific GSK3-β inhibitor, also resulting in the upregulation of the canonical Wnt signal.

Two types of nasal chondrocytes could be morphologically distinguished in the conditional Col2a1-*Cre*;*Apc*$^{15lox/15lox}$ E16.5 mouse embryos: some that were negative for β-catenin, expressing the nascent chondrogenic markers *Sox9* and *Col2a1* and some that stained positively for β-catenin due to inactivation of *Apc*. These latter cells displayed typical chondrocyte morphology and were embedded in an extracellular matrix. Interestingly, they did not express *Sox9* and *Col2a1* at the mRNA level and their matrix contained significantly less proteoglycans, suggesting that increased β-catenin activity in chondrocytes results in dedifferentiation. This finding was confirmed in a mouse metatarsal culture model. Activation of the canonical Wnt signal downregulated the expression of specific chondrocyte genes like *Sox9*, *Col2a1* and *Agg* at 24 hrs and strongly stimulated proteoglycan loss at 72 hrs. Prolonged culturing resulted in complete resorption of the cartilaginous bone explant.

Our *in vivo* and *ex vivo* data indicate that activation of the Wnt/β-catenin signaling pathway induces loss of the chondrocyte phenotype. This implies that modulators of the canonical Wnt signal might represent a potential pharmaceutical tool to repress chondrocyte dedifferentiation which occurs in the majority of cartilage degenerative diseases.

Conflict of Interest: None declared

Su-OP12

MYOSTATIN DEFICIENCY INCREASES CORTICAL BONE FORMATION INDEPENDENT OF CHANGES IN GROUND REACTION FORCES DURING NORMAL LOCOMOTION

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Myostatin (GDF-8) is a negative regulator of muscle mass and mammals lacking myostatin show a significant increase in muscle mass. We have previously shown that bone marrow stromal cells (BMSCs) isolated from mice lacking myostatin show increased osteogenic potential, and BMSCs also express the myostatin receptor, the type IIB activin receptor. These findings suggest that myostatin deficiency may directly increase bone formation independent of its effects on muscle mass. In order to further test this hypothesis we examined bone formation and in vivo locomotor forces in weight-matched normal and myostatin-deficient mice four months of age. Mice walked across a small custom-designed force plate and forelimb ground reaction forces were measured. Contact time, peak vertical and transverse ground reaction force, rate of loading, and impulse (area under the force curve) were collected from the force traces. Forelimbs of mice were embedded in methyl-methacrylate, and the length of fluorochrome labeled periosteal and endocortical surfaces measured from the ulna midshaft. Results showed that weight-matched normal and myostatin-deficient mice did not differ in any of the kinetic variables measured. Yet, mice lacking myostatin showed ~15% increase in periosteal mineralizing surface ($P < .05$) and ~20% increase in endocortical mineralizing surface ($P < .05$) compared to the normal mice. Although mice were similar in body weight, the myostatin-deficient animals showed increased forelimb muscle mass ($P < .001$). These data suggest that peak locomotor forces are unlikely to directly link the increased muscle mass of myostatin-deficient animals with increased bone formation and that other mechanisms, such as a direct effect of myostatin deficiency on osteoprogenitor cell differentiation, are likely. Funding for this research was provided by the National Institutes of Health (AR 049717).

Conflict of Interest: None declared

Su-OP13

IN VITRO OSTEOCLASTOGENESIS AND BONE RESORPTION DURING SPACEFLIGHT

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Serious effects on human health are experienced after long duration space missions. Prolonged exposure to microgravity seems to affect several physiological systems. Bone loss is considerable, with losses of 1–2% of bone mass per month in flight, occurring predominantly in the load bearing regions of the legs and lumbar spine. Microgravity induces an uncoupling of bone remodeling between bone formation and resorption that could lead to bone loss. Both processes are probably involved, but their relative importance and how they are orchestrated remain unclear. In order to fully understand the mechanisms underlying this bone loss we participated to the FOTON M3 mission launched on September 2007 that carried three experiments OSTEO, OCLAST and PITS developed by our team. For the first time the effect of microgravity directly on osteoclasts (OCs) was studied in vitro and our preliminary results indicate that OCs are directly affected. The OSTEO experiment has been conducted within bioreactors with a perfusion system, where the differentiation of precursors in mature OCs has been tested utilizing as a support a synthetic 3D bone-like biomaterial, skelite, that partially reproduces the chemical composition and physical structure of natural bone. The aim was to analyze the gene expression of osteoclast differentiated in microgravity, compared with ground controls. RNA extracted from the cells has been examined by RT-PCR. The preliminary results indicate that gene involved in osteoclast final maturation and activity, as integrin beta3, cathepsin K, MMP9 were several fold higher in microgravity in comparison with the same cultures on ground, while other genes were substantially at the same level. A microarray analysis is under way. In OCLAST and in PITS experiment mature OC were cultured on devitalized bovine bone slices in appropriate temperature conditions. The experiments started in orbit and were stopped after 4 days in microgravity. After landing the amount of collagen telopeptides was measured from OCLAST culture media, while from PITS samples, RNA was obtained for a genetic screening. After 5 days of space flight an increase in OC activity was found, proved by an increased number of excavated pits VS the ground control and by an increase in the expression of genes related to osteoclast activity. These results have been further confirmed by simulating microgravity conditions on ground with an ad hoc centrifuge mimicking weightless conditions. These preliminary data indicate osteoclasts precursors as direct target of microgravity conditions.

Conflict of Interest: None declared

Su-OP14

MYELOMA CELL-INDUCED COLLAPSE OF THE VASCULAR BONE REMODELING COMPARTMENTS LEADS TO OSTEOLYSIS AND THE GENERATION OF OSTEOCLAST-MYELOMA HYBRID CELLS

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Bone remodeling is a tightly coupled process where bone formation follows bone resorption to maintain the integrity of the skeleton throughout life. Bone remodeling occurs in a specialized vascular bone remodeling compartment (VBRC), separated from the marrow compartment by a cell wall. In the case of myeloma-induced osteolysis, bone formation does not follow and compensate for the increased bone resorption. This uncoupling is a major hallmark of multiple myeloma (MM). Here we report that VBRCs often are disrupted in MM and that this destabilization correlates with: 1) an uncoupling of bone resorption and bone formation, 2) the presence of bone osteolysis shown by X-rays, 3) a high density of MM cells 4) a direct cell contact between MM cells coming from the marrow compartment and osteoclasts, leading to the formation of myeloma-osteoclast hybrid cells.

Bone histomorphometric analysis of the VBRC, showed that MM patients having less than 75% erosion surface (ES) in VBRC show an increased ES/BS and a decreased osteoid surface (OS/BS) compared to patients with more ES in VBRC. The VBRC wall or its remains were always present at ES. Interestingly, collapse of VBRCs correlated significantly with the number of osteolytic lesions demonstrated by X-ray, as patients with no osteolytic lesions showed 80% of the ES in VBRC, while patients with more than 5 osteolytic lesions showed only 58% of the ES in VBRC.

Immunohistochemistry of MM cells and osteoclasts in intact or collapsed VBRCs revealed that only osteoclasts in collapsed VBRC had a direct contact with MM cells. These were more often focally positioned in the vicinity of the collapsed VBRCs (68% vs. 15%) compared to intact VBRCs.

In vitro, we mimicked the VBRC wall by establishing a confluent G0-arested mono-layer of MC3T3 cells and tested its response to MM cells. Co-cultures with MM cells disrupted the confluent layer of MC3T3 cells mimicking the destabilization of the VBRC wall.

Interface FISH for MM clone specific translocations within the osteoclasts, revealed that the disruption of VBRC is a critical event in the generation of osteoclast-myeloma clone hybrid cells that we demonstrated earlier.

In conclusion the VBRC is a micro-anatomical structure that is important for the integrity of the bone remodeling process and its collapse is a key event in the development of MM bone disease. The VBRC is disrupted directly by MM cells, favoring the development of osteolytic lesions and the formation of myeloma-osteoclast hybrid cells.

Conflict of Interest: None declared

Su-OP15

CANCER STRESS AFFECTS TRANSCRIPTOME OF BONE CELLS: METASTASES AND MULTIPLE MYELOMA GENERATE DISTINCT TRANSCRIPTIONAL FOOTPRINTS IN OSTEOCYTES IN VIVO

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Little is known of how osteocytes, the most abundant cells in bone, react to cancer stress. We have comparatively studied the effect of bone-residing and metastases of bone-remote cancers on osteocytes *in vivo*. While morphological changes in bone associated with these tumour types are indistinguishable, the changes in the transcriptome of osteocytes are specifically related to the tumour stress present. Screening roughly 22,000 genes in osteocytes prepared from cryosections of native, i.e., undecalcified, bone using laser-supported

microdissection, roughly 1,400 and 1,800 gene expression differences were observed between osteocytes dissected from normal bone compared with those associated with metastases and multiple myeloma, respectively. A group of genes affected by both showed clear specificity: The genes elevated in expression due to the stress exerted by metastases were repressed by multiple myeloma, and *vice versa*, indicating stress-specific footprints in the transcriptome of osteocytes. Functionally, the stressors seem to impose selective pressures on signalling pathways such as that of TGFbeta, a major player in bone biology. Our data show for the first time that the transcriptome of osteocytes *in vivo* becomes strongly affected by cancer stress generating gene expression footprints which, in contrast to comparable morphological changes, appear to relate to the nature of cancer and might thus become helpful in distinguishing different bone diseases.
References: Eisenberger S, Hoppe G, Pyerin W, Ackermann K. High quality RNA preparation for transcript profiling of osteocytes from native human bone microdissections. Anal. Biochem. 2004;**335**: 260–266.
Eisenberger S, Ackermann K, Voggenreiter G, Sültmann H, Kasperk C, Pyerin W. Metastases and multiple myeloma generate distinct transcriptional footprints in osteocytes *in vivo*. J. Pathol., in press.
Conflict of Interest: None declared

Su-OP16

IN VIVO AND IN VITRO BONE PHENOTYPE CHARACTERIZATION OF THE IMMUNODEFICIENT ADA-KO MOUSE

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Background: The bone marrow microenvironment is defined by close interaction between cells derived from mesenchymal and hematopoietic progenitors. It has long been established that the latter require the support of stromal elements to engraft, self-renew, and progress towards lineage commitment. In Adenosine Deaminase (ADA)-deficient patients, defects in both compartments manifest predominantly as immunodeficiency but also as bone growth abnormalities. ADA-ko mice retain many features associated with ADA deficiency in humans, including SCID and a profound metabolic defect. The study aims to investigate bone growth and mineralisation and to characterise the bone marrow microenvironment in the murine ADA-ko model.

Results: In order to understand the mechanism underlying the ADA-ko bone phenotype, bone architecture and density was analysed in vivo by peripheral quantitative computed tomography (pQCT). ADA-ko mice showed a specific bone phenotype in which the dominance of periosteal bone apposition was lost and the density of the trabecular compartment was reduced compared to controls. These features occured despite the preservation of appropriate bone material properties. The sRANKL/OPG ratio was decreased in the serum of ADA-ko mice compared to wt, indicating a possible in vivo reduction in osteoclasts formation. Conversely in vitro osteoclastogenic assays showed no difference in osteoclasts formation from M-CSF and RANKL stimulated bone marrow of ADA-ko mice compared to wt. Interestingly osteoblasts (OB) from calvariae of ADA-ko mice show a significant reduction in the proliferation rate compared to OB from wt mice. Viability in ADA-ko OB was reduced while the apoptosis was significantly increased. Viability of both wt and ADA-ko OB decreases dose dependently when exposed to increasing concentrations of the ADA substrate Adenosine (0–1 mM). On the other hand the proliferation rate of ADA-wt OB is significantly reduced when cultured in presence of the ADA inhibitor EHNA (100 mM). ADA-ko OB do not show any difference in the expression of specific osteoblastic markers such as runx2, alkaline phosphatase (ALP), osteocalcin (OC), OPG and RANKL, but ALP activity is decreased in ADA-ko OB cultures.

Conclusions: The results obtained in vivo and in vitro suggest that the ADA metabolism may represent an additional modulatory factor of bone remodelling and may help to understand the role of purine signalling in modulating bone cell activities.
Conflict of Interest: None declared

Su-OP17

THE IKK INHIBITORS CELASTROL AND PARTHENOLIDE INHIBIT BREAST CANCER CELL PROLIFERATION AND MIGRATION IN VITRO AND OSTEOLYTIC BONE METASTASIS IN VIVO

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NFκB activation is known to promote the proliferation of cancer cells and the development of metastases. Studies have shown that genetic inactivation of NFκB and IKK signaling reduce pro-inflammatory cytokine-induced signaling in cancer cells and prevent the development of metastases in mice. Here we studied the effect of the inhibitors of IKK, Celastrol (IKKα/β and TAK-1 inhibitor), BMS345541 (selective IKKα/β inhibitor) and Parthenolide (IKKβ inhibitors) on the proliferation and migration of Walker 256 (W256) cells in vitro and osteolytic bone metastasis in vivo. All compounds tested inhibited the proliferation of W256 cells (IC50 0.43 μM for Celastrol; 4.7 μM for BMS345541 and 8.5 μM for Parthenolide) and induced W256 apoptosis as evidenced by caspase-3 activation and nuclear morphology. Pretreatment of W256 with Celastrol (1 μM) and Parthenolide (10 μM) for 1 hour prior to stimulation with IL-1β, TNFα or TGFβ completely abolished the phosphorylation of IκB and prevented NFκB nuclear translocation. Celastrol, but not Parthenolide prevented the recruitment and binding of TAK-1 to RANK, one of the earliest events in this cascade. None of the compounds tested inhibited BMP-2 or TGFβ-induced Smad1/5/8 activation, a pathway which does not involve IKK activation. Both Celastrol (0.5 μM) and Parthenolide (2 μM) inhibited the migration of W256 in vitro and markedly reduced mRNA expression of MMP-9 (59%, $p < 0.01$) and urinary plasminogen activator (48%, $p < 0.05$). We investigated the effects of IKK inhibition on osteolytic bone metastases using an in vivo model of the metastatic breast cancer, the rat W256 cell model. 36 animals received intracardiac injection of W256 tumor cells (10^7 cells) followed by intraperitoneal injection of either vehicle, Celastrol (1 mg/kg/day) or Parthenolide (1 mg/kg/day). Animals were sacrificed 10 days post injection and both tibias and femurs were analyzed by numeric radiography and microCT. All vehicle treated animals (9/9 animals) presented with massive trabecular bone loss below the growth cartilage indicative of osteolytic bone lesions, whereas 78% of rats treated with Parthenolide (9/11 animals; $p < 0.01$) and 89% treated with Celastrol (8/9 animals; $p < 0.01$) were completely protected. In conclusion, Celastrol and Parthenolide are effective in preventing osteolytic bone metastasis. Therefore, small molecules inhibitors of IKK may represent a promising new therapeutic agent for treating both inflammatory and cancer associated bone diseases.
Conflict of Interest: Aymen Idris is a recipient of ECTS/Amgen Bone Biology Fellowship

Su-OP18

TNF-RELATED APOPTOSIS INDUCING LIGAND (TRAIL) INHIBITS PRIMARY BONE TUMOR GROWTH AND AUGMENTS SURVIVAL IN A HUMAN MODEL EWING SARCOMA

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Ewing's sarcoma is a small round-cell tumor typically arising in the bones, rarely in soft tissues, of children and adolescents. The development of multi-disciplinary therapy with chemotherapy, irradiation and surgery has increased current long-term survival rates in most clinical centres to greater than 50%. However, patients with clinically detectable metastases at diagnosis, or patients not responding to therapy and patients with disease relapse have a significantly poorer prognosis (20%). Among new therapeutic approaches, TNF-related apoptosis-inducing ligand (TRAIL) is a death ligand that possesses selective anti-tumor activity against a number of cancer cell lines without systemic toxicity.

In this study, we investigated the sensitivity of several human Ewing's sarcoma cell lines all expressing the EWS-FLI1 fusion gene (A673, SIM, TC-71, TC-32, SK-ES1, RDES) to TRAIL in terms of proliferation, apoptosis and receptor expression, and its potential preclinical application in the corresponding animal models developed in nude mice.

Results of in vitro proliferation assays showed that the cell lines exhibit differential sensitivity to TRAIL: highly sensitive cell lines (TC-71, A673, RDES: EC50 between 0.5 and 1 nM), weakly sensitive (TC-32, SK-ES1; EC50 = 10 nM) and resistant ones (SIM). The inhibition of cell proliferation in sensitive cells is caused by cell death induced by caspase 3 activation. RT-PCR analysis revealed that the cell lines differentially express TRAIL activator (DR4, DR5) and decoy (DcR1, DcR2, OPG) receptors, this differential expression being independent of the p53 status of the cells.

Using a human model of Ewing sarcoma induced by intra-muscular injection of human A673 cells in nude mice, TRAIL administered by non viral gene therapy inhibits the primary bone tumor growth (−86%, n = 8) leading to a significant 2-fold increase of animal survival 40 days after tumor induction. TRAIL also exerts a beneficial effect on mice survival in a model of lung metastases developed from intravenous injection of osteosarcoma cells, another primary bone tumor.

The overall results suggest that TRAIL may represent a good candidate for the development of new therapeutic strategies in Ewing sarcoma.

Conflict of Interest: None declared

Su-OP19

SKELETON REGULATES PANCREATIC FUNCTION VIA BONE MORPHOGENETIC PROTEIN-6 (BMP-6)

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Skeleton might act as an endocrine regulator of energy metabolism via osteocalcin (Lee et al, Cell, 2007). Systemic administration of BMP-6 restores the bone volume (BV) in osteoporotic rats (Simic et al, J Biol Chem, 2006) and lowers the blood glucose in Bmp-6−/− and non obese diabetic mice (Orlic et al, Calc Tiss Int, 2007). We hypothesized that BMP-6 released from the bone might mediate the endocrine function of the pancreas. We therefore revisited the phenotype of the Bmp-6 −/− mouse and found that the loss of Bmp-6 function exerts both an abnormal bone development and the reduced pancreatic function. Although long bones of the wild type (WT) and Bmp-6 −/− littermates are of a similar size and shape, BV is reduced up to 120% and 80% at embryonic day 15 and 17, respectively. Apart from the abnormal bone development, Bmp-6 −/− born mice have a reduced number of Langerhans islands with a lower serum insulin and a subsequent increase of the serum glucose levels. Exogenously injected BMP-6 (60 μg/kg/3 × week) reduced the blood glucose in 2 h and at 3 weeks of therapy increased the BV of Bmp-6 −/− mice to normal. To test whether the endogenous BMP-6 deposited in the bone matrix could be released into the circulation and affect the serum glucose level we treated Bmp-6 −/− and WT mice with 3 injections of phosphate (0.6 mg/kg i.v.) to stimulate the bone resorption. This resulted in the identification of BMP-6 in plasma of WT mice by liquid chromatography—tandem mass spectrometry and western blot analysis. We then exposed the WT mice to the glucose tolerance test at days 3, 5 and 7 following the phosphate overload, which resulted in a higher reduction of glycemia in animals with bone released BMP-6 in the serum as compared to the control WT mice ($P < 0.01$). Molecular analyses of tissue samples revealed that BMP-6 inhibited the hepatic glucose production and activated the expression of the key enzymes of the lipid metabolism. BMP-6 also increased the expression of IGF-I in the pancreas, liver, bone and the serum. We then showed that the co-administration of an IGF-I neutralizing antibody reduced the BMP-6 glucose lowering effect, suggesting that BMP-6 acts, at least in part, via an IGF-I related mechanism. These results suggest that BMP-6 has a developmental role in the bone and pancreas maturation, and that in the post natal life the skeleton regulates the glucose homeostasis via releasing BMP-6 from the bone matrix.

Conflict of Interest: None declared

Su-OP20

SILENCING LAMIN A/C DECREASES OSTEOBLAST DIFFERENTIATION AND STIMULATES OSTEOCLASTOGENESIS THROUGH ENHANCED RANKL SIGNALING

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Age-related osteoporosis is a condition of low bone mass and poor bone quality predisposing the elderly to fractures. Low bone turnover in senile osteoporosis results from impaired osteoblast proliferation and differentiation. However, the underlying molecular mechanisms are poorly defined. Recently, the Hutchinson-Gilford progeria syndrome, a disease of accelerated aging, has been linked to mutations in the lamin A/C gene providing novel insights into cellular senescence. Here, we test the hypothesis that the loss of lamin A/C in osteoblasts leads to impaired osteoblast differentiation and results in an altered interaction with osteoclasts. Human mesenchymal stem cells were cultured in osteogenic medium for 21 days. Lamin A/C was

continuously knocked-down using 50 nM specific siRNAs. Cell proliferation was assessed using a tetrazolium salt-based assay. The mineralization capacity was determined with alizarin red S staining. In addition, mRNA steady state levels of runx2, osteocalcin, RANKL and OPG were analyzed by real-time RT-PCR. Protein levels of RANKL and OPG were assessed using an ELISA. In co-culture systems, the ability of osteoblasts to support osteoclastogenesis was investigated by counting the number of tartrate-resistant acid phosphatase (TRAP)-positive, multinucleated cells. We found that expression of lamin A/C increased with osteoblast maturation. Lamin A/C deficiency significantly reduced osteoblast proliferation and differentiation by 30% and 34%, respectively ($p < 0.001$ and $p < 0.05$). The mRNA expression of runx2 and osteocalcin in mature lamin A/C-deficient osteoblasts was significantly reduced (−44% and −78%, $p < 0.05$). The RANKL/OPG ratio was significantly increased at mRNA (+200%, $p < 0.001$) and protein level (+162%, $p < 0.05$), resulting in an increased ability to support osteoclastogenesis as assessed by counting TRAP-positive multinucleated cells (+34%, $p < 0.05$). Our data indicate that the nuclear protein lamin A/C is important for mesechymal stem cell differentiation into mature osteoblasts and that lack of lamin A/C favours conditions for enhanced osteoclastogenesis. These data provide a novel connection between age-related osteoblast insufficiency and loss-of-function of lamin A/C, which is mutated in the progeroid syndrome HGPS.

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Conflict of Interest: None declared

Mo-OP21

SERUM HOMOCYSTEINE LEVELS AND BONE MATRIX QUALITY

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High serum homocysteine levels have been suggested to be a risk factor for osteoporotic fractures in elderly patients. Collagen is the most abundant protein of the organic matrix in mineralizing tissues. Collagen molecules undergo intermolecular cross-linkings which provide mechanical properties (strength and elasticity) to the bone organic matrix. Homocysteine is known to interfere with the cross-linking of the collagen and abnormally high serum levels might potentially affect the mechanical properties of bone.

From 200 patients with acute femoral neck fractures scheduled for hemiarthroplasty, we selected 9 females (age 70 to 95 years) with high (mean±SD, 28.1 ± 8.0 µmol/l) and 9 with low (mean ± SD, 7.0 ± 0.7 µmol/l) serum homocysteine levels. From these, the femoral heads were collected during surgery for determination of collagen cross links.

Fourier transform infrared imaging (FTIRI) was used to determine the ratio of non reducible pyridinoline versus reducible dehydro-dihydroxylysinonorleucine (Pyr/deH-DHLNL) collagen cross-links in 2-to-4-µm-thick bone sections from these human femoral heads. Biopsies were measured for the ratio of the relative areas of the absorption peaks at the wavelength of 1660 and 1690 cm^{-1} for Pyr/deH-DHLNL in forming, resorbing and interstitial trabecular bone areas.

In bone formation areas, patients with high homocysteine levels had significantly higher Pyr/deH-DHLNL ratios than patients with low serum homocysteine levels (+56%; $P < 0.05$) In bone resorption areas, the high serum homocysteine level group showed a higher Pyr/deH-DHLNL ratio (+38%) but not significantly. A similar trend was obtained in the interstitial trabecular bone area (+40%).

The results provide evidence that a high serum homocysteine level is associated with impaired collagen maturation and cross-linking. Further studies have to clarify if this might lead to an increased bone fragility.

Conflict of Interest: None declared

Mo-OP22

COLLAGEN'S CONTRIBUTION TO BONE STRENGTH

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In the present study the lathyritic rat animal model was employed to decipher the contribution of collagen cross-links to bone strength. 24 female Wistar (47-days old) rats of equal weight were divided into two groups: control and treatment (12 rats per group). They were kept in separate cages and fed a semi-synthetic diet containing 0.6% calcium and 0.6% phosphorus. β-aminopropionitrile fumarate (b-APN; 0.1% dry weight) was added to the diet of the treatment group. After 15 (6 rats per group) and 30 days, the rats in the two groups were sacrificed, and the L3-L6 vertebra were collected. Biochemical analysis of the borohydride-reduced tissues indicated decreases of 11% and 20% in the concentrations of the DHLNL cross-link, compared to the appropriate controls. Corresponding, marginal decreases were observed for pyridinoline (pyr) (1% and 6%), deoxypyridinoline (3% and 2%), and HLNL (1% and 4%). Two dimensional histomorphometric determination of trabecular bone (L5) structural parameters (BV/TV, BV/TS, Tb.N, TbPF) revealed no differences between the four groups with the exception of trabecular thickness (Tb.Th) at 4 weeks. Quantitative backscattered electron imaging (qBEI) analysis revealed no differences between the 4 groups in any of the mineralization outcomes monitored (CaMean, CaWidth, CaLow, CaPeak). Uniaxial compression tests of the vertebral bodies revealed a significant ($p < 0.05$) reduction in stiffness compared to the appropriate controls at the two time points, respectively. Similarly, maximal force or strength and energy to failure was reduced ($p < 0.05$). Analysis of the spatial variation of pyr and DHLNL collagen cross-links by Fourier transform infrared imaging (FTIRI) revealed differences similar to those observed by biochemical analysis, with the added information that the changes were observed only within the first 40 µm of forming trabecular surfaces as determined by histology and qBEI analysis. Nanoindentation tests indicated that the only areas where E modulus and hardness were reduced by 35% and 40%, were indeed in the same areas as seen with the FTIRI analyses. The results of the present study clearly indicate the importance of collagen in determining bone strength, even independent of amount and quality of mineral. Moreover, they emphasize the importance of collagen cross-links, a finding deserving further study especially in view of the recent clinical studies correlating fracture risk with serum concentrations of homocysteine.

Conflict of Interest: None declared

Mo-OP23

USE OF THIAZIDE DIURETICS AND RISK OF HIP/FEMUR FRACTURE: A POPULATION-BASED CASE-CONTROL STUDY AND THE FIRST-EVER META-ANALYSIS OF DOUBLE BLINDED RCTS

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Introduction: Over the past two decades, a majority of at least eight observational studies have reported inverse associations between use of thiazide diuretics and risk of hip fracture. Recent findings that all classes of antihypertensive drugs, and statins, may protect against fracture risk suggest unmeasured distortion in health care databases. Randomization, but also smoothing spline analyses can be used to determine whether such inverse association is more likely to reflect unmeasured bias or a causal relationship. Objective of this study was to evaluate the association between use of thiazide diuretics and the risk of hip/femur fracture.

Methods. First, we conducted a population-based case-control study using data from the UK General Practice Research Database (GPRD). Cases were patients with a first hip/femur fracture; controls were individually matched on practice/region, gender, year of birth and calendar time. Current use of thiazide diuretics was defined as a prescription in 90 days before the index date. We adjusted for medical conditions and drugs associated with falling or bone mineral density. Second, we conducted a meta-analysis of double blinded RCTs in which use of thiazide diuretics > 6 months was compared with placebo.

Results: The case-control study population included 22,247 cases and controls in the GPRD. Current use of thiazide diuretics was not associated with a reduced risk of hip/femur fracture (adj. OR 0.93 95% CI [0.86–1.00]). A higher cumulative exposure (> 40 gram hydrochlorthiazide eq.) did not change the risk of hip fracture (adj. OR 1.02 95% CI [0.89–1.17]. Smoothing spline plots did not support a duration of use or average daily dose relationship. In the meta-analysis (6 RCTs), 34 hip/femur fractures occurred among 2,564 thiazide diuretic users, whereas this was 35/2,866 in the placebo group, yielding a Mantel-Haenzel OR of 1.02 95% CI [0.64–1.62].

Conclusions. None of the exposure analyses in the case-control study was in line with a potential beneficial effects of thiazide diuretics on risk of hip fracture. The absence of beneficial effects of thiazide use on hip fracture risk in the meta-analysis of RCTs and the case-control study may indicate presence of undetected distortion in previous epidemiological studies, rather than a causal effect.

Conflict of Interest: None declared

Mo-OP24

EFFICACY OF BAZEDOXIFENE IN REDUCING NEW VERTEBRAL FRACTURE RISK IN POSTMENOPAUSAL WOMEN WITH OSTEOPOROSIS: RESULTS FROM A 3-YEAR, RANDOMIZED, PLACEBO- AND ACTIVE-CONTROLLED CLINICAL TRIAL

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Bazedoxifene (BZA), a novel selective estrogen receptor modulator (SERM), is currently in clinical development for the prevention and treatment of postmenopausal osteoporosis. We report the results of a 3-year, Phase III study that evaluated the effect of BZA therapy on the incidence of new vertebral fracture compared with placebo (PBO) and raloxifene (RLX) in postmenopausal women with osteoporosis. This study enrolled generally healthy postmenopausal women aged 55–85 years with lumbar spine (LS) or femoral neck (FN) T-scores ≤ -2.5 and no prevalent vertebral fractures *or* LS or FN T-scores ≥ -4.0 with prevalent vertebral fractures. Participants were randomized to receive 20 mg/d or 40 mg/d BZA, 60 mg/d RLX, or PBO and received supplemental calcium (1200 mg) and vitamin D (400 IU). The primary efficacy outcome was the incidence of new vertebral fractures after 36 months; secondary outcomes included non-vertebral fractures (NVFs). A total of 7492 subjects (mean age $\pm$ SD, 66.4 $\pm$ 6.7 years) were randomized and received ≥ 1 dose of study medication. At baseline, mean LS T-score was -2.4, mean total hip T-score was -1.4, and 56% of women had ≥ 1 prevalent vertebral fracture (mostly mild). The 3-year incidences of new vertebral fractures, based on Kaplan-Meier estimates, were 2.3%, 2.5%, 2.3%, and 4.1% in the BZA 20 mg, BZA 40 mg, RLX 60 mg, and PBO groups, respectively, with a relative risk reduction for new vertebral fracture of 42% ($P = 0.015$), 37% ($P = 0.031$), and 42% ($P = 0.012$), respectively, versus PBO. There was overall no treatment effect on NVFs. In a post-hoc analysis of women with FN T-score ≤ -3.0 or ≥ 1 moderate or multiple vertebral fracture ($n = 1772$), NVF incidence was 4.9%, 6.5%, 8.4%, and 9.1% in the BZA 20 mg, BZA 40 mg, RLX 60 mg and PBO groups, respectively. Relative to PBO, BZA 20 mg reduced NVF incidence by 50% ($P = 0.02$). Similar reduction was observed when both BZA doses were combined (40% reduction, $P = 0.03$). We conclude that BZA treatment significantly reduced the risk of new vertebral fractures, and in subjects at higher risk for fractures, was associated with a significant reduction in NVFs.

Conflict of Interest: H. K. Genant, Synarc, Stock; Amgen, GSK, Merck, BMS, Servier, Eli Lilly, SABs with Compensation
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J. R. Zanchetta, Eli Lilly, Pfizer, Wyeth, NPS, Amgen, MSD, Servier, Consultant; Eli Lilly, Wyeth, MSD, Pfizer, Roche, Lecturer
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Mo-OP25

COMPARATIVE EFFECTS OF TERIPARATIDE AND STRONTIUM RANELATE ON BIOCHEMICAL BONE MARKERS AND ILIAC CREST BIOPSIES IN POSTMENOPAUSAL WOMEN WITH OSTEOPOROSIS

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We compared the early osteoanabolic effects of teriparatide (TPTD) with those of strontium ranelate (SR) as measured by biochemical markers of bone turnover and iliac bone biopsies, in postmenopausal women with osteoporosis in a multicenter, open-label, randomized trial. Eighty patients (mean age: 64.6 yrs) were randomized to 20 mcg/d of TPTD (n = 40) or 2 g/d of SR (n = 40). Tetracycline-double labeled iliac crest biopsies were obtained from 57 subjects (TPTD:29; SR:28) who were treated for 6 months. Six biopsies from the SR group were not valid for analysis. Standard histomorphometric parameters at the cancellous, endocortical and cortical levels were measured. Serum biochemical markers of bone formation (P1NP, BSAP), and bone resorption (ß-CTx) were evaluated at 0, 1, 3 and 6 months. Table 1 summarizes MS%BS and % change of bone markers after 6 months. Other dynamic indices of bone formation were higher in the TPTD group than in the SR group, but there was insufficient evidence of a statistical difference. Cortical porosity was higher in the TPTD group (5.40% vs 4.14%; p = 0.037). In the TPTD arm, a rapid increase in P1NP was observed, while there was a slight decrease in the SR group (Figure). Patients reported adverse events more frequently in the SR group (70% vs 41%; p = 0.013). TPTD shows a more consistent and robust effect on bone formation activity than SR after 6 months of treatment.

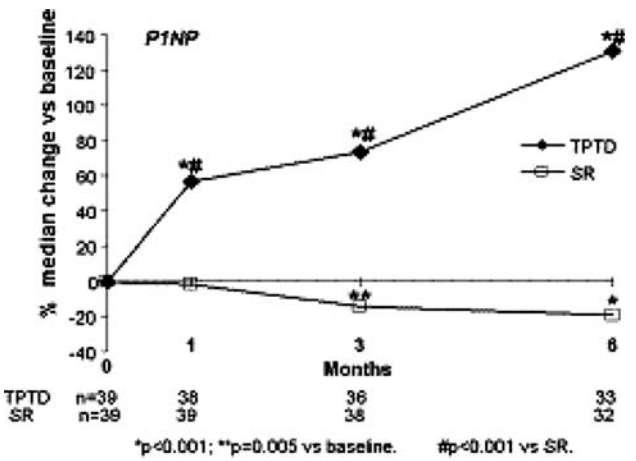

Table 1 MS%BS [mean (SE)], and median % change of bone markers

	TPTD	SR	p-value
MS%BS (Cn.)	7.73 (1.48)	5.25 (1.15)	0.219
MS%BS (EndoCt.)	17.22 (3.06)	9.70 (2.07)	0.052
P1NP (n)	131.26%* (33)	−18.75%* (n = 32)	<0.001
BSAP (n)	32.44%* (n = 32)	−1.10% (31)	0.005
sCTX (n)	110.80%* (n = 33)	−6.30% (32)	<0.001

Cn: Cancellous. EndoCt: Endocortical.*p<0.005 vs basely

Conflict of Interest: B. Sanz, H. Oertel and F. Marin are employees of Lilly.

Mo-OP26

A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY OF ODANACATIB (MK-822) IN THE TREATMENT OF POSTMENOPAUSAL WOMEN WITH LOW BMD: 18-MONTH RESULTS

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Cathepsin K, a cysteine protease abundantly expressed in osteoclasts, is necessary for bone collagen degradation. Odanacatib, a selective inhibitor of cathepsin K, has been shown to rapidly and reversibly decrease bone resorption in both preclinical and Phase I clinical studies.

A 1 + 1 year dose-ranging trial is ongoing in postmenopausal women with low BMD to evaluate the safety, tolerability and efficacy of weekly doses of placebo, 3, 10, 25 or 50 mg of odanacatib on BMD and biochemical indices of bone turnover. The primary hypothesis for the 1-year extension study was that odanacatib would increase lumbar spine BMD compared with placebo over 24 months. An interim analysis comparing BMD at 18 months of treatment with that at baseline was performed. Patients and investigators remain blinded to treatment allocation.

Postmenopausal women (N = 399) with BMD T-scores ≤ -2.0 and ≥ -3.5 at lumbar spine (LS), femoral neck (FN), trochanter, or total proximal femur were randomized to receive placebo or 1 of 4 doses of odanacatib. Mean age was 64.2 ± 7.8 years. Three hundred twenty women completed the 12-month base study and entered the 1-year extension.

Treatment produced dose-related increases in BMD from baseline. The highest dose of odanacatib tested (50 mg) resulted in a 4.8% increase from baseline in lumber spine BMD vs. 0.15% for placebo and a 2.4% increase from baseline in total hip BMD vs. a 1.2% decrease for placebo. The 50-mg dose resulted in a 49% reduction in uNTx/Cr vs. 2.3% for placebo and a 9% reduction in BSAP vs. a 4% increase for placebo.

The safety profile of odanacatib was generally favorable. There were no dose-related trends in any adverse experiences (AEs). The number of patients who discontinued the study due to AEs was the same for the placebo and 50 mg groups (9 patients), as was the number who discontinued due to AEs considered drug-related (4 patients). Rash was reported in 3.8% of those in the 50-mg arm and in 8.4% of those in the placebo arm.

In summary, 18 months of odanacatib treatment was generally well-tolerated and increased lumbar spine and total hip BMD in postmenopausal women with low BMD.

Conflict of Interest: A. Ince, N. Verbruggen, A. Rybak-Feiglin, C. daSilva, A. Santora, Merck and Co., Inc, Employees.
M. McClung, H. Bone, Merck and Co., Inc., Consulting Fees.

Mo-OP27

THE 10-YEAR PROBABILITY OF RECURRENT OSTEOPOROTIC FRACTURES IN POSTMENOPAUSAL WOMEN AFTER A WRIST FRACTURE, AND THE NUMBER NEEDED TO TREAT FOR 5 YEARS WITH AN ORAL BISPHOSHONATE TO PREVENT A SECONDARY FRACTURE

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Background: Wrist fractures are the most prevalent of the "osteoporotic" fractures in post-menopausal women, and herald future fractures. Little is known of the absolute risk of recurrent (2^0) fracture after a 1^0 wrist fracture, as compared with other 1^0 fracture sites.

Methods: 21,432 women age 45 y and older were referred to the Manitoba Bone Density Program for baseline hip DXA testing. Longitudinal health service records were assessed for the presence of fracture codes before (12.7 ± 2.4 y) and after (4.1 ± 2.3 y) BMD testing, allowing for 359,737 pt.y of observation. Within the cohort, 2,652 (12.4%) experienced a 1^0 fracture of the wrist, humerus, vertebra, or hip, of which 1,225 (46.2%) were wrist fractures. From the 5 yr. risk for 2^0 fracture (Kaplan-Meier method), estimates of the NNT with an oral bisphosphonate were made assuming a 20% risk reduction vs. no treatment.

Results: 10-year probabilities of a 2^0 fracture after 1^0 wrist fracture were 14.2% (95%CI, 9.1–14.3), significantly less than for other 1^0 fractures (spine 25.7%, hip 24.9%, humerus 23.7%; all $p < 0.001$ vs. wrist) but greater than in those without prior fractures at the time of BMD testing (10.8%, $p < 0.001$). The table shows the NNT estimates for 1^0 wrist vs. other 1^0 fracture sites (spine, hip, humerus), categorized by age 65 (older vs. younger) and femoral neck T-score (osteoporotic vs. non-osteoporotic). NNT's were consistently higher in women with wrist fractures than for those with other 1^0 fractures.

Conclusions: The risk of recurrent osteoporotic fracture following a wrist fracture is intermediate between the risk for women with no prior fractures and the risk for women with prior fractures of the vertebra, humerus or hip. The efficiency of oral bisphosphonate therapy following a wrist fracture is maximized by selecting women older than age 65 years with osteoporotic BMD.

Table 1 NNT for 5 y to prevent a recurrent fracture

Primary Fracture	Age < 65 yrs.	Age > 65 yrs.
	T-score above −2.5	T-score above −2.5
None	162 (143–187) *	73 (64–85)
Wrist	88 (63–147)	72 (54–110)
Others sites	46 (36–65)	37 (30–48)
	T-score below −2.5	T-score below −2.5
None	66 (51–93)	30 (26–35)
Wrist	57 (32–246)	31 (22–44)
Other sites	39 (26–83)	26 (22–33)

* NNT (95% CI)

Conflict of Interest: None declared

Mo-OP28

CAN FALL RISK BE INCORPORATED INTO FRACTURE RISK ASSESSMENT ALGORITHMS: A PILOT STUDY OF RESPONSIVENESS TO BISPHOSPHONATES

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Recent tools developed for the prediction of fracture risk, including the FRAX™ tool produced by the WHO, have largely excluded falls as a risk variable due to the uncertain efficacy of bone-active agents such as bisphosphonates to reduce fracture risk in such patients. We examined the interaction between reported falls history and a surrogate marker of falls risk, the sit-to-stand test(1), in a prospective, placebo-controlled, randomized trial of the bisphosphonate, clodronate, over 3 years.

5212 women aged 75 years or more and unselected for osteoporosis were recruited to the study. At entry, they were asked to report if they had sustained more than one fall in the previous month and also underwent physical assessments including their ability to rise from a chair (the sit-to-stand test; classified as not possible, with difficulty or without difficulty).

Multiple falls were reported by only 4% of women at entry who were older ($p = 0.0025$) than women without multiple falls but had similar prevalences of prior fractures, glucocorticoid use, rheumatoid arthritis, maternal hip fracture and smoking with no differences in BMI or femoral neck BMD. Inability or difficulty in rising from a standard chair, observed in 31% of the women, was associated with increased reports of multiple falls (OR 2.66, 95%CI 1.96–3.61). An unsuccessful chair test was also associated with older age ($p < 0.001$), a higher prevalence of prior fractures ($p = 0.014$), more frequent glucocorticoid exposure ($p < 0.001$) and self-reported rheumatoid arthritis ($p < 0.001$). Importantly, however, inability or difficulty in rising from a chair was associated with a higher BMI ($p < 0.001$) and femoral neck BMD ($p = 0.016$). Oral clodronate 800 mg daily was associated with a 24% reduction in osteoporotic fracture incidence (HR 0.76, 95%CI 0.63–0.93). The efficacy of clodronate was similar in women with an unsuccessful chair test to those with no difficulty in rising (HR 0.79 vs. 0.74 respectively, p-value for interaction with treatment > 0.30).

We conclude an indicator of the risk of falls does not significantly impact on the efficacy of clodronate in reducing the incidence of fracture. If confirmed in other studies with other agents, falls risk indicators could be incorporated into risk assessment tools designed to target skeletal therapies.

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Conflict of Interest: None declared

Mo-OP29

INCREASED INTEGRAL BONE MINERAL DENSITY (BMD) OF THE HIP WITH ONCE-YEARLY ZOLEDRONIC ACID 5 MG (ZOL): QUANTITATIVE COMPUTED TOMOGRAPHY DATA (QCT) FROM THE HORIZON-PFT TRIAL

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QCT of the proximal femur allows study of cortical and cancellous compartments and calculations of bone strength indices rather than

just density. HORIZON-PFT showed that once-yearly infusion of ZOL at baseline, 12 and 24 months decreased fracture risk and improved BMD vs placebo (PBO) in 7765 women with postmenopausal osteoporosis. In a sub-population we evaluated whether BMD change estimated by QCT was similar to that by dual-energy X ray absorptiometry (DXA), whether BMD change measured by QCT is similar for cancellous and cortical bone, and whether ZOL affects bending strength indices (BSI), compression strength indices (CSI) or ratio of total hip cortical bone volume. DXA of the hip was performed at baseline, Months 6, 12, 24 and 36. QCT of the hip was performed at baseline and Month 36. At baseline, 233 women had QCT of the hip, 179 of whom had evaluable QCT data at 36 months. Changes in total hip measures by DXA and QCT are similar and integral changes result from changes in cancellous and cortical bone. Apparent changes in cortical bone are more evident for cortical bone volume than cortical bone density. These changes in density result in large increases in CSI at the femoral neck and trochanter but not BSI. In conclusion, once-yearly ZOL results in increases in integral BMD of the hip vs PBO. Overall increase in integral BMD appeared to be driven by changes in cancellous bone and thickening of cortical bone, resulting in higher hip cortical bone volume. Most frequent AEs in ZOL patients were pyrexia, myalgia, and bone/musculoskeletal pain.

Table 1 Mean (±SD) % changes in DXA and QCT measures over 36 mo

Variable	ZOL (n = 93)	PBO (n = 86)	P value
Total hip BMD (DXA)	3.53 ± 4.24	−1.54 ± 4.83	<0.0001
Total hip BMD integral	2.86 ± 5.14	−3.15 ± 6.64	<0.0001
Total hip BMD cancellous	2.0 ± 9.89	−8.73 ± 21.28	<0.0001
Total hip BMD cortical	−0.43 ± 3.63	−1.43 ± 3.23	0.0541
Total hip cort bone vol	7.20 ± 12.95	−0.02 ± 13.07	0.0003
Femoral neck BSI	−2.25 ± 5.67	−2.21 ± 5.77	0.9674
Femoral neck CSI	4.91 ± 16.01	−3.70 ± 11.23	0.0001
Trochanter CSI	9.83 ± 12.97	−4.25 ± 13.62	<0.0001

Conflict of Interest: R. Eastell, Novartis, Consultant

Mo-OP30

INCREASED INCIDENCE OF ATRIAL FIBRILLATION AND ISCHAEMIC STROKE IN FRACTURE PATIENTS TREATED WITH ORAL BISPHOSPHONATES - A NATIONAL REGISTER STUDY

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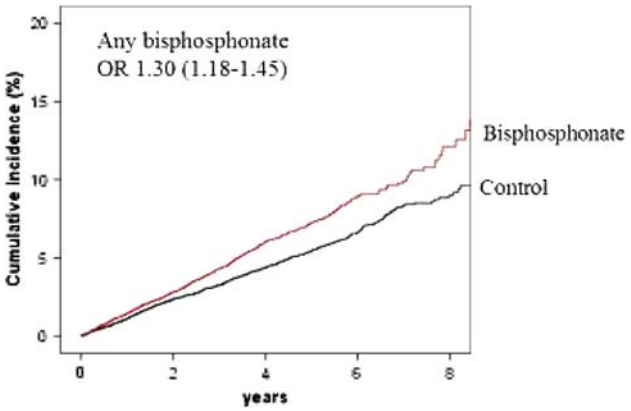
Increased risk of atrial fibrillation (AF) has been reported with zoledronate and suspected with alendronate. The timing of AF in relation does not suggest an acute phase effect and the mechanism is unknown.

Aim: To investigate the incidence of AF and stroke in patients exposed to bisphosphonates(*bis*).

Methods: Using national registers (1995–2005), fracture patients beginning *bis* (N = 15795) were matched with 31590 fracture patients of the same age, sex and fracture type. Probable AF was defined as first prescription of digoxin and/or diagnosis I48.9. Stroke was defined as I63. We used Cox proportional hazards analysis.

Results: The incidence of AF was low; 12.5/1000 person years (pty) in untreated fracture patients and 16/1000 pty in those exposed to *bis*. An OR of 1.30(1.18–1.45) was found for AF. Adjustment for age, sex and use of cardiovascular/antidiabetic drugs did not alter the estimate. Adjustment for Charlson comorbidity score (CCS) and no of co-medications reduced the OR to 1.19 (1.07–1.33). The adjusted risk of hospital treated AF [9.26 (3.82–22.42)] and ischaemic stroke [2.82 (1.55–5.15)] was higher in bis users. The excess risk of stroke was 1 case in 1500 pty.

Conclusion: Fracture patients exposed to oral bis had increased but low occurrence of AF and stroke, which remained significant after adjustment for co-morbidity based on medication use and CCS. Studies are needed to determine if unmeasured co-morbidity (eg lower BMD, smoking or sedentary lifestyle) explain these differences or if oral *bis* per se could be responsible.



Conflict of Interest: BA: Advisory board, Nycomed. Research grants Roche and the Novo Nordisk Foundation.

Mo-OP31

MICE LACKING NEURONAL NOS SHOW ACCELERATED BONE LOSS AFTER OVARIECTOMY

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Nitric Oxide (NO) is a highly reactive molecule synthesized by the nitric oxide synthases (NOS), that plays an important role in bone metabolism. Inducible NOS (iNOS) plays a role in inflammation mediated bone loss, whilst endothelial NOS (eNOS) is important in regulating osteoblast function and bone formation. The third NOS isoform, neuronal NOS (nNOS) is predominantly expressed in the brain and we have previously shown that nNOS knockout mice (nNOS-KO) have increased bone mass and decreased bone turnover. Here we studied the trabecular architecture of nNOS-KO mice, and studied their response to changes in estrogen levels.

Ten-week old female nNOS-KO and wild type (WT) mice underwent either bilateral ovariectomy (Ovx) or a sham operation. After the operation, slow release estrogen pellets or placebo pellets were inserted subcutaneously. Three weeks after the Ovx, the mice

were killed, and the proximal tibia were analysed using a Skyscan 1172 μCT scanner at a resolution of 5 μm. The number of animals per treatment group was at least 8, data are presented as averages ±SEM.

In keeping with our previous analysis using pQCT, the nNOS-KO mice had a substantially higher trabecular bone volume (BV/TV) than WT mice (19.7 ± 1.0% versus 13.9 ± 0.7%, $p < 0.001$). Although there was a small increase in trabecular thickness (5.4 ± 2.4%, $p < 0.05$), the increase in bone volume was mostly due to an increase in trabecular number (34 ± 7%, $p < 0.05$). This resulted in a higher level of trabecular connectivity in the nNOS-KO mice as indicated by the decrease in trabecular pattern factor (−26 ± 3.6%, $p < 0.001$). Ovx resulted in increased bone loss in nNOS-KO mice compared to WT mice (41.4 ± 2.4% versus 31 ± 2.9% decrease in BV/TV, $p < 0.01$), and after Ovx the BV/TV of nNOS-KO mice was no longer significantly different from that of WT mice (10.8 ± 0.51% versus 9.6 ± 0.40%). Treatment with 1 μg estradiol pellets partially prevented the Ovx induced bone loss (32 ± 2.1% versus 41 ± 2.4% in the placebo group, $p < 0.001$), while treatment with 10 μg estradiol pellets lead to a dramatic increase in BV/TV compared to both the placebo Ovx and the Sham control groups (258 ± 24% and 97 ± 13% increase respectively, $p < 0.0001$).

In conclusion, nNOS-KO mice have increased trabecular bone volume, but accelerated bone loss after ovariectomy. These results indicate that nNOS-derived NO plays a role not only in regulating basal bone volume and turnover, but also in mediating the bone loss after estrogen deficiency.

Conflict of Interest: None declared

Mo-OP32

PROGRESSIVE ANKYLOSIS GENE (ANK) REGULATES OSTEOBLAST DIFFERENTIATION

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Background/aims: The progressive ankylosis gene (ank) is a transmembrane protein that transports intracellular pyrophosphate to the extracellular milieu. Human mutations of ank lead to craniometaphyseal dysplasia, a disease, which is characterized by the overgrowth of craniofacial and long bones, suggesting that Ank plays a role in the regulation of osteoblast differentiation. The purpose of this study was to determine the role of Ank in osteoblast differentiation.

Methods: To determine the role of Ank in osteoblast differentiation, we suppressed Ank expression in osteoblastic MC3T3 cells using siRNA or studied the osteoblastic differentiation of bone marrow stromal cells isolated from the bone marrow of *ank/ank* mice, which express a truncated, non-functional Ank protein, or wild type littermates.

Results: MC3T3 cells mineralized within 20 days after the addition of ascorbate and eta-glycerophosphate. Ank expression was the highest at 3 days and declined afterwards, whereas alkaline phosphatase (APase) expression and activity increased on day 8 and was the highest between day 17 and day 20. Suppression of Ank expression in MC3T3 cells using siRNA led to a decrease of the expression of bone marker genes, including APase, type I collagen, bone sialoprotein, and osteocalcin. Suppression of Ank expression also affected the expression of the two transcription factors, which are master regulators of osteoblast differentiation, osterix and runx2. Osterix expression was downregulated in Ank expression suppressed MC3T3 cells, whereas runx2 expression in these cells was upregulated. Bone marrow stromal cells isolated from bone marrow of *ank/ank* mice and wild type littermates were cultured in the presence of 100 mug/ml ascorbate-2-phosphate for various days and analyzed by staining for APase enzyme activity and van Kossa to determine mineralized colonies. The number of APase activity positive and van Kossa stained colonies was markedly reduced in bone marrow stromal cell cultures from *ank/ank* mice compared to the number of APase activity and van Kossa positive colonies in bone marrow stromal cell cultures from wild type littermates.

Conclusions: These findings suggest that Ank is a positive regulator of differentiation events towards a mature osteoblastic phenotype and Ank appears to act upstream of osterix. Loss of Ank function in osteoblastic precursor cells results in an arrest of cells in a premature osteoblastic stage, in which they express high levels of runx2 and low levels of osterix.

Conflict of Interest: Supported by NIH/NIAMS grant R01AR046245–08 to T.K.

Mo-OP33

HETEROGENEITY BETWEEN CALVARIAL AND LONG BONE OSTEOCLASTS WITH RESPECT TO THE INTERNAL PH REGULATION

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Background: Osteoclasts are multinucleated cells specialized in bone resorption. Central to this is extracellular acidification by proton secretion, a process which changes the internal pH of the cell. Anion exchangers are thought to maintain the intracellular pH (pH_i) by removing bicarbonate and taking up chloride ions. We have assessed the presence of anion exchanger 2 (Ae2) in osteoclasts and found this exchanger to regulate the internal pH. From mice of which three out of five Ae2 transcripts were deleted we learned that without the Ae2a, b1 and b2 isoforms the long bone osteoclasts were inactive, resulting in osteopetrotic long bones. Calvarial osteoclasts, however, proved to exert a normal activity; the bones were not osteopetrotic. Since the c1 and c2 isoforms of Ae2 are only found in the stomach, our data suggest the use of another pH regulator by calvarial osteoclasts.

Objective: To evaluate whether osteoclasts at different bone sites make use of different anion exchangers or co-transporters to regulate their internal pH.

Methods: Osteoclasts were generated in vitro from bone marrow obtained from the different bone types (long bone and calvaria) with M-CSF and RANKL. In these osteoclasts the regulation of the internal pH was measured per single cell loaded with a fluorescent pH sensitive substrate. The cell was incubated with media in which chloride or sodium was depleted to discriminate between different anion exchangers and co-transporters. With QPCR the expression of mRNA for several acid loaders was analyzed for both types of osteoclasts.

Results: Ae2 is important for the internal pH regulation of osteoclasts generated from marrow of both types of bone. Yet, in addition to Ae2 we found that calvarial osteoclasts use a sodium-dependent acid loader to regulate the internal pH. With QPCR we assessed that mRNA expression of SLC4a4, a sodium-dependent co-transporter, is almost exclusively expressed in calvarial and not in long bone osteoclasts. Ae2 is equally expressed in both types of osteoclasts.

Conclusion: We propose that calvarial osteoclasts differ from those of long bones in their capacity to regulate intracellular pH. Long bone osteoclasts use Ae2 only, whereas calvarial osteoclasts employ in addition to Ae2 the sodium-dependent co-transporter SLC4a4 to regulate their internal pH.

Conflict of Interest: None declared

Mo-OP34

BIDIRECTIONAL REGULATION OF PEAK BONE MASS AND AGE-RELATED BONE LOSS BY THE TYPE 1 CANNABINOID RECEPTOR

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Osteoblasts and adipocytes are derived from a common precursor in the bone marrow and in age related osteoporosis there is a progressive reduction in bone mass with accumulation of bone marrow (BM) adipocytes at the expense of osteoblasts (OB). This has led to the suggestion that there is an age related switch in lineage commitment of BM stromal cells but the mechanisms underlying this process are poorly understood. Here we show that mice with deletion of the type 1 cannabinoid receptor ($CNR1^{-/-}$) have increased peak bone mass due to a reduction in osteoclast (OC) activity but develop age-related osteoporosis due to increased accumulation of adipocytes in BM. Skeletal phenotyping showed that both male and female $CNR1^{-/-}$ mice had a 20–25% increase in peak bone mass affecting the trabecular compartment at 3 months of age compared with wild type littermates ($p < 0.01$). Analysis of bone histomorphometry showed that the high bone mass was accompanied by marked decrease in OC number (52%) and resorption area (54%), whereas OB numbers were unchanged. Studies in vitro showed that OC formation was significantly reduced in OB-BM co-cultures prepared from $CNR1^{-/-}$ mice, due mainly to a defect in the ability of OB from $CNR1^{-/-}$ animals to support OC formation. In keeping with this, expression of RANKL mRNA was reduced by 30% ($p < 0.05$) in $CNR1^{-/-}$ OB, providing an explanation for the reduction in OC formation. We also found evidence for a direct reduction in RANKL and M-CSF induced OC formation in BM cultures from $CNR1^{-/-}$ mice indicating the OC precursors are less responsive to osteoclastogenic stimuli. Analysis of BMD in later life showed that by 6 months of age BMD was similar in $CNR1^{-/-}$ and wild type, whereas by 12 months $CNR1^{-/-}$ mice had developed severe osteoporosis, with a 67% reduction in trabecular BMD from 3 months of age, compared with a 22% reduction in wild type ($p < 0.01$). Histomorphometric analysis at this point showed a dramatic increase in marrow fat accumulation and a reduction in OB numbers. Expression of the adipogenic transcription factor PPARγ was increased in BM stromal cells from $CNR1^{-/-}$ mice and stromal cell cultures showed an increase in adipocyte differentiation and decrease in OB differentiation when compared to wild type. We conclude that CNR1 signalling inhibits bone accrual and peak bone mass yet protects against-age related osteoporosis by promoting osteoblast differentiation and inhibiting adipocyte differentiation in the bone marrow compartment.

Conflict of Interest: None declared

Mo-OP35

IDENTIFICATION OF A NOVEL LOSS-OF-FUNCTION MISSENSE MUTATION IN THE RANKL GENE THAT CAUSES OSTEOPETROSIS IN MICE

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To discover novel targets in bone diseases we have established a high throughput chemical-induced random mutagenesis approach in the mouse. Mutagenized progeny that display visible skeletal phenotypes are selected for the identification of the causal mutation using linkage genetic analysis. We have recently isolated a mouse mutant of osteopetrosis, which is characterized by loss of tooth eruption, abnormally increased bone density, and complete absence of osteoclasts. Genetic analysis using genome-wide polymorphic markers, SSLPs and SNPs, have led to the localization of the causal mutation in distal chromosome 14 at a genomic interval including the RANKL gene. Sequencing analysis of the RANKL coding region revealed a missense mutation, which caused a single aminoacid substitution in a highly conserved region of the extracellular domain. Functional characterization of the mutated inactive protein provides initial evidence that the mutation affects RANKL binding to its cellular receptor RANK or the decoy receptor OPG. In conclusion, we have generated a unique mouse model of osteopetrosis that closely resembles RANKL-mediated human autosomal recessive osteopetrosis. This mouse model constitutes a unique tool for applying therapeutic approaches in this type of osteopetrosis whereas analysis of the mutated protein could provide new possibilities for generating novel RANKL inhibitors.

Conflict of Interest: None declared

Mo-OP36

THE EFFECTS AND MECHANISM OF PROLACTIN ON OSTEOBLAST DIFFERENTIATION

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Background/aim: Hyperprolactinemia is one of the risk factor of decrease in bone mass which has been believed to be mediated by hypogonadism. However, the presence of prolactin receptor in human osteosarcoma cell line and primary bone cell culture from mouse calvariae supported the hypothesis of a direct prolactin (PRL) action on bone cells. Therefore, the aim of this study was to investigate the role of PRL and its signal transduction pathway in the regulation of bone metabolism via osteoblast differentiation.

Method: Human pre-osteoblasts (SV-HFO) that differentiate in a 3-weeks period from proliferating pre-osteoblasts (days 2–7) to extracellular matrix producing cells (days 7–14) which is eventually mineralized (days 14–21) were used. Concentration of PRL mimicked a lactating period (100 ng/ml) was used to incubate SV-HFO for 21 days in osteogenic medium.

Results: Human prolactin receptor mRNA and protein are expressed in SV-HFO. PRL significantly decreased osteoblast number (DNA content) which was not due to an increase in apoptosis. Calcium measurement and Alizarin red staining showed a decrease in mineralization by PRL while having no effect on alkaline phosphatases activity. Furthermore, PRL decreased RUNX2 and osteocalcin mRNA expression at a late stage of osteoblast differentiation suggesting an inhibiting effect PRL on osteoblast differentiation. The lower RANKL/OPG ratio in PRL-treated group compared with control group supported a reduced osteoclastogenis function of osteoblast which could, in turn, inhibit the proliferation and differentiation of osteoclast. The effect of PRL on mineralization was abolished when administered together with PI-3 kinase inhibitor, showing involvement of this signaling pathway in the PRL effect on mineralization.

Conclusion: The present study provides evidence of a direct action of prolactin on osteoblast differentiation and its function on osteoclastogenesis.

Conflict of Interest: Grant/Research Support : Royal Golden Jubilee PhD programme

Mo-OP37

THE NOVEL VITAMIN D ANALOG ZK191784 NORMALIZES THE DECREASED BONE MATRIX MINERALIZATION IN MICE LACKING THE EPITHELIAL CALCIUM CHANNEL TRPV5: A QBEI STUDY

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Mice lacking the renal epithelial Ca2+ channel TRPV5 (TRPV5−/−) display impaired renal Ca2+ reabsorption, hypercalciura, intestinal Ca2+ hyperabsorption—due to secondary hypervitaminosis D - but remain normocalcemic and normophosphatemic. It has been suggested that the chronic high 1,25(OH)2D3 levels might be responsible for reduced bone thickness and decreased bone matrix mineralization found in the TRPV5−/− mice. ZK191784 has been previously shown to act as an intestine-specific 1,25(OH)2D3 antagonist (Nijenhuis et al., Faseb J 2006). The present study was undertaken to evaluate the effects of the vitamin D antagonist on bone matrix mineralization.

8-week-old female TRPV5+/+ ($n = 12$) and female TRPV5−/− mice ($n = 12$) were treated for 28 days with or without 50 μg/kg/day ZK191784. Bone mineralization density distribution (BMDD) was assessed in femurs by quantitative backscattered electron imaging (qBEI). The analyses revealed that knockout animals had a significantly decreased average matrix mineralization compared to their wild-type littermates (CaPeak −4.8%, $P < 0.01$). In contrast, in the TRPV5−/− group treated with the ZK191784, the Ca Peak was found increased by 2.7% ($P < 0.05$) compared to non-treated TRPV5−/− and was non-significantly different from wild-type animals anymore. The homogeneity of mineralization (CaWidth) was found similar in all groups independently of genotype and/or treatment with ZK191784. Interestingly, the amount of lowly mineralized areas (CaLow), corresponding to areas of primary mineralization, was found highly increased in the TRPV5−/− group compared to the wildtype group (+58.8%, $P < 0.05$). This increase seemed also partially rescued in the knockout group treated with antagonist (−21.5%, n.s.) and therefore not significantly different anymore to the wildtype animals (+24.7%, n.s.)

Taken together, these data suggest that the Vitamin D antagonist ZK191784 normalizes the impaired bone phenotype in TRPV5−/− animals. These results support the significance of vitamin D and optimal control of calcium homeostasis for bone formation and matrix mineralization.

Conflict of Interest: None declared

Mo-OP38

REGULATION OF KLOTHO EXPRESSION BY ESTROGEN

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Background: Klotho is a glycoprotein predominantly expressed in the kidney, parathyroid gland, reproductive organs and choroids plexus in the brain. Studies have shown that decreased expression of the klotho gene in mice leads to multiple disorders, such as arteriosclerosis, skin atrophy, abnormal calcium homeostasis, and shortened life span. Up-regulation of klotho and TRPV5 by vitamin-D has been reported. Overlap in the expression pattern of estrogen receptors and klotho raises the potential for estrogen regulation of klotho expression.

Methods: We examined the potential regulation of klotho by estrogens by in vitro and in vivo studies. The kidney cell lines, MDCK or DCT, were cultured in DMEM supplemented with either 10% regular FBS or charcoal stripped FBS. The expression of klotho mRNA was determined by real-time PCR and klotho protein expression was examined by western blot. To determine the effects of estrogen on klotho expression in vivo, we used wild type (WT, $n = 4$) and aromatase deficient mice (ArKO, $n = 4$) treated with estrogen (20 ug/mouse 3 × /week) or vehicle for 3 weeks. RNA and protein were prepared from the kidneys for real time PCR and WB analysis, respectively. We also compared klotho expression in estrogen receptor alpha knockout mice to that of WT littermates. Finally, we have collected serum and urine from postmenopausal patients on or off estradiol ($n = 3$ per group), early, mid and late phase of menstrual cycle from 3 normally cycling young females, and 1 patient being treated for infertility before and after estradiol treatment.

Results: The klotho protein expression as detected by western blot (WB) was significantly higher in cells grown in csFBS compared to regular serum. However, the addition of estradiol at 10(−8)M in 10% csFBS restored klotho expression to the same level as cells grown in 10%FBS. Our results showed a significantly higher expression of klotho both at the mRNA and protein levels in ArKO animals compared to WT; however, ArKO mice treated with estrogen had WT levels of klotho. Protein extracts prepared from kidneys of estrogen receptor alpha mice had higher levels than wild-type littermates. Mass spectroscopy and western blot analysis of serum and urine klotho protein levels are in process.

Conclusion: Estradiol suppresses klotho expression in the murine kidney. Determination of the effect of estrogens on klotho expression in human females is underway.

Conflict of Interest: None declared

Mo-OP39

IDENTIFICATION OF NOVEL UNSUSPECTED MOLECULAR TARGETS OF NITROGEN-CONTAINING BISPHOSPHONATES THROUGH BARCODE TECHNOLOGY

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Nitrogen-containing Bisphosphonates (NBPs) are the most widely used class of drugs for the treatment of conditions associated with excessive bone resorption. The action of NBPs seems to involve the block of protein prenylation of prosurvival proteins, such as Ras; however, these effects may involve additional mechanisms that at present are still unknown. To identify biological gene targets of NBPs, we used the yeast Saccharomyces cerevisiae as a model organism, using a genome-wide, highthroughput screening of a collection of 5936 deletion heterozygotes, in which one copy of each gene has been deleted and substituted with an unique "barcode", a

stretch of 20 bp (TAG) which defines each mutant. The collection was grown competitively in the presence of a sub-lethal dose (10–3/10–4 M) of three different NBPs, i.e. risedronate, alendronate and ibandronate. Subsequently, the molecular TAGs were amplified by PCR and discriminated on microarrays. Strains carrying the deletion of genes encoding for the molecular targets of these drugs will grow slowly and will be out-competed from the population. The reliability of our approach has been demonstrated by the presence, among the deletion mutants that displayed a slower growth rate in the presence of the drugs, of the strain carrying the deletion for Farnesyl Pyrophosphate Synthetase gene, a well-known target of N-BPs. Moreover, we could identify two novel biological targets: TBCB (Tubulin cofactor B), involved in microtubules maintenance and ASK/DBF4 (Activator of S-phase kinase), required for entry into S-phase, whose heterozygous deletion protects yeast from NBPs-induced toxicity. We demonstrated that NBPs at high doses induce the protein levels of TBCB and the phosphorylation of DBF4 in our mammalian model (MCF-7 cells) as well. We reported some previously unknown effects of NBPs on microtubule dynamics, as seen through immunoflorescence and time-lapse microscopy and on microtubule organization as demonstrated by electron scanning microscopy and we hypothesized the upregulation of TBCB as the leading cause. We also document the role of DBF4 as a key-player in NBPs-induced citotoxicity, which might explain their effects on cell-cycle. The use of the BARCODE approach has proven to be useful to obtain new information on the molecular mechanism of action of NBPs and may help in designing more effective and selective drugs.

Conflict of Interest: None declared

Mo-OP40

VISUALISATION OF CELLULAR UPTAKE AND LOCALISATION OF BISPHOSPHONATE IN VIVO USING A FLUORESCENT ANALOGUE OF RISEDRONATE

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Bisphosphonates (BPs) are effective anti-resorptive agents due to their bone-targetting properties and efficient internalisation by bone-resorbing osteoclasts. However, it is still unclear exactly where BPs localise within the skeleton, and whether any non-osteoclast cells are directly affected by these drugs in vivo. We have used fluorescein-risedronate (FAM-RIS) to investigate cellular uptake and localisation of BP in newborn rabbits. Three day-old rabbits were subcutaneously injected with 0.5 mg/kg FAM-RIS and sacrificed 24 hours later. Using confocal microscopy, FAM-RIS uptake could be clearly detected in osteoclasts in vivo, both in histological sections of ulnae, and in osteoclasts purified ex vivo using immunomagnetic bead separation. In contrast, other bone marrow cells showed very little evidence of drug uptake. Flow cytometry demonstrated only a slight increase in mean fluorescence of BMCs in 3 out of 4 experiments (± 0.5 fold mean increase) in FAM-RIS-treated animals as compared to vehicle-treated controls. In comparison, ex vivo treatment of BMCs isolated from the same animals with 1 μM or 5 μM FAM-RIS for 2 hours resulted in an approximately 5–10 fold and a 20–25-fold increase, respectively, in mean fluorescence as compared to vehicle-treated cells, with intracellular drug uptake confirmed by confocal microscopy. Histological analysis of the ulnae and vertebrae revealed clear FAM-RIS labelling of bone surfaces in vivo, especially around the growth plate. More detailed analysis further revealed strong labelling around vascular channels within the bone matrix. Interestingly, osteocytic lacunae in close proximity to these vascular channels also showed evidence of FAM-RIS binding. Moreover, FAM-RIS was also localized to the lacunae of newly embedded osteocytes. This reinforces the possibility that BPs may exert direct effects on osteocytes in vivo through extracellular mechanisms, as previously suggested, although our findings suggest that only a small subset of osteocytes may be exposed to BP. In conclusion, these results show that osteoclasts are the only cell type in bone that internalise substantial quantities of BP in vivo, although FAM-RIS labelling of some osteocytic lacunae suggests that osteocytes could also be affected by BPs in vivo.

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Mo-OP41

EFFICACY OF ADDING TERIPARATIDE VERSUS SWITCHING TO TERIPARATIDE IN POSTMENOPAUSAL WOMEN WITH OSTEOPOROSIS PREVIOUSLY TREATED WITH RALOXIFENE OR ALENDRONATE

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Background: We sought to determine the relative efficacy of adding teriparatide 20 mcg/day (TPTD) versus switching to TPTD in patients previously treated long term with antiresorptive drugs.

Methods: 198 Postmenopausal women with osteoporosis previously treated for at least 18 months with alendronate (ALN) or raloxifene (RLX) were randomized to add TPTD or switch to TPTD for 6 months plus a 12-month treatment extension.

Results: Median bone marker increases were greater in the switch compared with the add groups and appeared to plateau in the add groups between 1 and 3 months but continued to increase in the switch groups. This difference was more marked in the ALN compared with that of previous RLX groups (Table 1). However, adding TPTD conferred significantly greater mean increases in BMD versus switching to TPTD in the ALN groups, and numerically greater increases in the RLX group. All regimens were safe and well tolerated. Increases from baseline in predose serum calcium were greater in patients switched to TPTD versus those who added TPTD, but these differences were small.

Conclusion: In general, greater bone turnover was achieved by switching from antiresorptive to TPTD, while greater BMD increase was achieved by continuing antiresorptive during TPTD treatment.

Table 1 Changes from baseline in bone markers and BMD

Variable	ALN switched toTPTD (N = 50)	TPTD +ALN (N = 52)	RLX switched toTPTD (N = 49)	TPTD+RLX (N = 47)
PINP (μg/L) month 1	38.0 (129.6%*)	22.0 (79.4*†)	46.0 (113.6%*)	34.0 (95.7%*)
PINP (μg/L) month 3	59.5 (192.8%*)	26.0 (83.3%*†)	66.5 (171.8%*)	53.0 (108.2%*†)
CTx (ng/ml) month 1	0.07 (41.2%*)	0.01 (4.4%†)	0.05 (15.6%*)	−0.01 (−5.6%†)
CTx (ng/ml) month 3	0.24 (144.8%*)	0.03 (30.0%*†)	0.24 (71.5%*)	0.10 (44.4%*†)
LS BMD (g/cm2) 6 month	0.012 (2.0%*)	0.032 (4.5%*†)	0.034 (4.2%*)	0.036 (4.5%*)
LS BMD (g/cm2) 18 month	0.034 (4.8%*)	0.060 (8.4%*†)	0.063 (8.1%*)	0.075 (9.2%*)
FN BMD (g/cm2) 6 month	−0.007 (−0.8%)	0.009 (1.4%*†)	0.003 (0.5%)	0.013 (1.8%*†)
FN BMD (g/cm2) 18 month	0.003 (0.9%)	0.020 (3.2%*†)	0.013 (1.8%*)	0.020 (2.8%*)

*P<.05 within group; †P<.05 b/w switch and add groups

Conflict of Interest: Sponsored by Eli Lilly and Company

Su-P001

DLK1/FA1 REGULATES THE CHONDROGENIC CONDENSATION AND EARLY CHONDROCYTE DIFFERENTIATION IN LIMB BUD MICROMASS CULTURES AND ITS EXPRESSION IS MODULATED BY TGF-BETA SIGNALING PATHWAY

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We have recently identified Dlkl/FA1 (delta like 1/fetal antigen1) as a novel surface marker expressed by proliferating epiphyseal plates chondrocytes during limb development (ECTS, 2008, Abdallah BM, et al., abstract no. 0381). Also, Dlk1 deficient mice exhibited cartilage malformations suggesting a regulatory role of Dlk1/FA1 in chondrogenesis. Thus, we examined the regulation of Dlk1/FA1 expression in response to different signaling pathways involved in cartilage development program. We employed the mouse embryonic limb mesenchymal micromass culture as an in vitro system that recapitulates the sequential stages of chondrogenesis. Real-time PCR, immunostaining and ELISA assays revealed that Dlk1/FA1 was expressed at stages of mesenchymal cell condensation (day 1–3), peaked during early chondrogenesis (day 3–6) in parallel with the expression of Sox9 and type 2a collagen (Col2a) and then dramatically down-regulated after day7 to disappear completely upon the expression of Collagen X (ColX) by hypertrophyic chondrocytes at day 10. BMP-2 or -4 treatments for up to 15 days accelerated the differentiation program of chondrocytes with an early up-regulation of late chondrocytic marker ColX and increased expression of Dlk1/FA1, Sox9 and Col2a1. Blocking Ihh signaling (an essential signal for maintaining proliferative chondrocytes) using cyclopamine promoted premature chondrocyte hypertrophy with an early stimulation and disappearance of Dlk1/FA1 expression (day 1–5). Interestingly, TGFβ 1 treatment enhanced chondrogenesis at early stage while abolished chondrocyte hypertrophy and inhibited matrix mineralization in parallel with marked down-regulation of Dlk1/FA1 expression during the whole time course of differentiation program (by 80% reduction). In contrast blocking TGFβ signaling using SB431542 (a selective inhibitor of ALK-2,-5 and -7 receptors) strongly inhibited the formation of mesenchymal cell condensation and chondrogenesis in parallel with marked stimulation of Dlk1/FA1 expression (by 4 folds). In conclusion, Dlk1/FA1 is a novel regulatory marker controlling early events of endochondral bone formation. The down-regulation of Dlk1/FA1 expression by TGFβ signaling, suggest a plausible mechanism by which the stimulatory effect of TGFβ on chondrogenesis is mediated at least in part by Dlk1/FA1.

Conflict of Interest: None declared

Su-P002

BONE MINERAL ACCRUAL AND GAIN IN SKELETAL WIDTH IN PRE-PUBERTAL SCHOOL CHILDREN IS INDEPENDENT OF THE MODE OF SCHOOL TRANSPORTATION—ONE-YEAR DATA FROM THE PROSPECTIVE OBSERVATIONAL PEDIATRIC OSTEOPOROSIS PREVENTION (POP) STUDY

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Background: Walking and cycling to school could be an important regular source of physical activity in growing children. The aim of this 12 months prospective observational study was thus to evaluate the effect of self-transportation to school on bone mineral accrual and gain in bone width in pre-pubertal children, both traits independently contributing to bone strength.

Methods: Ninety-seven girls and 133 boys aged 7–9 years were recruited as a part of the Malmö Pediatric Osteoporosis Prevention (POP) Study in order to evaluate the influence of self-selected school transportation for the accrual of bone mineral and bone width. Children who walked or cycled to school were compared with children who went by bus or car. Bone mineral content (BMC) was measured by dual energy X-ray absorptiometry (DXA) in the lumbar spine (L2–L4), third lumbar vertebra (L3) and hip, and bone width was calculated at L3 and femoral neck (FN). Changes during the first 12 months were compared between the groups. Subjective duration of physical activity was estimated by a questionnaire and objective level of everyday physical activity at follow-up by accelerometers worn for four consecutive days. All children remained in Tanner stage 1 throughout the study. Comparisons were made by independent student's t-tests between means, ANCOVA and Fisher's exact tests.

Results: There were no differences in baseline or annual changes in BMC or bone width when the transportation groups were compared. No differences were detected in objectively measured daily level of physical activity by accelerometer. All children reached above 60 minutes of moderate to intense daily physical activity per day, the international recommended level of daily physical activity according to the United Kingdom Expert Consensus Group.

Conclusions: The everyday physical activity in these pre-pubertal children seems to be so high that the school transportation contributes little to their total level of physical activity. As a result, the choice of school transportation seems not to influence the accrual of bone mineral or gain in bone size during a 12-month follow-up period.

Conflict of Interest: None declared

Su-P003

STUDY OF THE LOCAL VARIATIONS IN TRABECULAR MICROSTRUCTURAL PARAMETERS OF PROXIMAL HUMAN FEMUR AND ITS RELATIONSHIP WITH BIOMECHANICAL PROPERTIES

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The trabecular microstructural changes produced as a consequence of osteoporosis contribute significantly to a decline of the mechanical properties. A huge number of studies have found significant correlation between microstructural parameters of the entire specimen and their biomechanical properties. However, with the application of new methods, referred as Image Guided Failure Assessment (IGFA), it is possible to identify both the compressive failure regions and the failure mode of their different structures. The aim of this work is to determine the microstructural properties of the compressive failure regions of proximal human femur, and evaluate if these properties are a better predictor of sample failure than the averaged properties over the entire specimen.

Twelve cylindrical biopsies of human femoral head were retrieved during standard hip arthroplasty in patients with intracapsular hip fracture. The samples were introduced inside a Material Testing Stage, a system with incorporates stepwise micro-compression in combination with micro-computed tomographic (micro-CT) imaging (Trabeculae, Empresa de Base Tecnológica S.L.). The specimens were scanned with micro-CT for initial imaging. Following, the specimen was submitted to a controlled strain rate step. After a relax time, a new micro-CT imaging was carried out. This procedure was repeated until sample failure.

The failure regions presented a lower bone volume fraction (BV/TV, 12.8%), lower connectivity (Tb.Pf, 6.8%), and high relative prevalence of rod-like structures respect to plate-like structures (SMI, 9.1%) respect to non-failure regions. A good correlation was found between ultimate stress and a lineal combination of averaged microestructural variables (SMI, Tb.Pf and BS/BV) of the whole sample ($r2 = 0.7822$). This correlation improved when the averaged microstructural variables of the failure regions were selected ($r2 = 0.9171$).

It can be concluded that the microstructural parameters of the failure regions better predict the ultimate stress of the sample. It was also visually observed from 3D models that the failure mode for the plate-like structures was predominantly bending, starting in a region of the plate that was already perforated. The rod-like structures failed preferently in a buckling mode.

Conflict of Interest: None declared
José R. Caeiro Rey, Trabeculae, Consultor
Sonia Dapia Robleda, Trabeculae, Research Support
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Miriam Silva Covelo, Trabeculae, Research Support

Su-P004

ACTION MECHANISMS OF DIACERHEIN ON CATABOLIC PATHWAYS OF HUMAN OSTEOARTHRITIC CHONDROCYTES

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Aims: It has been proposed that diacerhein acts as a slow-acting, symptom-modifying and perhaps as a disease-structure modifying drug in the treatment of osteoarthritis (OA). This work was designed to simultaneously study the effects of diacerhein and that of conventional symptomatic drugs on the synthesis of inflammatory and structural mediators in OA chondrocytes and synoviocytes in culture.

Methods: Chondrocytes and synoviocytes were obtained from joint specimens of OA patients who underwent total knee replacement surgery. We used quiescent cells stimulated with 10 U/ML IL-1b, and studied the effects of diacerhein (10–5 M), celecoxib (CBX, 10–6 M), diclofenac (DCF, 10–6 M), meloxicam (MXC, 10–6 M) and indomethacin (IND, 10–6 M) in the release of prostaglandin (PG) E2 and nitric oxide (NO), the expression of cyclooxigenase (COX)-2, the accumulation of metalloproteinase (MMP)-1 and MMP-13, and the activation of the nuclear factor kappa B (NF-kB). Results: The activation of NF-kB binding induced by IL-1 was inhibited by diacerhein, both in chondrocytes and synoviocytes. All of the NSAIDs used in these experiments also inhibited the activation of NF-kB induced by IL-1, although IND only showed this effect on chondrocytes. Diacerhein did not revert the increase in COX-2 synthesis and in PGE2 release elicited by IL-1 in chondrocytes. Furthermore, in synoviocytes, diacerhein not only did not inhibit the release of these mediators but even super-induced their expression and synthesis. NSAIDs diminished until almost basal levels the PGE2 release, and, unexpectedly, these drugs also diminished COX-2 synthesis in both cell types. In relation to the increase in NO synthesis evoked by IL-1 in chondrocytes, only diacerhein was able to prevent it, while no effect was noted for the NSAIDs. Diacerhein did not modify the synthesis of MMPs induced by IL-1 in chondrocytes, but it decreased MMP-13 accumulation in synoviocytes.

Conclusions: Diacerhein inhibited the activation of NF-kB induced by IL-1 in both cell types and the synthesis of NO in OA chondrocytes, whereas it failed in the control of PGE2, COX-2 and MMPs in any of the cell types. Taken together these results would anticipate a weak clinical response to diacerhein in OA. In turn, the NSAIDs only inhibited NF-kB activation and PGE2 release in accordance with their presumed "symptom relieving" role in the treatment of OA.

Conflict of Interest: None declared

Su-P005

MANDIBULAR BONE MINERAL DENSITY IN AN EXPERIMENTAL MODEL OF OSTEOPOROSIS IN RABBITS

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Aims: To determine the reproducibility of densitometric measurements at mandibular level. To evaluate the mandibular bone loss in ovariectomized rabbits (OVX) treated with systemic glucocorticoids after surgery, and to compare the bone mass changes at mandibles with respect to bone mass variation in other skeletal regions.

Methods: Thirteen female NZW rabbits (8 months old; mean weight of 4.1 kg) were randomly allocated in two groups. Eight

animals underwent bilateral OVX and subsequent parenteral methylprednisolone administration (1 mg/kg/d) for 4 weeks to induce OP according to a previous protocol (OP group). Five animals were used as controls (Control group). Bone mineral density (BMD) were measured by dual energy X-ray absorptiometry (DXA) at baseline and 22 weeks after OVX (Hologic ® QDR-1000) at lumbar spine (L3–L4, LS), global knee (gK) and subchondral bone of the knee (sK). After sacrifice, mandibles of each rabbit were dissected, and repeated DXA measurements of the left hemimandible of each healthy rabbit were performed in 3 consecutive days. For each measurement, analysis was divided in 4 different zones: mandibular body (R1), ramus (R2), gonion (R3) and global mandible (R4). Statistical analysis: Reproducibility of mandibular measurements was calculated by coefficient of variation (CV). To evaluate BMD differences between groups and correlation between mandibular BMD and the other regions analyzed, Mann-Whitney and Spearman correlation test were used (SPSS, vs. 10.0). Results: The in vitro CV (%) of mandibles DXA in healthy rabbits after sacrifice was 1.10. Mandibular BMD in the different zones analyzed was (mean ± SD): 0.459 ± 0.009 mg/cm^2 at R1, 0.183 ± 0.013 at R2, 0.139 ± 0.008 at R3 and 0.335 ± 0.010 at R4, in control group; and 0.438 ± 0.015 mg/cm^2 at R1, 0.180 ± 0.014 at R2, 0.135 ± 0.013 at R3 and 0.333 ± 0.010 at R4, in OP rabbits (only significant at R1, $p < 0.05$). However, BMD showed a significant decrease in OP rabbits when compared to controls in LS, gK and sK ($p < 0.05$ in all) at week 22. There was no correlation between BMD at LS, gK and sK, and the four mandibular regions analyzed.

Conclusion: Reproducibility of mandibular DXA measurements in vitro was excellent. In our experimental model of OP in rabbits, through OVX and steroids administration, bone mass variation found at mandibular level was smaller than the one demonstrated in other skeletal regions, reaching statistical significance only at mandibles body. Correlation between bone loss at spine or knee and mandibular loss was not found.

Conflict of Interest: None declared

Su-P006

RNA INTERFERENCE TO MODULATE COMPOSITE TISSUE ALLOGRAFT TOLEARNCE BY GENE SILENCING OF JANUS KINASE 3—PRELIMINARY RESULT IN VIVO

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Background: Six cytokines: interleukin-2 (IL-2), IL-4, IL-7, IL-9, IL-15 and IL-21, all activate JAK3 because it selectively binds γc; this was an important observation that was pertinent to the development of a new immunosuppressant. On the other hand, RNA interference (RNAi) has recently emerged as a specific and efficient method to silence gene expression in mammalian cells by transfection of short interfering RNAs (siRNAs). We would like to use combined RNAi and JAK3 knowdown to induce the allotransplantation tolerance.

Methods: To silence JAK3 expression in vitro and in vivo, one dsRNA (RSS303439) will be tested to incorporate the JAK3 mRNA sequence. The expression vector containing the pre-miRNA expression cassette will be transfected into rat basophilic leukemia cell line, RBL-2H3, for transient RNAi analysis. After the transient RNAi analysis, use the Lipofectamine™ 2000 (Invitrogen) to transfect Stealth™ RNAi or siRNA into mammalian cells in a 24-well format. siRNA is then injected to allogenic group to modulate the skin allograft rejection.

Results: It is found that the allogenic group under siRNA regimen proves the lesser rejection reaction than without siRNA treatment in histology.

Conclusions: In this study, we would like to use RNAi technique to block the JAK3 expression. Therefore, we investigate to show that siRNAs are capable of specific, highly stable and functional silencing of gene expression in the T helper cells and determine when the golden time for the suppressive effect of the RNAi to block JAK3 is. Furthermore, we would like apply this immune preconditioning to our modified hind-limb allotransplantation in vivo.

Conflict of Interest: None declared

Su-P007

BONE MORPHOGENETIC PROTEIN MRNA EXPRESSION IN HUMAN PERIOSTEUM DURING FRACTURE HEALING

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Periosteum is an osteogenic flexible tissue. The presence of pluripotential mesenchymal cells in the under-surface of the periosteum in combination with growth factors regularly produced or released after injury provide this unique tissue with an important role in the healing of bone and cartilage. Bone morphogenetic proteins (BMPs) may be the main signal that regulates skeletal repair. They act on mesenchymal stem cells (MSCs) and osteoprogenitor cells to induce differentiation into osteoblasts and chondroblasts. In the present study, we assessed the expression of BMPs mRNA in the periosteum in cases with fractures vs normal samples. Twenty patients (median age, 40; range: 7–83) with fracture and fifteen normal individuals (median age, 35; range: 8–80) without fracture were included in the analysis. The expression of BMP2, BMP4 and BMP6 mRNA was quantitated with the use of real-time polymerase chain reaction (Q-RT-PCR). In both fracture and normal samples, BMP2 mRNA was expressed at high levels, followed by BMP6 and BMP4 mRNA; expression levels of BMP2 mRNA were very low. However, compared to normal samples, fracture samples were characterized by (i) significantly higher BMP2 mRNA expression and (ii) significantly lower BMP4 and BMP6 mRNA expression. Expression of BMP mRNAs was also higher in fracture samples in which the process of healing was ongoing for at least 20 days prior to sampling than those samples obtained before that time. Furthermore, BMPs mRNA expression patterns were age-dependent, in that higher expression was observed in fractures of patients with advanced age. In conclusion, our study indicates that the gene expression profile of BMPs in fracture healing follows the osteogenic hierarchical model in which BMP2 and BMP6 may play an important role in inducing osteoblast differentiation of MSCs, while other BMPs (in particular, BMP4) function later, during the differentiation process from osteoprogenitor cells to osteoblasts.

Conflict of Interest: None declared

Su-P008

HIGH FREQUENCY OF OSTEOPROGENITORS IN THE PORCINE AORTIC VALVE

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Background: Bone and other mesenchymal tissues are often observed in calcified aortic heart valves. The source of the cells responsible for elaborating ectopic mesenchymal tissues in the valve is still unknown. It is possible that the aortic valve contains mesenchymal progenitor cells that contribute to the formation of ectopic tissues in calcification. Aims: The objectives of this study were: 1) to demonstrate the multi-lineage potential of porcine aortic valve interstitial cells (VICs); and 2) to identify and measure the frequency of the mesenchymal progenitors and osteoprogenitors in freshly isolated and subcultured VICs. Methods: We cultured VICs in different induction media to test differentiation capacity. The frequency of the mesenchymal progenitors and osteoprogenitors were measured using colony forming unit-fibroblast (CFU-F) and colony forming unit-osteoblast (CFU-O) assays. Results: In osteogenic media, VICs expressed transcripts for Cbfa1, osteocalcin, osteonectin, bone morphogenic protein 2 and alkaline phosphatase (ALP), and formed three-dimensional nodules that stained positive for ALP activity, osteocalcin by immunostaining, and mineral by von Kossa. We found that VICs also have adipogenic, chondrogenic, and myofibrogenic differentiation capacity. By limiting dilution and clonal analyses, we found that primary isolated VICs have strikingly high frequencies of mesenchymal progenitor cells ($57.1 \pm 6.1\%$) and osteoprogenitors ($34.4 \pm 0.4\%$). The high frequencies were maintained for three to six population doublings but decreased after nine population doublings to $27.8 \pm 8.3\%$ and $5.2 \pm 2.1\%$ (for mesenchymal progenitors and osteoprogenitors, respectively) suggesting a limited self-renewal capacity in culture. Further, we identified a morphologically-distinct subpopulation that is highly enriched for osteoprogenitors, occurs at high frequency, self-renews, and elaborates bone matrix from single cells. Conclusions: These novel findings suggest that the aortic valve is rich in a unique multipotent mesenchymal progenitor cell population that is distinct from other vascular progenitor cells. The high frequency of the osteoprogenitors in the aortic valve might have significant implications in the pathobiology of aortic valve calcification.

Conflict of Interest: None declared

Su-P009

GENETICALLY ENGINEERED KSFRT MESENCHYMAL PROGENITORS CELLS IN COMBINATION WITH WHOLE BODY OPTICAL IMAGING PROVIDE AN EXCELLENT MODEL TO STUDY EFFECTS ON CELL FATE, BONE AND CARTILAGE FORMATION AND BIOMATERIALS

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Mesenchymal stem cells (MSCs) have great potential for application in tissue engineering of bone and cartilage, especially in combination with biomaterials. Stimulation of MSCs to differentiate into bone forming osteoblasts could greatly supplement current treatments for osteoporosis. The composition and structural properties of biomaterials can considerably influence MSC differentiation. At present, differentiation of MSCs in vitro or in vivo as well as osteogenic properties of biomaterials are poorly understood. Therefore, we have created a model, which allows fast and reproducible production of stable cell lines, by introducing a FLP Recombinase Target (FRT) site into the genome of the KS483 cell line. This site is used for the insertion of DNA to enable gene function studies by either overexpression or knock down of a gene or by insertion of reporter genes to visualise and quantify specific biological processes. E.g., insertion of a luciferase 2 gene has enabled us to follow cell fate in vivo by whole body bioluminescent imaging after subcutaneous implantation in nude mice. After 20 weeks, luciferase expressing tissues were isolated for immunohistochemical analysis. We could identify cartilaginous tissue in 1 of the samples, while in the others the cells had retained an undifferentiated mesenchymal character. These results indicated that without further stimulation, the KSFrt cells do not differentiate into bone in vivo. Therefore, KSFrt-Luc2 cells and KSFrt cells overexpressing Runx2 were seeded onto biphasic calcium phosphate (BCP) scaffolds prior to subcutaneous implantation. First results show, that while the KSFrt-Luc2 cells start to form bone after 8 weeks, the KSFrt-RunX2 cells already have formed bone after 3 weeks, indicating that proper stimulation can significantly accelerate bone formation by KSFrt cells in vivo. Therefore, these cells provide an excellent model to investigate bone inducing properties of biomaterials. To facilitate these studies, we have generated an osteogenic reporter cell line by introducing an osteoblast specific collagen I promoter driving the expression of the luciferase 2 gene into the genomic FRT site. Preliminary results indicate, that luciferase expression increases during osteogenic differentiation in vitro. This cell line will be used to test the effect of (new) drugs and different biomaterials in medium throughput in vitro, followed by in vivo conformation of the results using whole body optical imaging.

Conflict of Interest: None declared

Su-P010

DEVELOPMENTAL CHANGES OF HISTOMORPHOMETRICAL AND PHYSICAL PROPERTIES OF RIBS IN CRITICAL POSTWEANING TIME IN PIGLETS

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The most critical time after weaning is the short period when feed is changed from liquid to solid. The recent developmental study of postweening piglets revealed fluctuations in bone mineralization. The aim was to determine the differences in structural properties of skeletal system development on ribs model between 30 ($n = 6$) and 35 ($n = 6$) day old male piglets. Bone length, weight and the mean cross section area (A) were assessed. For examination of bone mineral density (BMD) and bone mineral content (BMC) the dual-energy X-ray absorptiometry (DEXA) method and NORLAND XR 43 apparatus were used. The fragments of bone from the head of the ribs through the neck to a distance of 5 mm past the tuberculum were taken from each piglet. Pictures from a laser scan of H + E stained bone sections were analyzed by graphical analysis software ImageJ 1,38 u. The following parameters were analyzed: fractal dimension of trabecular bone (D), trabeculae border line length, trabecular bone volume (BV/TV %), number, total and mean area of osseous lacunae. Bone length, weight and A increased thorough 5 days but not significantly. D and trabeculae border line length were significantly higher in 35 day old piglets ($P = 0.008$ and $P = 0.009$, respectively). BMD ($P = 0.004$), BMC ($P = 0.011$), number ($P = 0.016$) and total area ($P = 0.031$) of osseous lacunae decreased compared with younger piglets. Moreover, mean area of osseous lacunae and BV/TV

% did not differ between piglets during ageing. Present study showed that 5 days during postweaning time is too short to observe visual changes in physical parameters but restricted feeding led to form less mineralized bone according to BMD and BMC. However, at this limit time on microscope there were observed significant changes indicating intensive bone turnover after weaning.
Conflict of Interest: None declared

Su-P011

NON-INVASIVE COMPARISON OF POLY LACTIDE CARBONATE IMPLANTS WITH DIFFERENT ADDITIVES ON BONE FORMATION IN OVARIECTOMISED RATS

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Introduction: Biodegradable orthopaedic fixation devices are often required to be implanted in poor quality bone. Ideally the bioabsorbable implant should not only degrade with replacement by bone but the material should also stimulate bone formation surrounding the implant.

Biodegradable materials implanted in the rat tibia can be evaluated with in vivo micro-CT, a non-invasive method for longitudinal monitoring of bone changes. This study investigates biodegradable materials of the poly(DL-lactide-co-glycolide) (PLGA)/calcium carbonate (PLC) group The purpose of this study was to longitudinally evaluate bone formation associated with biodegradable materials implanted in the proximal tibia of ovariectomised (OVX) rats.

Materials and Methods: OVX Sprague-Dawley rats ($n = 12$) were used in this study. Two classes of implant material were used, polyethylene as negative control and PLGA preparations. Implants were inserted into the right tibia of each rat one month after OVX. A baseline micro-CT scan of the right tibia was performed at 1 week before the implant was inserted. Follow-up tibial scans to the same region were performed at 1 month, 2 months, 3 months 6 months, 9 months and 12 months. A volume of interest (VOI) around the implant was selected and three-dimensional model reconstruction was performed. Bone volume fraction (BV/TV [%]) was measured from the VOI.

Results: Three-dimensional reconstruction of the VOI revealed different patterns of bone formation associated with the implant materials. At each observation time point, there was more bone associated with the implants of the PLC group. The bone around the polyethylene appeared to change little for the 12 months of the study. Interestingly, at the 12 month time point with little bone change for the polyethylene implants, a significant increase of BV/TV ($p < 0.05$) was identified around the PLC implants.

Discussion: We have shown that a biodegradable material can promote bone formation in the proximal tibia of OVX Sprague-Dawley rats. As the healing tissues are increasingly able to take mechanical loads, ideally the implant degradation must continue until completion over a reasonable period allowing healing to occur and eventual replacement of the implant by native bone. Currently, we are comparing the in vivo micro-CT results with the end point results obtained from conventional bone histomorphometry in order to better understand the mechanism of increasing bone in the presence of PLC.
Conflict of Interest: Smith & Nephew Inc, Grant Research Support

Su-P012

NODULAR CELL FORMATION IN SMOOTH MUSCLE CELLS CULTURE FROM RABBIT AORTA: A SUBPOPULATION WITH INCREASED OSTEOGENIC POTENTIAL?

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Background: Calcification and ossification have been described in artery wall of end stage renal disease, atherosclerosis, diabetes mellitus and aging. Controversy exists about the cellular type and mechanisms involved in this phenomenon.

Methods: Fresh rabbit thoracic aorta was studied. Medial layer was separated from adventicia and intima by rubbing and washed with 2 mg/ml collagenase. Small pieces of tissue were obtained by cutting the specimen. Explant culture was mantained for several weeks in growth medium DMEM supplemented with 10% fetal bovine serum, 10000 u/10000 mcg/ml Penicillin/Streptomycin and L-glutamin. Immunocitochemistry and scanning electron microscopy was made to the cells obtained in culture.

Results: Cultures became confluent at about 4 weeks after seeding. A population of cells organized in aggregates, that we called nodules. Antibody against alpha smooth muscle actine stained positive almost 100% of the cells in a filamentous pattern. Cells uderwent calcification after long-term culture (von Kossa staining) more intense in nodular regions. Scanning electron microscopy showed a higher amount of rough endoplasmic reticulum in nodular cells, that localized around amorphous matrix (nodule centre) that appears like calcium deposits.

Conclusions: In media layer of thoracic rabbit aorta exist cells with smooth muscle phenotype that aggregates in nodules, with tendency to calcify in culture and an increased rough endoplasmic reticulum, with a 3-D location around acellular amorphous matrix that reminds calcium deposits, suggesting that these are a subpopulation of cells involved in arterial calcification.
Conflict of Interest: None declared

Su-P013

OPTIMIZED BONE REGENERATION BASED ON SUSTAINED RELEASE FROM 3D SCAFFOLD

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Background/aim: Recent research focus has moved to the application of cells, growth factors and biomaterials in combination. However, one common problem is that the existence of a large burst over a narrow time period during the early stage of release. Therefore, modify the performance of scaffolds in bone healing is a challenge. The aim of this study is to examine whether the PLGA/HAp scaffolds loaded with BMP-2 through electrospinning can controlled sustained release and thus improves bone healing.

Materials and Methods: The composition of the emulsion of the four different experimental named F1–F4 respectively. In Vivo Experiments, critical size defects are made in Nude mice. About 1.25 mg of scaffold with around 1 ng of BMP-2 is used in each implant. After 1, 2, 4, and 6 weeks, the tibia bone fractures were examined by soft X-rays. Serum was taken out for biochemical assays 1, 2, 4, and 6 weeks after implantation of all scaffolds. The serum BMP-2 concentration and alkaline phosphatase (ALP) activity was determined.

Results: X-ray shows F1 group were as sharp as the post-operation case and there was no significant bone regeneration after 4 weeks. In contrast, those from F2–F4 groups indicated the formation of new bone after 4 weeks. BMP-2 concentration the F2, F3 and control experienced similar time profiles and showed their maximal concentrations after 4 weeks, while F4 got the highest serum BMP- 2 concentration after 2 weeks and dropped dramatically in the following weeks. In ALP concentration also revealed that F4 showed the highest ALP activity over the initial 2 weeks, and after 2 weeks, ALP activity decreased to a very low level.

Conclusion: BMP-2 is easy to get digested by enzyme once it is exposed to serum in vivo. Sustained release provides the best strategy to maintain high level of BMP-2 in the local area and that is the main motivation to design the release profile of scaffolds. The bioactivity of BMP-2 was well maintained in vivo and better performance of bone healing was observed in this study. Comparing the results of all samples after 4 weeks, F4 demonstrates the best performances. Hence, it can be concluded that the HAp and BMP-2 encapsulated fibrous scaffolds (F2–F4) are promising as BMP-2 delivery devices for bone regeneration and it is better to maintain high concentration of BMP-2 within 2 weeks to facilitate bone formation.

Conflict of Interest: A financial interest:Ministry of Economic Affairs, R.O.C
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Su-P014

PHENOTYPIC COMPARISON OF INTERVERTEBRAL DISC CELLS WITH ARTICULAR AND NASAL CHONDROCYTES: TOWARDS A PHENOTYPIC SIGNATURE FOR ANNULUS FIBROSUS AND NUCLEUS PULPOSUS CELLS

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Incidence of low back pain, which is generally attributed to the degeneration of intervertebral disc (IVD), is dramatically increasing. In the last decade, there has been a surge of interest in applying tissue-engineering principles to treat spinal disorders associated with the IVD. However the basic tissular, cellular and molecular characteristics of intervertebral disc, which are essential prerequisites for the development of tissue engineering, are still poorly defined. The aim of the present work was to further characterize IVD cells by comparing the phenotype of Annulus fibrosus (AF) and Nucleus pulposus (NP) cells to that of articular and nasal chondrocytes. IVD from 4–5 weeks-old New Zealand White Rabbit were histologically characterized by Alcian blue (AB) and Masson's trichrome (MT) staining as well as by type II collagen immunostaining. The comparative level of transcripts coding for Type I (COL1A1), II (COL2A1) and V (COL5A1) collagens, aggrecan (AGC1), Sox9, Ihh and MGP were assessed by real-time PCR in AF and NP cells as well as in articular (AC) and nasal chondrocytes (NC). AB staining reveals an increasing content of sulfated GAG from the outer part to the inner part of the AF. Conversely, MT evidences an inverse distribution for total collagen that was abundant in the outer AF and decreased towards the inner AF and the NP. Finally, whereas the immunostaining for type II collagen was negative in the outer AF, it appeared strongly positive in the inner AF and the NP.

Differential expression of the various markers were observed. Whereas AGC1 was mostly expressed in NP, COL2A1 was predominantly expressed in AF. COL2A1/COL1A1 and COL2A1/AGC1 ratios in IVD cells were significantly different from those in AC and NC. Of particular interest, COL5A1, COL1A1 and MGP were strongly expressed in AF but barely detectable in NP cells. As expected AC and NC expressed COL2A1, AGC1 and MGP. Sox9 and Ihh were expressed in all cell types without any significant difference.

The present study demonstrates that a combined evaluation of aggrecan, type II and V collagens and MGP transcripts may potentially allow to distinguish cells from IVD, articular and nasal cartilage. In view of a stem cell-based tissue engineering approach for IVD regeneration, expression of these markers could become instrumental in monitoring and, eventually, triggering stem cell differentiation towards IVD cells.

Conflict of Interest: None declared

Su-P015

HYPOXIA EXERTS DIFFERENTIAL EFFECTS ON THE CHONDROGENIC AND OSTEOGENIC DIFFERENTIATION OF HUMAN ADIPOSE-DERIVED MESENCHYMAL STEM CELLS

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Human adipose tissue-derived stem cells (hATSC) have recently been contemplated as potential reparative cells for cartilage and bone tissue engineering. Chondrogenic differentiation of hATSC can be induced by the combination of enriched culture medium and a three dimensional (3D) environment. Given that bone is vascularized and cartilage not, oxygen tension has been suggested as a differential regulatory factor for osteogenic and chondrogenic differentiation. Our work aimed at determining whether hypoxia affects differentially the chondrogenic and osteogenic potential of hATSC. HATSC were cultured in chondrogenic or osteogenic medium for 30 days respectively in 3D (pellets) or monolayer, and under low (5%) or normal (20%) oxygen tension. Cell differentiation was monitored at the level of mRNA by real-time PCR (type II collagen, aggrecan and osteocalcin). The chondrogenic differentiation was further evaluated by Alcian Blue and immunohistological staining for the detection of sulphated glycosaminoglycans (GAG) and type II collagen respectively. The osteogenic differentiation was also assessed by the production of a mineralized matrix (Red alizarin staining) and alkaline phosphatase activity (ALP) measurement. Real-time PCR analysis indicated that type II collagen expression was markedly induced by hypoxia in both media, whereas aggrecan expression was induced by the presence of chondrogenic medium whatever the oxygen tension. The presence of both GAG and type II collagen in the matrix was detected only when hATSC were exposed to chondrogenic medium and hypoxic condition. Osteocalcin mRNA expression, mineralization and ALP activity were enhanced in the presence of osteogenic medium. An early decrease of the osteocalcin mRNA level was however observed in hypoxic condition compared to the typical pattern obtained in normoxia. The mineralization and ALP activity appeared to be weaker in hypoxic condition. Our data indicates that a 5% hypoxia seems to favor the chondrogenic differentiation as evidenced by an early expression of the

chondrogenic markers, whereas it seems to alter the osteogenic potential of hATSC. Our results highlight the differential regulatory role of hypoxia on the chondrogenic and osteogenic differentiation process of hATSC. These data could help us exploit the potential of tissue engineering and stem cells to replace or restore the function of traumatized or degenerated osteoarticular tissues.

Conflict of Interest: None declared

Su-P016

CALCIUM PHOSPHATE BIOMATERIALS FOR A LOCAL-DELIVERY OF BISPHOSPHONATE

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Calcium phosphate (CaP) biomaterials such as calcium deficient apatite (CDA) or b-tricalcium phosphate (b-TCP) have been used as bone substitutes. At present, we investigate the capacity of these CaP to release locally drugs. Bisphosphonates (BP) are antiresorptive agents largely used in clinical treatment in bone disorders. Our laboratory has reported that the reaction of CaP powders with aqueous zoledronate can result in different association modes between the two components (1).

Through a in vitro osteoclastic model (2), this study was designed to evaluate the in vitro bioactivity of two innovative bone drug delivery system (DDS), based on different association of zoledronate on PCa (CDA or b-TCP) matrices.

7.5 mg and 28 mg of zoledronate were respectively loaded on 100 mg of CDA and on 200 mg of b-TCP. Powder samples were then compacted to obtain 10 mm diameter pellets.

5.106 of total rabbit bone cells were seeded on dentin slices in the presence of BP-loaded materials. Pellets of materials were prepared by mixing zoledronate-loaded CaP powder in zoledronate-free CaP powder in different ratio. Pellets of pure vehicle were used as control. The resorption activity of osteoclasts, measured by image analysis, was estimated by the total resorbed surface and the total number of pits formed on dentin slices.

We observed a dose-dependent effect of both systems on the inhibition of resorption activity, according to the amount of zoledronate loaded on CaP.

These data demonstrate that both DDS were effective for the release of Zoledronate at doses which inhibit osteoclastic resorption.

This study shows that the two biomaterials (CDA and b-TCP) are good candidates for releasing bioactive BP molecules. Whether such a DDS could be promising for the local prevention of pathological bone resorption in appropriate osteoporotic animal models (rats, ewes) would be paid further investigation.

1 Josse S, et al. Biomaterials. 2005 ;26:2037–2080

2 Grimandi G, et al, Microsc Res Tech. 2006;69:606–12

Conflict of Interest: None declared

Su-P017

Influence of Mechanical Stimulation and Perfusion on 3-D BMSC Cultures

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Background/aims: Until now, there has been no in vitro model that duplicates the environment of bone marrow. The purpose of this study was to analyze the proliferation and differentiation behavior of bone marrow stromal cells under the influence of perfusion and mechanical loading.

Methods: Cells of 7 donors were pooled and 10^6 BMSC were seeded on a xenogenic spongiosa disc and transferred into a bioreactor system. Cell culture was continued under 3 different conditions: Continuous perfusion (group A), 10% cyclic compression (group B) and static controls (group C). After one day, one, two, and three weeks, proliferation (MTS-assay), differentiation (protein, osteocalcin, ELISA) and Tenascin-C and Runx-2 (RT-PCR) were evaluated.

Results: In groups A and B, proliferation was enhanced after 2 (48.6 ± 19.6 and $44.6 \pm 14.3 \times 10^3$ cells) and 3 weeks (46.6 ± 15.1 and $44.8 \pm 10.2 \times 10^3$ cells) compared with controls (26.3 ± 10.8 and $17.1 \pm 6.5 \times 10^3$ cells, ANOVA, $p < 0.03$). There was an upregulation of Runx-2 in both stimulated groups after 1, 2, and 3 weeks (perfusion: 1 week: 5.2 ± 0.7; $p < 0.01$, 2 weeks: 4.4 ± 1.9; $p < 0.01$, 3 weeks: 3.8 ± 1.7-fold; $p = 0.013$; mechanical stimulation: 1 week: 3.6 ± 1.1, $p < 0.01$, 2 weeks: 4.2 ± 2.2, $p < 0.01$; 3 weeks: 5.3 ± 2.7-fold, $p < 0.01$). Mechanically stimulated cultures contained the highest amounts of osteocalcin at all observed time intervals (1st week: 294.5 ± 88.4, 2nd week: 294.4 ± 73.3, 3rd week: 293.1 ± 83.6 mg/g protein, $p \leq 0.03$).

Conclusion: The main stimulus for cell proliferation in a 3-dimensional culture of BMSC is continuous perfusion. Mechanical stimulation fosters osteogeneic commitment of BMSC. This study has impact on understanding the physiological stimuli that influence BMSC in 3-dimensional cell culture and within the bone marrow.

Conflict of Interest: This study was supported by an institutional grant (HilF).

Su-P018

APPLICATION OF LEAST SIGNIFICANT CHANGE (LSC) IN INTERPRETATION OF SERIAL BONE MINERAL DENSITY AND BONE SIZE MEASUREMENTS IN CHILDREN AFTER LIVER TRANSPLANTATION

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LSC is a new valuable tool for clinical interpretation of serial bone mineral density (BMD) measurements in adults. In children its use is more complicated due to growth. However, LSC allows to discriminate between real and apparent (due to precision error) change and also to check, if observed change was smaller than expected in normals, the same or bigger, what is crucial in pediatry.

The aim of the study was assessment of bone development by densitometry in children after liver transplantation.

Dual energy X-ray absorptiometry were used and bone mineral density, bone mineral content and bone area were measured in whole skeleton. LSC was derived from 33 measurement of pediatric whole body phantom. Measurement were done in period of 6 months. Expected rates of bone changes were calculated basing on previous collected reference data. Examination of patients were done before liver transplantation, 6, 12 and 18 months after LTx.

17 cholestatic children, 12 girls and 5 boys, initial age 1–2.4 yrs, qualified for liver transplantation. After LTx immunosupressive

treatment was introduced (tacrolimus—serum level 8–19 ng/dl and prednisone—0.1 mg/kg body weight).

In children 6 month after LTx accretion of bone mass and size was faster than expected in normals. 12 month and 18 month after LTx accretion of bone mineral density was still the same as in normals, however accretion of bone mineral content and bone area was slower than expected in normals.

It seems, that low bone mass and size remain after LTx and bone deficiency has not been compensated.

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Conflict of Interest: None declared

Mo-P019

ACTIVITY OF HUMAN OSTEOBLASTS ON A 3D COLLAGEN GAG SCAFFOLD

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Background: Our lab has examined osteogensis in vitro on a 3D collagen GAG(CG) scaffold with the intention of developing a novel bone graft substitute. This study aims to investigate osteogenesis in the scaffold using the human hFOB 1.19 cell line as a model for osteogenesis. In this study Transforming Growth Factor beta (TGF) & conventional osteogenic factors were compared to induce osteogenesis on the scaffold.

Methods: hFOB 1.19 cells were seeded onto scaffold sections & initially cultured for 14 days to allow cellular infiltration. Four media groups were compared (standard, osteogenic, standard + TGF & osteogenic + TGF) & further incubated for 7,14&21 days @37C. Cell viability was assessed using alamar blue & cell number was recorded using the Dispase digest method. A histological assessment was carried out staining with H&E, Alizarin red & Von Kossa. Gene expression using real time RT PCR examined the early & late bone formation markers, alkaline phosphatase(ALP) & osteocalcin(OC).

Results: Viability was maintained on the scaffold up to 35 days. Groups containing osteogenic media and/or TGF yielded higher cell metabolic reduction of alamar blue dye and a higher cell number ($p < 0.023$). H&E showed that cells fully infiltrated scaffolds irrespective of media & the porosity appeared to be more reduced in groups containing TGF indicating matrix deposition. Staining for mineralisation however, was minimal for both alizarin red & Von Kossa in groups exposed to osteogenic media. Relative gene expression for ALP of 2.3–2.8 times greater in TGF exposed groups compared to standard media alone at day 7 post exposure ($p < 0.016$) after which, although ALP activity increased, no significance difference was detected between media groups at 14 & 21 days post exposure. OC expression fluctuated over time. An increase in expression in groups containing factors was observed at day 21 post exposure however this trend was not significant.

Conclusion: In this study osteogenesis using human cells, was induced on a CG Scaffold. Osteogenic media and/or the presence of TGF yielded greater cell numbers & a reduction in porosity indicating possible matrix deposition. Although mineral staining appeared weak, bone formation markers were expressed at the gene level. ALP expression was greatest following initial exposure, Oc levels began to rise only later in the incubation; indicating osteogenesis of a maturing osteoblastic phenotype. Ongoing work involves immunostaining to identify spatial bone specific matrix proteins within the scaffold.

Conflict of Interest: None declared

Mo-P020

Abstract withdrawn

Mo-P021

INTRATHYROIDAL BONE TISSUE AND FOLLICULAR THYROID CARCINOMA

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The development of the thyroid gland in close contact with organs of mesenchymatous origin may lead to the finding of normal thyroid tissue in the muscles or the fatty tissue of the neck. Fat, cartilage or muscles may also be found within the thyroid. However, the presence of true bone tissue within the thyroid is extremely rare.

The aim of the study was to describe the case of a patient who suffered from multinodular goiter and developed a follicular thyroid carcinoma and ectopic intrathyroidal bone tissue.

A female patient, aged 45 years, suffered from multinodular goiter. At the age of 19 years the presence of a calcified area in the area of the thyroid was detected ultrasonographically. The patient developed hyperthyroidism. In the thyroid scan multiple cold and hot areas were found. Antithyroid drugs and propranolol were administered. The patient had multiple nodules within the thyroid. One of the nodules increased in size. A near total thyroidectomy was performed. On histology a follicular carcinoma and metaplastic bone tissue was observed. Therapeutic radioiodine 100 mCi 131I was administered. The patient is now well.

The presence of degenerative changes in multinodular goiter is quite common. Hemorrhagic areas, cholesterol crystals, fibrous tissue or areas of colloid may be found amongst foci of thyroid tissue. The finding of calcified areas is quite common. However, the presence of bone tissue is very rare. Bone tissue with an area of extramedullary hemopoiesis has been found in a thyroid nodule (Pontikides et al, Thyroid 13: 877–880, 2003). The ectopic bone tissue found in the patient described may represent a fetal remnant, or calcification of degenerative tissue in an area of multinodular goiter.

Conflict of Interest: None declared

Mo-P022

GROWTH EFFORT RULES BONE MINERAL COMPOSITION

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Antlers are costly bone secondary sex structures of the deer family that grow and are shed every year. During the first years of life, body growth takes precedence over antler growth. Thus, under natural conditions most young males fail to reach their full potential for antler size. However, does the age affect antler composition under ad libitum conditions? Our aim was to study age-related variations in macro- and micromineral contents on antlers of Iberian red deer (Cervus elaphus hispanicus).

Mature antlers were obtained during 5 years from the same 11 specimens kept in outdoor captivity at the IREC-UCLM Farm (Albacete, SE Spain). Samples from the burr and restricted to the cortical section were obtained by collecting bone shavings. For mineral content determination, dry samples were wet digested (HNO3-HCl) and diluted. Concentrations of several macro- and microminerals were quantified with ICP-OES.

The antlers contains on average 19.5 ± 0.1% Ca, 9.57 ± 0.06% P, 0.688 ± 0.008% Na, 0.502 ± 0.005% Mg, 856 ± 18 mg/kg S, 577 ± 19 mg/kg K, 384 ± 6 mg/kg Sr, 90 ± 7 mg/kg Si, 36 ± 4 mg/kgFe, 49.7 ± 0.7 mg/kgZn, 2.8 ± 0.1 mg/kgB, 1.0 ± 0.2 mg/kg Cu, 1.2 ± 0.2 mg/kg Se, 0.85 ± 0.051 mg/kg Mn, 0.48 ± 0.02 mg/kg Co, 0.53 ± 0.08 mg/kg Mo, and 0.13 ± 0.03 mg/kg Ni. Distributions of Ca, Se, K, and Na showed an age-dependent trend (P for trend < 0.001). Contents of Ca and Se increase with age up to 4 years (juveniles), but decrease thereafter (adults). In contrast, K and Na showed an inverse trend.

Presence of mineral components in antlers of Iberian red deer is influenced by age, since some minerals are shifted to other physiological functions which prevail over antler growth. Ca in antlers of yearlings is affected by total milk protein yield, whereas there is an inverse relationship with antler K content. As the animal ages, body-growth slows, and more Ca, previously diverted to internal skeletal formation, is now available for antler production. High levels of K during highly demanding events of Ca (early body growth) will prevent elevated Ca losses. Once Ca necessities for internal skeletal diminish (later growth stages), K levels may decrease as well. High levels of Na are also required for maximizing resistance to diseases, adverse effects that may be acute during the infancy. Selenium enhances the immune function. Immunity costs, and therefore selenium expenditures, are much higher during lactation and early body growth.

Conflict of Interest: None declared

Mo-P023

ASSOCIATION BETWEEN MACRO AND MICRO MINERAL COMPOSITION, AND INTRINSIC MECHANICAL PROPERTIES, DXA, AND CORTICAL THICKNESS, IN HUMAN FEMURS AND DEER ANTLER BONE

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Current research in bone mechanical quality is increasingly considering, apart from simple bone mass density, additional sources of variation such as remodelling rate and histology. A source of variation in bone mechanical and structural quality has been found recently in the combination of all minerals that can be measured in the mineral phase in healthy deer antler bone. Antlers provide a unique model for studying bone biology because they are grown and cast every year, so that no remodelling seems to take place, and no surgical procedure is needed to access them. Previous studies by our group have shown that bone mineral profile in antler is variable within standard conditions between regions of bone created at times of different physiological exhaustion. There is also a difference in chemical composition of high and low quality bones considering external (length and weight) and internal (cortical thickness) structural criteria. In addition, there is an association between intrinsic mechanical properties such as stiffness and bending strength and the mineral profile of antlers after the effect of ash content and porosity has been removed. No such study has been conducted so far on human bones. The present study aimed at examining antlers and human femurs of two different mechanical qualities: broken antlers from 2005 cold spring vs intact antlers from the previous, mild year, and human femur necks from osteoporotic vs osteoarthritic elderly people. In addition mechanical and chemical variables were measured in specimens from human long bone shafts. Mechanical quality was measured in material properties such as impact energy, Young's modulus of elasticity E, bending strength and bending work, and also structural properties that affect mechanical performance, such as cortical thickness, DXA density, and shaft diameter. There was an association between chemical composition and mechanical properties. Bone chemical composition may explain bone quality and mechanical performance more fully than traditional ash or calcium effects. This would have considerable importance for bone biology and medicine.

Conflict of Interest: None declared

Mo-P024

RAPID 3D CHARACTERIZATION OF CARTILAGE, BONES AND BIOSCAFFOLD TO SUBMICRON SPATIAL RESOLUTION, WITH A NOVEL MICROCT

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Introduction: The current study is focused on developing a new rapid non invasive 3D imaging technique for bone quality, bioscaffold and related drug efficacy evaluation. Bone quality evaluation is critical in patients suspected with osteoporosis or those treated with synthetic scaffolds for restoration of bone. Similarly success of synthetic scaffolds depends much on the micro architecture of the scaffold. It requires experienced personnel and time consuming sample preparation techniques for evaluating bone quality in osteoporosis, cartilage thickness in osteoarthritis or the 3D micro architecture of resorbable bioscaffolds with conventional histology and conventional X-ray micro tomography system (microCT). In addition, histology for cartilage evaluation can take up to a few weeks. Moreover the biggest deficiency of Conventional microCT is the lack of contrast and resolution to detect fine microstructures on bones and low Z bioscaffold materials. It is also not possible to image cartilage without contrast enhancing agents. In the current work we have used a novel microCT system for rapid virtual histology in 3D on cartilage, bones and bioscaffolds to submicron spatial resolution, without contrast agents nor the need for time consuming histology sample preparation steps.

Materials and Methods: The key to this novel microCT lies in proprietary X-ray detector optics capable of submicron spatial resolution and high contrast imaging of low Z materials such as cartilage and organic/polymer based scaffolds. Rat knee joint with intact cartilage and a synthetic scaffold made of PLGA with predefined macro architecture and micro porosity were used in addition to femur and joints from animal models

Results & Discussion: Examples of rapid bone quality and resorption pit assay in osteoporosis, quantitative evaluation of cartilage thickness in osteoarthritis research using murine model and 3D imaging to submicron resolution of pore structures and connectivity

in a novel porous resorbable bioscaffold used for bone and dental implants will be illustrated. Comparison with MRI and histology will also be shown.

Conclusion: The fast turn around time, submicron resolution and the fact there is no contrast agent involved, makes this new imaging technique an exciting tool for monitoring drug efficacy of bone degenerative conditions & 3D microstructure characterization of bone cells-scaffold interactions.

Conflict of Interest: None declared

Mo-P025

DOWNREGULATION OF OSTEOGENIC BMP AND CARTILAGE MATRIX DEGRADING MMP GENE EXPRESSION IN DELAYED COMPARED TO STANDARD BONE HEALING

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The aim of this study was to compare the temporal expression pattern of factors involved in cartilage and bone formation and endochondral ossification during standard and delayed bone healing for a more in-depth understanding of the molecular basis of disturbed bone healing. A tibial osteotomy was performed in 2 groups of sheep ($n = 30$ each) and stabilized with either a rigid external fixator leading to standard healing (group I) or with a mechanically critical one leading to delayed healing (group II). Hematoma/callus tissue was harvested 4, 7, 14, 21 and 42 days postop. qPCR was employed to determine the expression patterns of BMP2, BMP4, BMP7, Nog, Coll II, Coll Xa1, MMP9 and MMP13. qPCR analysis of BMP2 and BMP4 demonstrated a peak in expression at day 42 in both groups, but the temporal increase in expression was more pronounced in group I leading to considerably higher expressions of BMP2 ($p = 0.065$) and BMP4 ($p = 0.002$) at day 42. Whilst a similar temporal increase in expression of BMP7 and Nog was found in group I, group II showed a relatively constant expression of BMP7 and Nog over the healing course resulting in a significantly lower BMP7 expression ($p \leq 0.026$) from day 14 to day 42 and a significantly lower Nog expression ($p = 0.004$) at day 42 compared to group I. Coll II and Coll X expressions increased over the healing course, but there was no differential expression between the groups. The expression of MMP9 and MMP13 also temporally increased in both groups, but group II showed a significantly lower expression ($p \leq 0.004$) of both MMPs at day 42. This study demonstrated that gene expressions of BMP2, BMP4, BMP7, Nog, MMP9 and MMP13 were considerably lower in delayed compared to standard osteotomy healing. Among the BMPs, BMP7 showed the most markedly differential expression. The first evident difference in BMP7 expression between both groups was found at day 14 suggesting that exogen substitution in the context of a therapeutic approach should be postponed. Whilst there was no differential expression of Coll X between standard and delayed healing, we found a differential expression pattern of both MMP9 and MMP13 suggesting that there might be a failure in endochondral ossification in delayed healing. These results suggest that downregulation in gene expression of osteogenic BMPs and cartilage matrix degrading MMPs may account for a considerable delay of bone healing. This study was supported by the German Research Foundation (DFG SFB 760).

Conflict of Interest: None declared

Mo-P026

FABRICATION AND BIOMEDICAL APPLICATIONS OF ROD-LIKE NANOPARTICLES

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Background/aims: In the report is described the fabrication of nano-size rod-like calcium-phosphate nanomaterial that have led to the synthesis of high aspect ratio particles on nanometer length scales. The elongated structure of these materials often result in inherent chemical, electrical, magnetic, and optical anisotropy that can be exploited for interactions with cells and biomolecules in fundamentally new ways.

Methods: The synthetic procedures that have been developed to fabricate nanorods of hydroxyapatite with a narrow nano particle size distribution is based on precipitation from aqueous solution [S.D. Litvinov et al., Biomaterialen, 7 (3), 186 (2006)]. The material was prepared by ionic counter-diffusion of Ca^{2+}, $[PO_4]^{3-}$ $[HCO_3]^{-}$, OHH^{-} -ions in the bulk collagen fibers [S.D. Litvinov, Eur. J. Drug Metab. Pharmacokinet., 23(2), 346–349 (1998); Stomatologiia (Mosk), 80(3), 7–12 (2001)]. Is known that collagen manifests itself as a characteristic transverse banding of collagen with the period $c = 640$–700 Å, related to the alternation of "dense" and "friable" parts of structure; this banding is visible in electron micrographs. The "friable" parts of collagen structure contain vacancies, or nanotanks of $\sim$3–4 nm in section and 35–37 nm long; these nanotanks are free of collagen macromolecules, and may be filled by inorganic calcium phosphate during the fabrication procedure.

Results: The produced nano-size materials were characterized by high-resolution and scanning electron microscopy, X-ray diffraction, Raman and infra-red spectroscopy, high-resolution NMR spectroscopy. It was found that the XR reflection and IR absorption lines are significantly broadened, and NMR spectra are significantly narrowed as it is usually observed in materials built of nanometer-size particles. Biotransformation of material was studied after its implantation to different defects of bones of both experimental animals and in clinics. It was observed complete regeneration of target tissue correspondding to the normal state of tissue corresponding to its anatomic status.

Conclusion: From these facts may be concluded that hydroxyapatite nanoparticles produced by template synthesis in the bulk collagen fibers plays a role of some catalytic agent accelerating the regeneration process in studied tissues—from bone, tooth to cartilage and parenchyma.

Conflict of Interest: None declared

Mo-P027

CHARACTERISTICS OF MINERAL PARTICLES IN THE CALLUS DURING FRACTURE HEALING IN A SHEEP MODEL

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Bone healing is a complex process which involves the development and subsequent resorption of a fracture callus. The amount, size and orientation of the mineral particles with respect to the organic matrix are important determinants for the mechanical properties of the callus. The development and change in these parameters during bone healing are unknown. The aim of this study was to investigate mineral particle characteristics of the mineralized/hard callus and cortical bone, during osteotomy healing in a sheep tibia stabilized with a standard fixator. Longitudinal sections of the healing callus at different time points (2, 3, 6 and 9 weeks after osteotomy) were investigated by histology and scanning small angle X-ray scattering (s-SAXS). This method provides quantitative assessment of mineral particle thickness and orientation and has previously been used to study mineral particles in bone but not in callus tissues during healing. Regions of interest were selected in which the intramembranous and endochondral ossification occurred as well as cortical bone, based on Safranin-O-von Kossa stained histological analysis. Results showed that both the mean mineral particle thickness (T-parameter) and the degree of mineral alignment (ρ parameter) were found to increase significantly ($p < 0.01$) during the healing process. In the region adjacent to the bone cortex, the crystal orientation was observed to change gradually from a randomly distributed situation (at 3 weeks) to a more homogenous arrangement aligned with the trabecular structure (at 9 weeks). Interestingly, the mineral particle thickness within the cortical bone region had the tendency to decrease. This may be due to remodeling activity in the original cortex even far away from the osteotomy site. This was confirmed by backscattered electron imaging analysis showing clearly areas of lower mineral content (areas of new bone formation / primary mineralization) in this region. Since mineral particle size and orientation are known to influence mechanical properties of mineralized tissues, these observations give new insights not only into the process of mineral deposition process in the healing callus but also remodeling processes in the cortical bone.

Conflict of Interest: None declared

Mo-P028

BONE MINERAL ACCRUAL IN PRE-PUBERTAL BOYS IS NO HIGHER IN BOYS WITH AN ACTIVE THAN IN BOYS WITH A PASSIVE TRANSPORTATION TO SCHOOL—TWO YEAR DATA FROM THE PROSPECTIVE PAEDIATRIC OSTEOPOROSIS PREVENTION (POP) STUDY

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Background: Daily walking and cycling to school has been recommended as an important source of regular physical activity and one way to implement a general physical active life style. The aim of this two years prospective observational study was to evaluate the effect of self-transportation to school on bone mineral accrual in pre-pubertal boys.

Methods: 133 boys aged 7–9 years were recruited to the Malmö Prospective Paediatric Osteoporosis Prevention (POP) Study. Bone mineral content (BMC, g) was measured by dual energy X-ray absorptiometry (DXA) in the total body, lumbar spine (L2–L4) and femoral neck at baseline and after 24 months. Boys who walked or cycled to school ($n = 57$) were compared with boys who went by bus or car ($n = 24$). 52 boys who changes transportation level during the study period were excluded in the comaprison. Annual changes during the 24 months follow-up were compared between the groups. All children remained in Tanner stage I throughout the study. Level of everyday physical activity was estimated by accelerometers worn for four consecutive days. Comparisons were made by independent student's t-tests between means and ANCOVA with adjustment for differences in age at baseline and growth (changes in length and weight).

Results: There were no group differences at baseline in age, anthropometry or BMC. Even if there were groups differences in annual changes in unadjusted total body BMC (walking and cycling versus car and bus 154,6 (SD 33.8) versus 129.0 (31.3), $p < 0.001$) and L2–L4 BMC (2.2 (0.7) versus 1.8 (0.6), $p < 0.05$), these differences disappeared after adjusting for differences in age and growth (age at baseline and annual changes in weight and length). There were no group differences in level of physical activity. All boys reached above 60 minutes of moderate to intense daily physical activity per day, the international recommended level of daily physical activity according to the United Kingdom Expert Consensus Group.

Conclusion: A physical active transportation to school for two years is in pre-pubertal boys not associated with a higher accrual of bone mineral than a physical inactive mode of transportation. One explanation could be that the everyday physical activity in these pre-pubertal boys is so high that the school transportation contributes little to their total level of physical activity.

Conflict of Interest: None declared

Mo-P029

CALCIFIED TISSUES INVOLVED IN OSTEOINTEGRATION AND DENTO-ALVEOLAR ANKYLOSIS

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Introduction: The aim of the study is to compare the interactions established between the osseous alveolar and the dental calcified tissues (ankylosis), and between the alveolar tissues and hydroxyapatite-covered implants, as well as to ascertain the chronology of both processes.

Material and Methods: The first study consisted in the simultaneous implantation of five types of implants coated with hydroxiapatite by pulsed laser deposition. Three male Beagle dogs aged 24 months, with and average weight of 18 Kg were used. The samples were implanted in the tibial dyaphisis of the animals and covered by periostium. The second experiment consisted in the provocation of an ankylosis in twenty female beagle dogs aged 24 months and 18 Kg average weight. The second maxillary molars ligament was removed after surgical teeth extraction, both from the

tooth and of the alveolar bone. The natural teeth were replaced and secured by way of a surgical suture of the gum. The animals were sacrificed respectively one, two and three months after the surgical procedure in both experiences.

The samples were submitted to a scheduled procedure of embedding in plastic polymers without prior decalcification, in order to perform scanning microscopy with secondary and backscattered electrons (BS–SEM) and histology.

Results: The calcified tissues involved in osteointegration and in dental ankylosis follow the same chronological pattern. During the first month, osteoclasts initiate the healing process. At the second stage, chondroid tissue is deposited in the repair area forming calcified trabeculae that allow the deposition of new calcified tissue layers, mainly woven bone. Finally, the chondroid tissue-woven bone mass is progressively substituted by lamellar bone that appears first around the vascular spaces and finally, at the third month as newly formed haversian osteons.

Discussion and Conclusions: The calcified tissues observed both in the osteointegration and in the dento-alveolar ankylosis processes are the same ones previously descrived in the endomembranous cranial bone growth (Goret-Nicaise et al 1988), cranial sutural closure (Manzanares et al, 1988), fracture healing (Franch et al 1998), dental eruption (Pilipili et al, 1995, 1998) and distraction osteogenesis (Lafuente et al, submitted). The tissues involved in osteointegration and ankylosis, as well as in the previously described regeneration processes belong to the endomembranous developmental pattern.

Conflict of Interest: None declared

Mo-P030

DENTAL CALCIFIED TISSUES REACTION TO AN OSTEOCONDUCTIVE IMPLANT SURFACE. INITIAL RESULTS

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Introduction: the aim of this communication is to present the initial results of the reaction of the dental calcified tissues to a new dental implant surface, studied in an animal model. The surgical method for the implant placement was designed in order to obtain a stable contact between the implant surface and the dental tissues, both calcified and pulpar. The surface showed osteoconductive properties in previous studies, both in vitro and in vivo.

Material and Methods: Four implants were inserted the central incisive germs in two pigs, immediately post-extraction of the premolar teeth from maxillary bones, 3 with surface treatment and 1 without surface treatment. The curved shape of the incisive permitted the implant placement through the incisive alveolar space, thus allowing the contact of the implant surface with the dentin and the pulp of the incisor. The animals were sacrificed 90 days after the operation. The analyses of the implant-tissue interaction were performed by Backscattering-Scanning Electron Microscopy (BS–SEM) and by histology, following the procedure described by Manzanares et al (1998).

Results: The experimental surgical method proved its feasibility in allowing the contact between the dentin and the pulp tissues and the implant witouth causing bone fractures, dental lesions or the loss of the implant. The implant surface contacted the tooth organ inner tissues in the two experimental series. The implants submitted to the surface treatment were completely integrated into the dental calcified tissues, mostly with dentin. Our observations showed a consistent high percentage (95 to 100%) of direct contact between the treated implant surface and the dentin tissue. Moreover, the histological analysis of these samples proved that the dental pulp tissues were vital: no pulp damage was observed. The untreated surface implant did not show any of the precedent features: neither implant-dentin surface contact, nor pulp tissue vitality was observed.

Conclusions: The surgical method for implant placement proved to be feasible and effective. In addition, the findings obtained about the contact between implants and dental organs indicate interesting properties for dental calcified tissues integration of the implants.

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Conflict of Interest: None declared

Mo-P031

DIFFERENCES IN THE HEALING PATHWAYS: RAT MODEL VS. SHEEP MODEL

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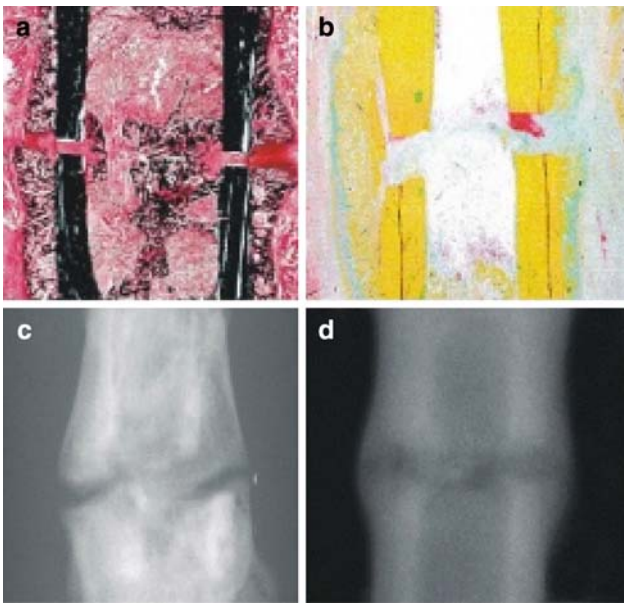
Background: Frequently faced is the selection of an appropriate animal model for fracture research and the ability to transfer it to clinical relevance. Unknown are the differences in healing patterns in these models. These differences in healing could act as limitations to the outcome and appropriate selection of the animal model. In an attempt to establish some consensus on the proper use and design of experimental animal models in musculoskeletal research, this study aims to highlight and compare the healing patterns in two commonly used animal models, the ovine with rodent models.

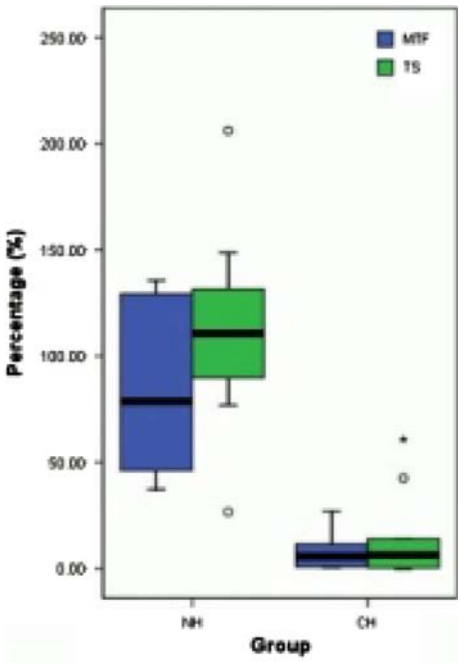
Methods: Qualitative histological, radiographic and microCT observations were made on samples from a 1.5 mm (rat) and 3 mm (sheep) osteotomy fracture healing model. The specimen samples were obtained as part of a larger study where the animals had a mid diaphysis osteotomy, fixated with an external fixator and allowed to heal with normal weight bearing. At 2, 6 weeks (rats) and 4, 12 weeks (sheeps), the animals were sacrificed and healing patterns were observed using image analysis.

Results: It is observed that during the early phase in healing, as seen from images a and c (rat, 2 weeks), the healing pathway is largely due to the endosteal pathways. On the contrary, in images b and d (sheep, 4 weeks), the healing pathway is largely following the periosteal pathway. No visual differences were noted in the two models during the late phase of healing.

Conclusion: Observations from this study show that the healing pathways during the early phase is different in rats and sheep. This has strong implications in choosing the right animal model for osteosynthesis strategies and fracture healing studies.



Conflict of Interest: None declared



Conflict of Interest: None declared

Mo-P032

CRITICAL SIZED BONE DEFECTS LEAD TO DELAYED BONE HEALING IN RATS

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Background: As an attempt to characterize the deviations in normal healing patterns, the initial aim of this study was to establish a critical size bone defect model that would result in a delayed bone healing. Methods: 16 male rats were divided into 2 groups, NH (1 mm gap, normal healing) and CH (4 mm gap, critical healing) with 8 animals in each group. The animals underwent femoral osteotomy and stabilized with an external fixator. Time from surgery to sacrifice was 6 weeks (NH) and 8 weeks (CH). Femoral ends of the fractured and contralateral, were embedded into custom made measuring containers and torsionally loaded until load failure using a testmachine. The maximum torque at failure (MTF) and torsional stiffness (TS) of the operated femur, reported as a percentage of the values from the intact contralateral side, were determined and compared. Qualitative radiographical examinations were performed. Results: After 6 weeks (NH) and 8 weeks (CH), TS and MTF of the fractured femurs were compared between the groups. The TS and MTF for the NH group was significantly higher than the CH group ($p < 0.002$ and $p < 0.001$ respectively), shown in Figure. Complete consolidation was observed in the NH group and to a lesser extent in the CH group. Conclusions: We have shown that using a critical sized bone defect it was possible to retard the healing process in rats. A lower mechanical competence of the healing callus was observed in mechanical testing (TS, MTF). Radiographics showed that the CH group did not achieve a full callus consolidation. Therefore a delayed healing model was achieved. Using this model, it is possible to explore the influences of biological and mechanical stimulations in complicated cases of fracture healing.

Mo-P033

A NEW IN VITRO TEST METHOD TO QUANTIFY MECHANICAL COMPETENCE OF THE EARLY SOFT CALLUS

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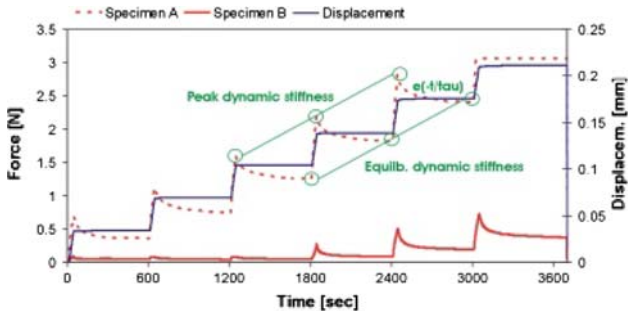
Background: The current gold standard to quantifiably assess for fracture healing outcome is to measure the late callus stiffness. We hypothise that the mechanical competence of the early callus could also acts as an indicator to the quality and progress of the healing tissue. However, no knowledge of this exists. Therefore, as our first aim, we develop an invitro test method to quantify the mechanical competence of the early soft callus tissue. Methods: 4 female adult rats, specimen A ($n = 1$, 12 months, old) and specimen B ($n = 3$, 12 weeks, young) underwent a femoral osteotomy and were stabilized with a fixator. The animals were sacrificed at 10 days and femurs isolated with the fixator intact. Muscle tissues were removed from around the callus. A pin was manually screwed into the proximal and distal ends of the femur. The free ends of the pins were fastened into a custom built axial compression test setup. Incremental displacements were applied in six steps of 35 microns, each with a 600 second relaxation time. Force and displacement were measured. The peak and equilibrium dynamic stiffnesses and the relaxation time constant (tau) were determined for the specimens. Results: Using the current test setup, it was possible to stably fixate the rat femur. During each incremental displacement of the femur, the callus responded instantaneously with a rapid increase in force (first phase) followed by a slower force relaxation (second phase). The peak force measured at each displacement step was consistently higher for specimen A compared to specimen B. Discussion: It was possible to measure with high sensitivity the stiffness of the early soft callus tissue. Mechanical

parameters that are typical of a viscoelastic material were calculated to assess for the callus comptence.



Conflict of Interest: None declared

Mo-P034

EFFECTS OF LOW AND HIGH PHOSPHORUS DIET ON BONE MICROSTRUCTURE IN RATS

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Back ground: Low and high phosphorus (P) intake cause the increase and decrease of BMD, respectively. But the change of bone microstructure as bone quality is not cleared. This study was investigated the effects of low and high P diet on bone microstructure of trabecular bone in male rats.

Subjects: Eighty male rats aged 10 week-old were divided into 3 groups (LP, CP and HP) randomly. CP rats were fed standard laboratory food (Ca/P = 1.24). LP rats and HP rats were fed low (Ca/P = 3.73) and high (Ca/P = 0.41) P food, respectively. They were bred for 12 weeks and allowed at libitum feeding and drinking; temperature, 23 ± 1°C, humidity, 55 ± 5%. After this experiment, tibias of all rats were dissected out. The bones were weighted and measured the length. Micro CT scanning (Hitachi Medico Co. Ltd.) was used to determine morphological indices of bone volume and architecture in the metaphysical region of the proximal tibia. This region spanned 2 mm, with the first slice starting 0.5 mm proximal from growth plate. Bone volume (BV), tissue volume (TV), trabecular thickness (Tb.Th), trabecular number (Th.N), trabecular separation (Th.Sp), trabecular Space (Th.Spac), connectivity density (Conn.D) and the structural model index (SMI) of trabecular bones were measured with bone analysis soft, TRI BON 3D (Ratoc Co. Ltd.). In statistical analysis, One-way ANOVAs followed by Tukey-Kraymer tests evaluated differences in bone quantity and micro architecture of Cont, LP, and HP rats. A significance level of $p = 0.05$ was set. This study was carried out in accordance with the Guide for Animal Experimentation, Kio University and the Committee of Research Facilities of Laboratory Animal Science, Kio University.

Results: BV, BV/TV, Tb.Th, Th.N, and Conn.D in HP rats were significantly lower than those in CP rats. Th.Sp, Th.Spac and SMI in HP group were significantly higher than those in CP groups. Tibia length, weight, Tb.Th, Th.Spac in LP rats was higher than those in CP group. Conn.D and SMI in LP rats were significantly lower than those in CP rats.

Conclusion: BV in LP group was increased and BV in HP group was decreased significantly, compared with CP group. In HP group, the decreases of BV and BV/TV were caused by the reductions of Tb.Th, Th.N, and Conn.D, and increase of Th.Sp and Th.Spac. On the other hand, in LP rats, BV was increased in spite of increase of Th.Spac and decrease of Conn.D. This was suggested that Tb.Th caused the increase of BV rather than Tb.N.

Conflict of Interest: None declared

Mo-P035

EVALUATION OF BONE HEALING IN RABBIT FEMORAL DEFECTS FILLED WITH A NEW CALCIUM PHOSPHATE GLASS CERAMIC POROUS

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Bone tissue has a great potential for regeneration and can restore its original structure and function completely. However, bone regeneration of an osseous defect is not always complete. A variety of graft materials and bone substitutes have been developed to facilitate and promote bone regeneration. In particular, calcium phosphate glasses are specially suitable since their chemical composition resembles that of the bone material phase.

The purpose of this study was to analyze the influence of bioactive glass in the system P2O5-CaO-Na2O-TiO2 on bone healing in surgically created defects in rabbit femurs.

18 New Zealand rabbits were included in this study. In each rabbit femoral condyle, a cavitary defect was made and the implants were placed in these sites. 6 animals were sacrificed in each time (1, 4 and 12 weeks after implantation). Tissue blocks containing the implant sites were processed for histological and SEM evaluation.

Qualitative histology: At 1 week, the results showed that glass presented a good in vivo biocompatibility. At 4 weeks, angiogenesis and bone ingrowth was clearly observed in the histological sections predominantly over the outer surface of the cylinder. At 12 weeks, the growth of newly formed tissue through the inner pores of the glass was observed colonizing the defect towards its centre.

Quantitative histology: At 4 weeks, the glass presented a lower total trabecular area than the control. However, after 12 weeks, significant differences were found between control and glass. In relation to the depth of bone ingrowth, no statistically differences were found at 4 week; At 12 weeks, an increment was observed in the case of glass in comparison with the values obtained for control group. On the other hand, the glass has shown no capacity for resorption, because at 12 weeks remained stable.

The image analysis by SEM showed the differences in macroporisity that characterized these implants and their slow capacity of resorption. SEM observations also revealed the formation of a calcium-phosphate rich layer on the periphery of glass which was stable in the short term, and acted as an osteoconductive mold for new bone formation.

Finally, this calcium phosphate glass that we developed showed good biodegradability and osteoconductivity without adverse inflammation. The resulting data indicated that the incorporation of Ti2O into the glass network enhances the chemical stability.

Conflict of Interest: None declare

Mo-P036

ALTERED GENE EXPRESSION IN EARLY OSTEOCHONDROTIC LESIONS

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Osteochondrosis is a condition involving defective endochondral ossification and retention of cartilage in subchondral bone. The pathophysiology of this condition is poorly characterised, but it has

been proposed that the fundamental defect is failure of chondrocyte hypertrophy. The aim of the current study was to characterise phenotypic changes in chondrocytes associated with the initiation of osteochondrosis. Early lesions were induced in an equine model of osteochondrosis by feeding foals a high energy diet for 8 or 15 weeks. Lesions in articular-epiphyseal growth cartilage were examined histologically and by quantitative PCR (qPCR) analysis of expression of a number of genes representative of pathways that regulate chondrocyte behaviour during endochondral ossification. There were more cells present in clusters (indicative of proliferation) in the lesions compared to normal articular cartilage. Expression of matrix metalloproteinase-13 mRNA was found to be increased 36-fold at 8 weeks and 113-fold at 15 weeks in lesions compared to normal cartilage from the same joint. Expression of type I collagen and Runx2 mRNA were also significantly increased in the lesions. MMP-13, Runx2 and vascular endothelial growth factor (VEGF) are highly expressed by chondrocytes undergoing hypertrophy. Expression of VEGF in the OC lesions tended to be increased, though not significantly. Type X collagen expression, which is associated with chondrocyte hypertrophy, was significantly increased in lesions from horses at 15 weeks. Type II collagen and aggrecan expression were not significantly different. Neither was there any difference in the expression of connective tissue growth factor, Sox9 or fibroblast growth factor receptor 3 mRNA indicating these factors are not involved in the establishment of OC lesions. The histological and gene expression studies together suggest that the defect in osteochondrosis is not failure to undergo hypertrophy, but possibly inappropriate proliferation. This research was funded by grants from the Australian Research Council and Racing Victoria.

Conflict of Interest: None declared

Tu-P037

STRUCTURE AND CHEMICAL COMPOSITION OF APATITE CRYSTAL IN HARD TISSUE OF CONODONT FOSSIL FROM SILURIAN TO CARBONIFEROUS

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The conodont apparatus served as a feeding structure. There have been few studies that have examined the ultrastructure and properties of hard tissue found in conodont fossils. The outer layer of tissue in conodont fossil was the enamel homologues and the inner layer was dentin or cartilage. The purpose of the present study is to examine the nature of apatite crystals found in the hard tissue of conodont fossils. The tooth apparatus of conodont fossils (Yokokurayama Formation, Silurian, Kochi, Japan, Contact Beechweed Limestone, Devonian, Indiana, USA, Grassy Creek formation, Upper Devonian, Missori, USA, and Hushpuckney shale, Carboniferous, Missouri, USA) were used in this study. The specimens were observed and analyzed using a transmission electron microscopy (TEM, JEM 100CX, JEOL), a scanning electron microscopy (SEM, S-2380N, Hitachi and JSM-6340, JEOL), a laser Raman microprobe spectrometry (Labspec, Horiba), and an electron-probe microanalyzer (EPMA, JXA-8200, JEOL). The backscattered electron image of SEM observation revealed that the crystals were highly calcified and needle-shaped. The hard tissue of condont consisted of 2 layers. The surface layer was more calcified than the inner layer. The small tubules were observed in the inner layer. The hard tissue of the condont consisted of 2 layers where the organization varied the size of crystal. Higher magnification showed that the crystals were observed in the lattice of (100) and the central dark lines were not present. Ca, P, and F were detected in the crystal using the EPMA. The Ca/P ratio was 1.74 ± 0.06. The weight % F was 3.92 ± 0.22. By Raman spectrum analysis, the peak of 970 cm^{-1} was detected, which was from PO_4^{3-}. Our results indicate that the apatite crystal in conodont was not hydroxyaptite but was fluoraptite. It is considered that the surface layer of conodont was enameloid and the inner layer was dentin or osteodentin. This study was performed under the cooperative research program of Center for Advanced Marine Core Research (CMCR), Kochi University (06B004, 07A005, 07B020).

Conflict of Interest: None declared

Tu-P038

AUTOLOGOUS MSCS AND OSTEOPROGENITOR CELLS FOR TREATMENT OF NON-UNIONS

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Non-unions represent one of the most severe complications in bone healing. The aim of this study was to examine the therapeutic potential of locally transplanted mesenchymal stem cells (MSCs) or osteoprogenitor cells (OPCs) in non-unions for improvement of healing. 76 rats were investigated histologically (day 14) and biomechanically (day 56). In the control group (C group), only biomechanical testing was performed. Autologous MSCs were harvested, cultured in DMEM or treated with osteogenic medium. A femoral osteotomy was stabilized with an external fixator. Except for the C group, a non-union was induced by thermal destruction of the periosteum around the gap and bone marrow removal. Two days post op, these animals received an injection of DMEM in the gap containing MSCs (MSC group), OPCs (OPC group) or no cells (NU group). The femora were analyzed histomorphometrically, immunohistochemically for collagen type II, and biomechanically by torsional testing. Histological analysis at day 14 demonstrated no bony bridging of the gap. The MSC and OPC group showed a greater callus area and a significantly larger cartilage area ($p \leq 0.03$) compared to the NU group. Collagen type II immunostaining revealed the greatest immunopositive area fraction in the OPC group. In the MSC and OPC group, the periosteal callus was larger, but the tissue distribution was similar compared to the NU group. The torsional stiffness at day 56 of the MSC and OPC group showed a higher torsional stiffness than the NU group with statistically significant differences in the OPC group ($p = 0.02$). However, compared to the C group the torsional stiffness of the NU and both cell groups was significantly lower ($p < 0.01$). Locally applied MSCs and OPCs slightly improved the healing of non-unions in a rat model. The MSCs were less effective compared to the OPCs, which significantly enhanced the torsional stiffness of the healing bone. The less than expected healing improvement of both cell treatments may be related to an unfavourable microenvironment not supporting cell viability at the time of application. A further explanation for the superior outcome of the OPCs might be that the in vivo milieu led to a more fibroblastic differentiation of the MSCs whereas the more differentiated OPCs may be protected by microscopically visible matrix at the transplantation time point. This study was supported by a grant of the AO Biotechnology Advisory Board, AO Foundation, Davos, Switzerland.

Conflict of Interest: None declared

Tu-P039

EFFECT OF PEPTIDE REGULATORS ON STRUCTURAL AND FUNCTIONAL STATUS OF OSSEOUS TISSUE IN AGEING

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Post-menopausal osteoporosis is one of the key issues of public healthcare in the developed countries. Medico-social significance of postmenopausal osteoporosis is stipulated by its complications, first of all collum femoris and spinal fractures, which infringe the quality of life and drastically increase the death rate. Hence, the search for new effective means of treatment of system osteopososis is a burning issue of gerontology.

Our study was aimed at evaluating the effect of peptide bioregulators on the structural and functional status of osseous tissue in a post-ovariectomy osteoporosis model in rats. 100 mature female Wistar rats aged 4–6 months with body weight of 200–230 g were randomly subdivided into 8 groups, each consisting of 10 rats, and received the studied substances intramuscularly in different doses, the control being made up of 2 groups of 10 rats—ovariectomized animals not treated with substances, and non-operated animals injected with physiological NaCl solution. The following peptide bioregulators were used in the study: substance extracted from cartilages of young calves, in the dose of 1 mg and 0,03 mg per rat, and peptide medication T-31 (H-Ala-Glu-Asp-OH) in the dose of 10 μg and 0,3 μg per rat. To model the post-menopausal osteoporosis, bilateral ovariectomy was performed. Mineral density of the osseous tissue (MDOT) was evaluated using a two-photon X-ray densitometer «PRODIGY». Study results pointed out the reliable efficacy of cartilages extract and T-31 peptide in maximum dosages in case of their administration from the 30Ih day since ovariectomy operation. The strongest effect was observed in case of cartilages extract administration in the maximum dosage (1 mg per rat): after a month of observation MDOT was reliably increased, remaining on the same level after 2 months since the beginning of the experiment. The administration of T-31 in the maximum dose beginning immediately after ovariectomy caused a reliable increase in MDOT after 30 days. However, in a month after the completion of the medication course (2 months after the surgery) MDOT was reliably reduced as compared to the initial level.

Thus, peptide bioregulators show good prospects as a means of prevention and treat¬ment of post-menopausal osteoporosis.

Conflict of Interest: None declared

Tu-P040

INHIBITORY EFFECTS OF BIOACTIVE GLASS 45S5 ON OSTEOCLAST FORMATION AND ACTIVITY

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Background: Loss of bone due to infections, congenital defects, trauma cancer and resections is a major clinical bone. Especially in the craniofacial region, defects may cause reconstructive difficulties. Current approaches for tissue repair include surgery and bone grafts but there are sometimes limitations associated with these therapies. One tissue engineering approach is the use of natural or synthetic bioactive materials. However, there are still several problems related with the use of biomaterials for bone replacement, e.g. poor biomechanical durability, lacking capacity for integration with the surrounding tissues and negative effects on the bone remodeling process. Thus, the chemical properties, ion release and surface characteristics of biomaterials must be analyzed in relation to cellular responses such as cell adhesion, gene expression and cell differentiation. Bioactive glass (BG) is a ceramic bone-replacement material consisting of calcium, phosphorus, sodium oxide and silicon. BG particles undergo corrosion until completely dissolved and the process depends on composition and size of glass particles. Deeper knowledge biomaterial-to-cell interactions at molecular levels will improve our ability to use biomaterials and predict treatment outcomes in patients.

Aim: In the present study, we have addressed the question how 45S5 BG particles and the corrosion products may regulate osteoclast differentiation and bone resorption.

Introduction: Effects of BG conditioned cell culture medium or BG particles on bone resorption were analyzed as 45Ca release from mouse calvarial bones, or in the osteoclast pit assay. Effects on osteoclast phenotypic gene expression was analyzed with real-time PCR and osteoclast formation in mouse bone marrow cultures and RAW264.7 cells

Results: A dose-dependent inhibitory effect on osteoclast formation and bone resorption was demonstrated when cell were incubated with either the conditioned medium or with BG particles.

Conclusion: Results in the present study implies that BG used in patients may inhibit osteoclast formation and bone resorption in close proximity to the biomaterial.

Conflict of Interest: None declared

Tu-P041

LOWER MINERAL CONTENT IN BONE MATRIX OF CHILDREN, ADOLSESCENTS AND YOUNG ADULTS: NORMATIVE DATA ON ILIAC BONE MINERALIZATION DENSITY DISTRIBUTION

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Mineral content is a key determinant of the stiffness, strength and toughness of bone matrix. In a reference data study using trabecular bone from adults, we found a remarkably small variation in bone mineralization density distribution (BMDD) from 25 to 90 years of age. Here we present reference data for BMDD in trabecular bone of younger subjects. BMDD was analysed in iliac bone biopsy samples ($n = 54$) from individuals aged 1.5 to 22.9 years using a validated quantitative backscattered electron imaging method. The BMDD of each sample was derived from digital grey-level images (resolution 2 μm). Four parameters characterizing the BMDD were evaluated: CaMean, the average mineral content; CaPeak, the most frequent mineral content; CaWidth, the width of the distribution reflecting the heterogeneity of the mineralization and CaLow, indicating the amount of bone with low mineralization, which typically represents bone undergoing primary mineralization. None of the four BMDD parameters varied with age from 1.5 to 22.9 years ($r^2 < 0.04$, $P > 0.15$), but significant differences to results in adults were found. CaMean and CaPeak were both 5.6% lower in the young group as compared to adults, whereas CaLow was 39% higher ($P < 0.0001$ for

each parameter). CaWidth was similar between groups. The lack of age-dependency of BMDD parameters during development may be explained by the fact that iliac bone biopsy is performed at a site approximately 1 cm below the iliac crest growth plate. Assuming a steady growth rate during most of the examined age range, the fixed distance to the growth plate means that the age of the bone tissue obtained by iliac bone biopsy remains constant and is independent of the chronological age of the individual. After the termination of growth, tissue age at the site of biopsy increases rapidly, and secondary mineralization can be completed. This may explain the difference in BMDD parameters between the young cohort and the adult group. In summary, this study suggests that age-independent BMDD reference data can be used to examine deviations from normal mineralization in children and adolescents with bone disorders.
Conflict of Interest: None declared

Tu-P042

THREE-DIMENSIONAL GEOMETRY DETERMINES THE TISSUE FORMATION KINETICS IN VITRO

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Tissue formation is determined by uncountable biochemical signals between cells, but also physical parameters are discussed to exhibit significant effects. Beyond this level there is still no quantitative analysis of how geometry affects tissue growth, which is of high significance for tissue engineering.

We established an in vitro model system, which allows quantitative description of new tissue formation in vitro in relation to physical parameters of a three-dimensional scaffold, namely pore size and geometry. Hydroxylapatite plates containing triangular, squared, hexagonal and round channels in different sizes normalized to the perimeter were used. Tissue formation was quantified over a time period of six weeks and fluorescence microscopy was used to visualize cellular organization. Mathematical modeling was applied to explain the mechanisms if tissue formation.

Here we show that the local growth rate of tissue formed by osteoblasts in three-dimensional substrates is strongly influenced by geometry of channels. New tissue formation starts preferentially in the corners leading to a network formation, which always keeps a round central opening, regardless of the original shape. A mathematical model, which deals with numerical solution, can describe this growth behaviour. Curvature driven growth leads to a growth rate proportional to local curvature, which occurs when surface tension is important. This implies that cells within the tissue surface are able to sense and react to radii of curvature much larger than the size of the cells themselves. Despite of these local variations, it was surprising, that channel geometry does not affect but channel perimeter significantly affects new tissue formation kinetics. The smaller the channel size, the higher the amount of tissue formed within the channels, displayed as projected tissue area. Doubling the perimeter from 3.14 to 6.28 mm reduced the amount of tissue at a defined time point to half or less. Furthermore, fluorescence microscopy revealed the development of mechanical forces within the new formed tissue in a way, which reminds of the "purse-string" formation found in embryonic wound closure.

The fact, that physical parameters lastingly influence new tissue formation provides a new aspect for tissue engineering and may support the optimization of the architectural design of bone implant materials to maximize the amount of new (bone) tissue formation in vivo.
Conflict of Interest: None declared

Tu-P043

MECHANICAL INDUCTION OF A DELAYED UNION IN SHEEP—A MODEL FOR INVESTIGATIONS OF THE PATHO-PHYSIOLOGICAL HEALING CASCADE IN BONE

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The aim of this study was to mechanically produce a standardized model for an experimental delayed union in sheep. This model could serve for investigations of the patho-physiological healing processes occurring in disturbed fracture repair. A tibial osteotomy was performed in 40 sheep and stabilized with either a rigid (group I) or mechanically critical (group II) external fixator. At weekly intervals, the 3-d interfragmentary movements (IFM) and ground reaction forces (GRF) were measured. After sacrifice at 6 weeks ($n = 16$), 9 weeks ($n = 16$) and 6 months ($n = 8$), radiographs were taken and the tibiae were tested biomechanically. IFM were significantly larger ($p \leq 0.048$) in group II throughout the healing period except for 42 days postoperatively compared to group I. Maximum unloading of the operated limb in group I was reached at 2 weeks postoperatively, but GRF returned to their preoperative values within 9 weeks. Maximum unloading in group II was measured at 3 days after surgery with no return to the preop values. Radiographic and mechanical observations showed significantly inferior bone healing in group II at 6 and 9 weeks compared to group I. At 6 months, 5 of the sheep showed radiological bridging of the osteotomy, although the strength of the tibiae was still inferior to group I at 9 weeks. That means, in the long term group all sheep showed a severely delayed healing with 3 sheep even developing a hypertrophic non-union. This study describes the mechanical induction of a critically delayed osteotomy healing in sheep by insufficient fixation stability. The mechanical induction of critical bone healing using an external fixation device is a reasonable attempt to investigate the patho-physiological healing cascade without suffering from any biological intervention. The analysis of a long term group with a healing time of 6 months in large animals is inevitable to surely differentiate between the development of a delayed or a non-union. In this study the delayed union was successfully induced by mechanical instability. Some sheep reacted to the instability by developing a hypertrophic non union. Therefore, the presented ovine model offers the option for investigations of

1) the patho-physiological healing processes occurring in fracture repair leading to delayed or even non-union and
2) new therapeutic approaches, arising from the identified differences in healing patterns.

This study was funded by the German Research Foundation (DFG SFB760).
Conflict of Interest: None declared

Tu-P044

DOES THE CELLULAR COMPOSITION OF THE INITIAL FRACTURE HEMATOMA ACCOUNT FOR SCARLESS BONE HEALING?

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The fracture hematoma (FH) is essential for the initiation of the healing cascade, but the cellular composition of the hematoma remains largely unknown. In this study, the immune cell populations in the FH were characterized and quantified. Results were compared with those gained from a soft tissue hematoma (STH). An osteotomy of both tibiae was performed in 6 sheep. In addition, a soft tissue trauma was induced in the M. gracilis. The hematomas were harvested 1 and 4 hours postop. Preop, peripheral blood was taken as a reference. The cells were prepared for FACS-analysis and labeled with CD45, CD5, CD2, CD8, CD4, CD21, CD14, WC1, CD25. FH: T-cell percentage in the peripheral blood and in the 4 h FH was similar and higher than in the 1 h FH, whilst the B-cell percentage was lower in the 1 h and 4 h FH compared with blood. In contrast the monocyte/macrophage percentage was highest in the 1 h hematoma (Table 1). In all investigated animals, the fraction of the cytotoxic T-cells positive for the CD25 marker showed similar courses with a significant increase ($p = 0.031$) between 1 and 4 hours. FH vs. STH: Comparing the amount of granulocytes present in the FH and STH 1 h post osteotomy, a significantly lower percentage ($p = 0.031$) was found in the FH. In the 4 h FH, a distincly higher percentage of B-cells ($p = 0.063$) was found compared to STH. Furthermore, the percentage of the T-helper cell subset was considerably higher in the FH compared to STH 1 h postop ($p = 0.063$). Fracture healing begins with the formation of a hematoma and an inflammatory response. The initial decline in T-cell/B-cell populations might be caused by the low oxygen conditions the cells have to adapt to in the hematoma. The increase seen in the macrophage population may be due to the chemotactic recruitment of these effector cells. The temporal raise of CD25 positive cells represents the increasing activation of T-cells. Granulocyte-lymphocyte percentages as well as the lymphocyte subpopulations differ between FH and STH. These results clearly suggest that the healing process in FH and STH takes different courses in this early phase which may contribute to the scarless regeneration of the fractured bone. This study was supported by the German Research Foundation (DFG SFB760).

Table 1

	T-cells	B-cells	Monocytes
Blood	58.3(40.0–62.3)	12.3(8.0–13.4)	5.3(3.9–7.9)
FH1h	49.8(31.3–59.8)	6.0(4.3–7.3)	6.5(2.8–9.9)
FH4h	55.5(47.3–59.5)	6.8(5.2–7.5)	4.1(2.9–5.1)

Median(25–75 percentiles)

Conflict of Interest: None declared

Tu-P045

BONE REMODELING MARKERS IN PATIENTS WITH EARLY ARTHRITIS

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Osteoporosis frequently complicates Rheumatoid Arthritis (RA) evolution. The system RANK/RANK-L/OPG, that regulates maturation and activation of osteoclasts, has been recently proved to play a key role in the pathogenesis of RA and osteoporosis.

Aim: To describe the bone remodelling markers serum levels in a cohort of patients with early arthritis, and to analyze the relation of theses markes with bone mineral density (BMD) variations and the RA disease activity.

Patients and methods: We studied 58 patients (62% fulfilling RA classification criteria, 38% undifferentiated arthritis). Forty patients were female and 21 of them postmenopausal. The mean age was 51 [41–65] years, and the disease duration at first visit 6.5 (5–9) months. During a follow-up of 2 years four structured visits were performed collecting demographic, clinical, disability data and basic blood tests. Serum samples were also obtained and frozen at −80°C for ELISA measurement of RANK-L, OPG (R&D Systems) and ß-crooslaps (Roche Diagnostics). Dual-energy X-ray absorptiometry measurements, (Hologic QDR 4500 Elite) were performed at lumbar spine, hip and ultradistal, distal and meadforearm in the first and last visits.

Results: Clinical, biochemical and densitometrical data obtained are presented in the table number 1 where data are shown as median and interquartilic range.ß-crooslaps serum levels inversely correlated with BMD variation at lumbar spine region (r: 0.4; $p = 0.0002$) and ultradistal forearm (r: 0.27; $p = 0.03$). ß-crooslaps serum levels also correlated with DAS28 (r: 0.32; $p < 0.0001$) and HAQ (r: 0.22; $p = 0.003$). A slight correlation between OPG and DAS28 (r: 0.15; $p = 0.04$) was also observated.

Conclusion: Results of this study suggest a discrete association between ß-crooslaps serum levels and the disease activity, the functional capacity and the bone mass loss in early onset arthritis patients.

Table 1

	RANK-L (pg/ml)	OPG (pg/ml)	ß-cross (ng/ml)	F.alcal (UI/l)	DAS28	HAQ
B	1649	6103	159	70	4,2	1,125
F	1086	5932	116	70	3,1	0,75
p	n.s.	n.s.	<0,05	n.s.	<0,0001	<0,0001
	Lumbar	Femoral N	Hip total	Forearm U	Forearm D	Forearm M
VD	−7	−5	7	−4	−1	−2
P	0,01	0,009	<0,001	0,002	0,004	n.s.

VD: yearly variation for BMD (mg/cm2)

Conflict of Interest: None declared

Tu-P046

NUTRITIONAL NEONATAL PROGRAMMING OF SKELETAL SYSTEM PROPERTIES IN SHEEP

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Nutritional and metabolic exposure during critical periods of prenatal and neonatal development in animals and humans may induce long-term effects on health status in later life. The aim of this study was to investigate the effects of neonatal treatment with β-hydroxy-β-methylbutyrate (HMB) during the first three weeks of life on programming of skeletal development in sheep. The study was

performed on 12 male sheep divided into two groups. While the control group was treated with placebo, the lambs from HMB group received calcium salt of β-hydroxy-β-methylbutyrate at the daily dose of 0.1 g/kg of body weight. The assessment of growth hormone (GH), insulin-like growth factor-1 (IGF-1) and biochemical bone turnover markers in serum was performed in 21 and 130 day old animals. After the slaughter, volumetric bone mineral density (vBMD), morphological and mechanical properties were determined in femur and lumbar vertebrae. Neonatal treatment with HMB increased serum concentrations of bone-specific alkaline phosphatase, osteocalcin, GH and IGF-1 in 21 day old lambs by 125.2%, 93.8%, 71.8% and 70.9%, respectively ($P \leq 0.05$). C-terminal telopeptide of type-I collagen (CTX) concentration was increased in 130 day old animals from HMB group by 33.1% ($P = 0.03$). Furthermore, HMB administration improved bone weight, vBMD and bone morphological and mechanical properties of femur and lumbar spine ($P < 0.05$). In conclusion, this study showed long-term beneficial effects of neonatal treatment with HMB on programming of the peripheral and axial skeleton properties that were mediated by transient improvement of the somatotrophic axis function and bone metabolism acceleration.
Conflict of Interest: None declared

Tu-P047

THE EFFECTS OF COMBINED TREATMENT WITH ALPHA-KETOGLUTARATE (AKG) AND BETA-HYDROXY-BETA-METHYLBUTYRATE (HMB) DURING PRENATAL LIFE ON SKELETAL PROPERTIES IN PIGS AT SLAUGHTER AGE

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Improved nutrition during pregnancy induces positive long-term effects on skeletal system properties in the offspring. The aim of this study was to investigate effects of alpha-ketoglutarate (AKG) combined with calcium salt of beta-hydroxy-beta-methylbutyrate (CaHMB) treatment of sows during last two weeks of pregnancy on bone mineral density, geometrical and mechanical properties of femur in the offspring at slaughter age. The study was performed on pigs born by 24 sows. Two weeks before delivery, pregnant sows were divided into four groups ($n = 6$ per group). Four groups of sows were administered orally with placebo (Control group), AKG (AKG group), CaHMB (HMB group) or AKG and CaHMB (AH group), respectively. Placebo ($CaCO_3$) and CaHMB were administered at the dosage of 0.05 g/kg of BW/day while the dosage of AKG was set at 0.4 g/kg of BW/day. At the age of six months, the offspring obtained from sows were slaughtered and femur was isolated for analysis. Furthermore, bone-specific alkaline phosphatase (BAP) was evaluated in newborn and 90 day old pigs. Maternal treatment with AKG and CaHMB increased weight, volumetric bone mineral density of the trabecular and cortical bone, cross-sectional area, second moment of inertia, mean relative wall thickness, cortical index, maximum elastic strength and ultimate strength of femur in the offspring when compared to the controls ($P < 0.05$). Treatment with AKG and CaHMB increased serum concentration of BAP in newborn and 90 day old pigs ($P < 0.05$). Analysis of bone weight, cross-sectional area and second moment of inertia of femur showed the highest values of these parameters in the AH group when compared to all other groups ($P < 0.05$). In conclusion, this study showed prenatal nutritional ability to program skeletal system properties in pigs with the use of AKG and CaHMB suggesting additive effects of these substances on bone tissue.

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Conflict of Interest: None declared

Tu-P048

EFFECTS OF BISPHOSPHONATE TREATMENT OF OSTEOARTRITIS IN A GUINEA PIG MODEL

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Dunkin Hartley guinea pigs spontaneously develop osteoarthritis (OA) at approximately 3 months of age. The effect of bisphosphonate (Risedronate, BP) on the development of OA in Dunkin Hartley guinea pigs was investigated. Our hypothesis is that treatment with BP would decrease bone turnover and thereby reduce formation of new subchondral bone resulting in a reduced load on the articular cartilage, thus arresting the development of OA. Fifty-six 3-month-old male Dunkin Hartley guinea pigs were randomized into 7 groups. Three groups received Risedronate (30 μg/kg) injections s.c. 5 times a week for 6, 12, or 24 weeks. The remaining four groups served as baseline or controls receiving vehicle. The left hind limb was removed from the anesthetised animals before they were perfusion fixed. The right knee was embedded in MMA and 7-μm-thick frontal sections were cut through the central part of the condyles, where the articular cartilage is not covered by meniscus. The sections were stained with Safranin O, TRAP, or von Kossa. The OA was scored with the Osteoarthritis Research Society International (OARSI) score. The articular surface of the left proximal tibia was stained with Indian ink, and the area of the articular cartilage lesion was obtained from images of the medial tibial plateau. Using TRAP sections, osteoclast covered surfaces were counted in the medial epiphysis with CAST grid software. Digital images of the von Kossa stained sections were obtained and combined trabecular and cortical bone volume fraction (bone density) and subchondral plate thickness were determined using custom software. The OARSI score did not differ between the BP and control groups at any timepoint. The area of the articular cartilage lesions of the BP-treated animals was significantly higher after 6 weeks, but did not differ at the later timepoints. The combined OARSI and area score did not differ at any timepoint. The bone density was higher for the BP-treated animals, but significant only at 6 and 12 weeks. The OARSI score was significantly correlated with bone density when all animals were pooled. The subchondral plate was significantly thicker in BP-treated animals than in control animals at all timepoints. The BP treatment significantly suppressed the fraction of osteoclast covered surfaces at all timepoints. In conclusion, treatment with Risedronate did not influence the development of OA in Dunkin Hartley guinea pigs. Funding: Danish Rheumatism Association.
Conflict of Interest: None declared

Tu-P049

DEVELOPMENT OF OSTEOARTHRITIS AND ITS RELATIONSHIP TO MENISCAL OSSIFICATION IN DUNKIN HARTLEY GUINEA PIGS

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Dunkin Hartley guinea pigs is a well established animal model of spontaneous development of osteoarthritis (OA). It has been observed that the menisci of these rodents ossify at a very early age and well before the OA develops. Our hypothesis is that this meniscal ossification may play a role in the pathogenesis of OA in these animals. Ten male Dunkin Hartley guinea pigs to each of the ages 2, 6, 9, and 12 months were anaesthetized and the left hind limb was removed before the animals were perfusion fixed. The meniscus of the left tibia was dissected free and embedded in MMA. Horizontal 7-μm-thick sections were cut at every 140 μm through the entire meniscus and stained with Goldner trichrome. For all sections the amount of fibrous cartilage, hyaline cartilage, and bone was determined using CAST grid software. The articular surface of the left proximal tibia was stained with Indian ink, and the areas of the articular cartilage lesions were determined from digital images of the tibial plateaus. The right knee was embedded in MMA and 7-μm-thick frontal sections were cut through the central part of the condyles, where the articular cartilage is not covered by meniscus, and stained with Safranin O. The degree of OA was scored using the Osteoarthritis Research Society International (OARSI) score. At the medial condyle, no articular cartilage lesions were found in the 2-month-old animals, whereas the older animals showed increasing OARSI score. At the lateral condyle, early stage OA was found in five 12-month-old animals, whereas no cartilage lesions were found in the younger animals. The meniscal bone volume/total meniscal volume (ossified meniscus fraction) increased with age from 26% to 38% (medial), and from 16% to 28% (lateral). The severity of the articular lesions were significantly correlated to the medial ossified meniscus fraction ($r^2 = 0.6$, $p < 0.0001$). In conclusion, the present study showed that ossification of the medial meniscus is correlated to the development and progression of OA in Dunkin Hartley guinea pigs. However, additional factors may also play a role in the pathogenesis of OA. Nevertheless, these findings may question whether the OA developed in this animal model is comparable to human primary OA, where ossification of the meniscus rarely occurs.
Funding: Danish Rheumatism Association.
Conflict of Interest: None declared

Tu-P050

IDENTIFYING TYPICAL PATTERNS OF BONE HEALING TO UNDERSTANDING ENDOGENOUS TISSUE FORMATION

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Healing of bone is mechanically and biologically controlled leading to an intricate pattern of different tissue types within the fracture callus. Computer models based only on differentiation rules have tried to predict this succession of tissue patterns. A major obstacle for further refinement of these models is the lack of quantitative data that can be used to quantify these "differentiation rules". The aim of this study is to identify these different tissue types and extract their position and size from histological sections of sheep-tibia healed under two different loading conditions. The results are two characteristic healing paths that describe how tissue evolves with time as a function of position within the callus. The data for the analysis stem from an animal study on 64 healthy female Merino sheep, which underwent a mid-shaft tibia osteotomy [1]. The two bone ends were stabilized by either a rigid or semirigid fixation, resulting in two different loading conditions. The healing process was monitored by means of longitudinal histological sections at 2, 3, 6 and 9 weeks postoperatively, which were stained with Safranin Orange/von Kossa and Safranin Orange/Fast Green. The sections were photographed and digitized. The following tissue types could be discriminated: cortical bone, marrow, haematoma, fibrous tissue, fibrocartilage, hyaline cartilage and newly formed woven bone. The volume fraction, the position and the shape of the different tissue types were quantified in each image and averaged. Standard deviations of these quantities are significantly larger for semirigid fixations. The result is a description of the healing path for the two different loading conditions. Under both loading conditions the healing pattern is characterized by the reduction of the haematoma in the gap. Cartilage forms first external to the osteotomy in between the ends of the newly formed woven bone, later cartilage develops subsequently towards the cortical region of the gap while reducing in size. In addition an increasing bone density at the periosteal side of the cortical ends is being perceived.

[1] H. Schell, D. R. Epari, J. P. Kassi, H. Bragulla, H. J. Bail and G. N. Duda (2005). "The course of bone healing is influenced by the initial shear fixation stability." J. Orthop. Res. 23(5): 1022–1028.
Conflict of Interest: None declared

Tu-P051

THE CONTENT OF BONE MORPHOGENETIC PROTEINS (BMPS) IN PLATELETS VARIES BETWEEN DIFFERENT PLATELET DONORS AND THE RELEASE OF BMP-2 AND BMP-4 IS HIGHLY PH DEPENDENT

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Background: Platelet rich plasma and platelet derivates influence mesenchymal cells during wound and fracture healing processes. Recent studies on mesenchymal cells, stimulated with platelet concentrates, demonstrate consistent effects on cell proliferation; however, the effects on osteoblast differentiation and bone formation have been ambiguous. Most bone morphogenetic proteins (BMPs) influence bone formation, but particularly BMP-2 seems to be pivotal for the initiation of fracture healing. We demonstrated recently the existence of BMPs in supernatants of lysed platelet buffer preparations and that BMP-2 was found only in acidic buffers.

Methods: The content of BMP-2, BMP-4, BMP-6, and BMP-7 was analyzed in supernatants of lysed platelet concentrates (1.2–2 giga cells/mL) from 31 healthy platelet donors, 16 males and 15 females, age 21–67 years. We also investigated the pH-dependent release of BMPs in lysed platelet buffer preparations at pH 4.3 and pH 7.4.

Results: A considerable variation was found among different platelet donors. Some individuals had detectable concentrations of all four BMPs, while some did not have any detectable levels of any BMP. BMP-2 and BMP-4 were significantly more common in acidic preparations in comparison with neutral preparations; however, the pH of the platelet preparations did not influence the release of BMP-6 and BMP-7. None of the observed differences were age- or gender-dependent. The variation between platelet donors may be of importance considering the ambiguous results presented in previous studies on osteoblast proliferation and

differentiation when various platelet derivates have been used. Variable BMP concentrations could likely have influenced the outcome of both in vivo and in vitro studies. The impact of acidic buffers on the release of BMP-2 and BMP-4 may reflect the conditions during the initial stage of wound and fracture healing and the milieu at the site under activated osteoclasts in bone tissue. If fresh fractures up-regulate the synthesis of BMPs in megakaryocytes and platelets, or if osteoporosis affects the BMP content in platelets, remains to be investigated.

Conclusion: We suggest that clinical studies, comprising platelet concentrates, should involve a characterization of the BMP content.
Conflict of Interest: None declared

Tu-P052

MATRIX STIFFNESS CAN FACILITATE CALCIFICATION BY VALVE INTERSTITIAL CELLS

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Background: Aortic valve calcification (AVC) is among the most common causes of heart disease. The disease progresses from valve thickening to dystrophic calcification involving the mineralization of valve tissue and/or ossification, associated with the expression of bone-related transcripts and proteins by valvular interstitial cells (VICs) and the deposition of bone matrix. Remodeling of the extracellular matrix (ECM) is often associated with AVC, but there is a limited understanding of its biomechanical impact on valve calcification. Hypothesis: ECM remodeling may alter the mechanical properties of the ECM and consequently regulate-pathologies (i.e. calcification and/or ossification) of VICs. Aims: (1) To demonstrate the ability of VICs to form ossified bone nodules in vitro, (2) to determine if matrix stiffness regulates calcification and ossification by VICs, and (3) to investigate the role of cytoskeletal proteins in mediating mechanically-regulated pathogenesis. Methods: Primary porcine aortic VICs were cultured on tissue culture plastic or topographically-similar stiff and compliant collagen matrices. We compared the morphology, proliferation, calcification and ossification by VICs by (immuno)staining, RT-PCR, and biochemical analyses. Results: VICs formed bone nodules after 3 weeks in culture on plastic. The nodules formed by VICs stained positive for alizarin red S, von kossa, alkaline phosphatase, and osteocalcin, and the VICs expressed Cbfa-1 protein. When VICs were cultured on compliant collagen matrices, they formed bone nodules more rapidly than on plastic. The bone nodules also displayed calcium accumulation and alkaline phosphatase activity, and expressed bone-related transcripts (bone morphogenetic protein 2, osteonectin, osteocalcin) and monomeric a-smooth muscle actin (SMA). In contrast, VICs on stiff collagen matrices formed fewer bone nodules ($p < 0.05$) and expressed abundant filamentous a-SMA and F-actin stress fibres. Treatment with Swinholide A (which preferentially disrupts SMA stress fibres) had no effect on VICs cultured on compliant matrices, but further reduced the number of bone nodules on stiff matrices. Conclusions: These data suggest that stiff collagen substrates promote myofibrogenic phenotypes of valve cells, as indicated by the expression of filamentous a-SMA, whereas compliant substrates promote bone-liked phenotypes of VICs, as indicated by the expression of bone transcripts and bone nodules.
Conflict of Interest: None declared

Tu-P053

SYNERGISTIC UP-REGULATION OF PLATELET DERIVED GROWTH FACTORB RECEPTOR IN CARTILAGE DEFECTS REGENERATION BY HYPERBARIC OXYGEN AND CHONDROCYTE-PLATELET DERIVED GROWTH FACTOR DELIVERY TREATMENT : AN IN VITRO AND IN VIVO STUDY

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The objectives of this study were (1) to investigate the influence of hyperbaric oxygen (HBO), platelet-derived growth factor-bb (PDGF-bb), and their combined effects on rabbit chondrocytes in vitro (2) using alginate as a matrix to develop a chondrocyte-PDGF-bb delivery system and investigate the synergistic effects of this system and HBO treatment on the healing of rabbit articular cartilage defects. In vitro, cells were divided into four groups: (I) Control (II) HBO (III) PDGF-bb (IV) HBO combined with PDGF-bb treatment. All hyperoxic cells were exposed to 100% O2 at 2.5 atmospheres absolute (ATA) in a hyperbaric chamber for 120 minutes per 48 hours. Cell growth was measured by increase in cell number. mRNA expression of PDGF α receptor (PDGFR-α) and PDGF βreceptor (PDGFR-β) were detected by reverse transcription polymerase chain reaction (RT-PCR). The concentration of PDGF-bb released from the delivery system was quantified by enzyme-linked immunosorbent assay (ELISA) and its bioactivity to chondrocyte was tested by increasing in cell number. In vivo, the cartilage defects were grafted with chondrocytes and PDGF-bb suspended in alginate. After eight weeks, the repaired tissues were sent for histological and histochemical examination with a standardized scoring system and evaluated by Safranin-O staining, Type II collagen staining and PDGFR-βexpression. Our data suggested that HBO or PDGF-bb treatment increased cell growth. HBO plus PDGF-bb treatment had a synergistic effect on cell growth as compared with HBO or PDGF-bb treatment, respectively (Fig. 1). PDGF-bb delivery lasting for more than 16 days and increased cell growth in vitro (Fig. 2). HBO or PDGF-bb treatment up-regulated mRNA expression of PDGFR-α (Fig. 3) and PDGFR-β (Fig. 4). Combined treatment with HBO plus PDGF-bb was synergistic in up-regulating mRNA expression of PDGFR-βthan HBO or PDGF-bb treatment, respectively (Fig. 4). In vivo, scoring results showed that HBO combined with PDGF-bb treatment significantly increased the cartilage repair. Safranin-O staining (Fig. 5) and Type II collagen staining (Fig. 6) confirmed the hyaline-like cartilage regeneration in the repaired tissues. In situ up-regulating of PDGFR-βexpression partially explain the synergy effect between HBO combined with PDGF-bb treatment (Fig. 7). Accordingly, alginate chondrocytes-PDGF-bb delivery system plus HBO offers a potential treatment method for cartilage injury.
Conflict of Interest: None declared

Su-P054

Abstract withdrawn

Su-P055

CROSS SECTIONAL STUDY OF PATIENTS WITH HYPOPHOSPHATEMIC RICKETS

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Aim: Phenotype presentation of patients with Hypophosphatemic Rickets.

Methods: Patients registered with the ICD10 diagnosis code DE83.3, disorders of phosphorous metabolism, were identified by search in The Danish National Patient Registry. Their medical journals were studied and patients with biochemically confirmed Hypophosphatemic Rickets (HR) were invited to participate. Furthermore family members were screened and invited in case of HR. Patients underwent clinical and dental examination, dental X-ray, DEXA-scan (Hologic), skeletal X-ray, blood- and urine samples. Renal phosphate wasting was quantified by calculations of Phosphate Fractional Tubular Reabsorption (TRP%) and Tubular Reabsorption Treshold of Phosphate per GFR (TPO4/GFR). The phosphatonin, Fibroblast Growth Factor 23 (FGF23), is believed to play a key role in the renal phosphate wasting, the cornerstone of HPR. Intact human FGF23 will be measured by the ELISA assay purchased from Kaino's Laboratories, Tokyo, Japan. Genetic analyses in the following genes; PHEX-, FGF23-, DMP1, CLCN5- and SLC34A3 will be performed.

Results: Until now, we have studied 40 adult patients (18+y) and 16 children with HR from 23 different families. HR was previously undiagnosed in 13 adults and 1 child found by family screening. In the adult group, 13 undiagnosed and 5 HR patients had never received medical treatment. BMD lumbar Z-score was 2.1 ± 2.6 SD and BMD hip Z-score was 0.8 ± 1.5 SD, both significantly elevated compared to Hologic ref., (95CI:[1.3–3.0] and [0.3–1.2], respectively). There was no difference between treated and never treated adults ($P = 0.9$ and $P = 0.6$ respectively). TRP% and TPO4/GFR were 76.7 ± 12.8% [ref. 80–95%] and 0.55 ± 0.14 mmol/l [ref. 0.8–1.5 mmol/l], respectively. TRP% and TPO4/GFR was higher among adults never treated compared to treated, but not statistically significant ($P = 0.054$ and $P = 0.12$, respectively). Six different PHEX-gene mutations are detected till date. Within each family we observed a distinct variation in severity of HR. We await the results of the serum FGF23 analysis for a possible correlation to renal phosphate wasting.

Conclusion: We observe a distinct variation in severity of HR within each family, suggesting variable degree of penetrance and we confirm previous findings of higher BMD in the lumbar spine in HR patients.

Conflict of Interest: None declared

Su-P056

SUBCUTANEOUS SWELLINGS OF BOTH LEGS: AN UNUSUAL PRESENTATION OF PRIMARY HYPERPARATHYRODISM

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Introduction: Classical primary hyperparathyroidism often presents as an asymptomatic disorder but it may be associated with skeletal and renal complications. The skeletal disease, described historically as osteitis fibrosa cystica is characterized by subperiosteal resorption, bone cysts and brown tumours of the long bones. Overt hyperparathiroid bone disease is now seen in <5% of patients with hyperparathyroidism.

Case report: A 48-yr-old man was referred to the hospital with a three months history of malaise, muscle fatigue, polyuria and polydipsia. He had observed painless swellings in the anterior face of both legs from a few years ago. Along the last three months these swellings became to increase of size and they would be tenderness. Indeed he notified a costal pain in the right lateral superior zone of the thorax that increased with direct pressure and movements. On physical examination, he appeared well. There was a slight bulging in the referred thoracic region. On the anterior face of both legs there were several warm and painful swellings.

Investigation showed serum calcium 13.3 mg/dl, serum phosphorus 1.1 mg/dl, serum alkaline phosphatase 591 IU/L, iPTH 1024 pg/ml, 24 hr urine calcium 514.5 mg, 24 hr urine phosphorus 1190.7 mg, urine N-telopeptide 344 nM BCE/mM, 25 OH-Vitamin D 42.4 ng/ml, 1–25 OH-Vitamin D 81.9 μg/ml. The skeletal radiographs, CT and MRI showed an intramedullary mass in the right 5th rib, intracortical osteolytic shadows in tibia and fibula with periostic reaction and dishing of the leg edge; there were also osteolytic shadows in spine and pelvis. Skeletal scintigraphy showed an increased uptake in the same bones. Cervical ultrasonography and MIBI scintigraphy revealed a great right parathyroid mass.

The right thyroid lobe was resected together with the parathyroid mass (5.5 × 4.8 × 1.5 cm) and another parathyroid gland as well as the right 5th rib lesion. The anatomical diagnoses were parathyroid adenoma and rib brown tumour respectively. Serum calcium and iPTH decreased until 8.6 mg/dl and 65 pg/ml and respectively. Currently the patient is asymptomatic; legs swellings persist but they are painless.

*Images of subcutaneous lesions, radiographic and pathological studies will be provided.

Conflict of Interest: None declared

Su-P057

EXPRESSION OF BONE MORPHOGENETIC PROTEINS AND THEIR RECEPTORS IN NORMAL HUMAN KIDNEY AND RENAL CELL CARCINOMA

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Background/aims: Bone morphogenetic protein (BMP), a member of the TGF-beta superfamily, is involved in development, morphogenesis, cell proliferation and apoptosis. The BMPs are produced as precursor proteins, which are cleaved forming a mature active protein. Mature BMP binds heteromeric complex of serine kinase receptors BMPR-IA/IB and BMPR-II which then phosphorylates and activates Smad 1, 5 and 8 transcript factors. Different members of BMPs family have been investigated in malignant disease of various organs. Dysregulation of BMP expression has been reported in tumorigenesis. Previous results have shown dysregulation of some BMPs and their receptors in renal cell carcinoma, too. We investigated the protein expression of bone morphogenetic protein-6 and-7 and their receptors BMPR-IB and BMPR-II in patients with different type of renal cell carcinoma as well as in healthy kidney tissue.

Patients and Methods: After nephrectomy due to renal cancer, tissue samples of renal cancer were taken and frozen at −80 degrees. Normal tissue sample was taken from the same kidney and served as control. Pathohistological analysis of renal tissue confirmed two types of renal cell carcinoma (RCC), RCC-clear cell and RCC-chromophobe type. For Western blot analysis we used goat polyclonal antibodies, anti-BMP-6, anti-BMP-7, anti-BMPR-IB and anti BMPR-II (Santa Cruz Biotechnology, USA). For normalizing we used beta-actin (R&D Systems). The bands were visualized and quantified using Kodak 1D image analysis software.

Results: The protein expression of BMP-6, BMP-7, BMPR-IB and BMPR-II was detected in normal kidney tissue and in renal cell

carcinoma tissue. RCC-clear cell and RCC-chromophobe type showed different level of protein expression toward expression in normal kidney tissue. The expression of BMP-6 and -7 was lower in RCC-clear cell as well as in RCC-chromophobe type than in normal kidney tissue. RCC-chromophobe type showed higher expression of BMPR-IB and BMPR-II than normal kidney tissue. Unlike RCC-chromophobe type, RCC-clear cell showed decreased expression for BMPR-IB and BMPR-II.

Conclusion: In the present study the correlation between the expression of bone morphogenetic proteins as well as their receptors and subtype of renal cell carcinoma was found.

Conflict of Interest: None declared

Su-P058

LYMPHOCYTES AND SYNOVIAL FLUID FIBROBLASTS SUPPORT THE OSTEOCLASTOGENESIS THROUGH RANKL, TNF-ALPHA, AND IL-7 IN AN IN VITRO MODEL DERIVED FROM HUMAN PSORIATIC ARTHRITIS

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Psoriatic arthritis (PsA) is an inflammatory joint disease, characterized by extensive bone resorption whose mechanisms have not been fully elucidated. Thus, in the present study we investigated the involvement of RANKL, TNFα and interleukin-7 (IL-7) in the osteoclastogenesis of PsA patients. In vitro osteoclastogenesis models, consisting of unfractionated and T-cell-depleted mononuclear cells from peripheral blood (PBMCs) and synovial fluid (SFMC) of 20 PsA patients as well as from healthy donors were studied. Freshly isolated T and B cells from PBMCs and T cells and fibroblasts from SFMCs of PsA patients were subjected to RT-PCR to detect the levels of RANKL, TNFα and IL-7. The osteoclastogenesis was studied in the presence of RANK-Fc, anti-TNFα, and anti IL-7 functional antibodies.

We demonstrated that lymphocytes and fibroblasts support the OC formation of PsA through the production of osteoclastogenic cytokines. In particular, OC formation was completely abolished in unstimulated T-cell-depleted PBMC cultures, and reduced of about 70% in unstimulated T cell-depleted SFMC cultures. Freshly isolated T cells from PBMCs and SFMCs of PsA patients overexpressed RANKL and TNFα, while fibroblasts from synovial fluid produced only RANKL. We showed that the presence of RANK-Fc and/or anti-TNFα functional antibodies reduced the OC formation. Moreover, T and B cells from PBMCs as well as T cells and fibroblasts from SFMCs express IL-7 mRNA. Finally, the anti-IL-7 functional antibody significantly reduced osteoclastogenesis.

Our results suggest that fibroblasts, B and T lymphocytes by producing RANKL, TNFα and IL-7, support the OC formation, contributing to the aggressive bone resorption in PsA patients.

Conflict of Interest: None declared

Su-P059

OSTEOGENIC POTENTIAL ALTERATION BY OSTEOBLASTS OBTAINED FROM HUMAN PERIODONTITIS PATIENTS: ROLE OF TRAIL

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Periodontal disease (Pd) is characterized by extensive alveolar bone loss, which occurs as a consequence of an impaired bone remodelling. Bone remodelling process is regulated by a correct balance between osteoclast and osteoblast (OBs) activity. Alveolar bone loss could be due to an increased osteoclast resorption or to a decreased osteoblast activity or both. Although it is already known the osteoclast dependent increase of bone resorption in Pd, it is still poorly understood osteoblast behaviour. In the present study we hypothesize that the activity and surviving of OBs could be altered in patients affected by Pd. Thus we studied OBs obtained from alveolar bone fragments of patients affected by Pd. The results were compared with osteoblasts obtained from healthy donors used as control.

We demonstrated that OB markers are weakly expressed compared to control ones. In particular we show that Alkaline Phosphatase activity, collagen I synthesis as well as mineralized nodules formation, the typical features of osteoblast differentiation, are significantly lower in OB from Pp. Furthermore OBs from Pp are more sensitive to the apoptotic effect of TNF-related apoptosis-inducing ligand (TRAIL) and express lower amount of decoy receptor 2(DcR2) leading to a higher and faster OB death in the presence of TRAIL. Moreover TRAIL levels were higher in Pp serum samples.

In conclusion, we demonstrated that the altered osteoblastic phenotype and the increased rate of TRAIL-induced OB apoptosis in Pp could be an additional mechanism contributing to alveolar bone loss in periodontal disease.

Conflict of Interest: None declared

Su-P060

Abstract withdrawn

Su-P061

BISPHOSPHONATE INDUCED-METAPHYSEAL SCLEROTIC BANDS IN THE FEMUR INVOLVED BY FIBROUS DYSPLASIA. A COMPARATIVE STUDY BETWEEN AFFECTED AND UNAFFECTED TISSUE

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Intravenous pamidronate is currently used in the treatment of children with fibrous dysplasia (FD) of bone. While relief of bone pain is the most obvious benefit of this therapy, other effects, including increase of radiographic density and reduction in size of individual lesion, have not been established. Of note, no obvious histologic effect of treatment on the dysplastic lesions has been observed. Histomorphometric results in FD tissue of patients treated with intravenous pamidronate, or untreated, have been reported to be similar. We report here on a patient with McCune Albright Syndrome presenting with precocious puberty, typical cafè au lait spots and FD of the right proximal femur. At the age of 4, extensive involvement of the long bones of the right limb and a varus deformity of the right proximal femur were apparent on X-ray examination. For this reason, a treatment with intravenous pamidronate was started. An X-ray performed at the age of 5 revealed that horizontal sclerotic bands, the radiographic hallmark of bisphosphonate therapy, were formed in the metaphysis of the

long bones of the limbs. At the age of 6, the patient was admitted for a diaphyseal fracture of the right femur and X-ray revealed that the sclerotic bands were increased in number. At the age of 10, a distal femur osteotomy was performed because of a 3 cm limb lenght discrepancy and radiograhic examination of the knee revealed well formed and clearly discernible metaphyseal bands only in the normal left distal femur and proximal tibia and fibula. Contact microradiography and histology of two bone samples obtained at surgery demonstrated that the metaphyseal bands occurred only in the unaffected bone. No cartilage tissue was detected in the bands. Histomorphometric analysis of static parameters of bone structure, formation and resorption revealed i) marked excess of unmineralized osteoid and increased bone resorption in FD bone compared to unaffected bone, and ii), for the affected bone, values similar to those previously reported for both pamidronate treated and untreated age matched FD patients. Our data indicate that intravenous pamidronate does not affect remodeling in FD lesional tissue. The absence of cartilage within the available metaphyseal bands may indicate that, once formed for the temporary interruption of growth plate cartilage resorption at the time of pamidronate infusion, they are normally remodeled in the intervals between the infusions.

Conflict of Interest: None declared

Su-P062

IMMUNOASSAYING OF BONE MORPHOGENETIC PROTEIN-9 AND ITS RECEPTORS IN LIVER AND PANCREAS OF DIABETIC WISTAR RATS

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Background data: In type 2 diabetes, elevated glucose concentration results from impaired secretion of insulin and insulin resistance in target tissues like muscle and liver. It is possible that liver generates hepatic insulin-sensitizing substance (HISS) which enhances glucose uptake in peripheral tissues, but the nature of this putative hormone remains obscure. BMP-9 molecule identified by Chen et al1 is very strong candidate for HISS, with an observed effect that was comparable to that seen with insulin. Moreover, purified recombinant BMP-9 was shown to cause a sustained lowering of plasma glucose concentrations in normal (C57BL/6) and diabetic (db/db) mice. In addition to mimicking the action of insulin, BMP-9 also stimulated insulin release in Wistar and Zucker diabetic rats. Despite to encouraging results of BMP-9 as potential HISS, questions remain regarding physiological role of BMP-9 in glucose metabolism.

Aim: To explore immunolocalisation of BMP-9 and BMP receptors (BMPR-IA, BMPR-IB and BMPR-II) in liver and pancreas of diabetic Wistar rat (N = 8) and also in control group (N = 8).

Methods: Diabetes was induced by single streptozotocin administration. Glucose concentrations were controlled in regular bases and animals were sacrificed by the end of the fifth week. Immunolocalisation was performed using anti-BMP-9, anti BMPR-IA, anti-BMPR-IB and anti BMPR-II goat polyclonal antibody (Santa Cruz Biotechnology, CA, USA).

Results: BMP-9, BMPR-IA, BMPR-IB and BMPR-II are expressed in both normal and diabetic organs. BMP-9 activity as well as activity of BMPR-IA, BMPR-IB and BMPR-II was observed as cytoplasmic staining in hepatocytes, epithelial cells of bilial ducts and in Kupfer cells. In pancreas, activity of BMP-9, BMPR-IA, BMPR-IB and BMPR-II was detected in epithelial cells of pancreatic ducts and also in epithelial cells of Langerhans islets.

Conclusions: BMP-9 and BMP receptors are expressed in the diabetic rat liver and pancreas. Further determination of the expression levels of the BMP-9 and it's receptors in organs mostly affected by chronic hyperglycemia will better explain this protein's possible protective function in diabetes.

1. Chen C et al. An integrated functional genomics screening program reveals a role for BMP-9 in glucose homeostasis. Nat Biotechnol. 2003 Mar;21(3):294–301.

Conflict of Interest: None declared

Su-P063

EXPRESSION OF BONE MORPHOGENETIC PROTEIN-9 IN ADULT HUMAN LIVER

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Background and aims: Bone morphogenetic proteins (BMPs) are expressed in a tissue specific manner and control the development and homeostasis of tissues in organisms ranging from drosophila to humans. Because these family members play such an important developmental role in regulating tissue growth and differentiation one must be careful to be certain that similar effect are also seen in adult organism. BMP-9 is specifically expressed in the liver and receptors for BMP-9 have been identified on liver cells, as it was presented on animal models. Our previous investigation revealed positive immunoassaying for BMP-9 and BMP receptors (IA, IB and II) on healthy and cirrhotic human liver. Positive activity of BMP-9 was observed as intracellular cytoplasmic staining in hepatocytes, bile duct epithelium and Kupfer cells of healthy and cirrhotic livers. With regard to afore mentioned it is important to quantify expression of BMP-9 in normal and cirrhotic liver in order to better understand possible protective function of this protein in the organism of an adult.

Patients and Methods: Tissue samples of healthy and cirrhotic livers were taken from autopsy cases and frozen at −80 degrees. For Western Blot Analysis liver tissue was homogenized and protein was obtained using Lyses buffer. After transferred to nitrocellulose the blots were incubated overnight at 4 degrees with anti-BMP-9 goat polyclonal antibody (Santa Cruz Biotechnology, CA, USA). BMP-9 was detected on the developed film and scanned into Adobe Photo Shop. The intensity of each band was determined using Kodak 1D image analysis software.

Results: Western Blot analysis revealed high expression of BMP-9 in normal and also in cirrhotic liver. BMP-9/beta actin ratio revealed no significant difference in levels of expression between normal and cirrhotic tissue samples.

Conclusions: The expression of BMP-9 was maintained in cirrhotic liver, compared to healthy organ. Less number of functional hepatocytes in cirrhotic liver showed similar level of the BMP-9 expression compared to normal liver tissue, which suggests good compensatory response of diseased organ.

Conflict of Interest: None declared

Su-P064

FOLLOW-UP OF ARTERIAL CALCIFICATIONS IN RATS WITH CHRONIC RENAL FAILURE: A QUANTITATIVE IN VIVO AND IN VITRO MICROTOMOGRAPHY ANALYSIS

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Vascular calcification (VC) is a prominent feature of cardiovascular disease in patients with chronic renal failure (CRF). We previously demonstrated that VC in rats with adenine-induced CRF can be detected and visualized by in vivo X-ray microtomography (micro-CT) [1].

In the present study, we used this method to assess the onset and evolution of the calcification process. Although micro-CT basically is an imaging technique, this study also evaluated the analytical performance of micro-CT in the detection and quantification of the amount of calcified tissue.

Male Wistar rats (n = 108) were divided in different experimental groups to be studied at given time points. To induce CRF the rats were fed two different high adenine diets for 4 weeks. VC was assessed by weekly scans of the living animals (Skyscan 1076, Kontich, Belgium). The results of the in vivo image analysis were compared with histological data (Von Kossa staining and scoring) and with bulk calcium content measured by atomic absorption spectrometry (AAS). VC started focally, was initially seen histologically after 3 weeks and could be detected by in vivo micro-CT after 4 weeks of CRF (8/9 animals). Further follow-up on high phosphate diet induced a pronounced increase of VC with a median calcium content of 32.9 mg/g tissue (median in control animals: 0.17 mg/g) at week 8. In several animals, density of the ectopic calcifications became comparable to that of bone as measured by in vivo micro-CT.

As confirmed by traditional destructive methods, micro-CT proved to be effective for the follow-up of aortic calcifications at all different time points. "Positive" animals, with ectopic calcifications higher than 5 mg/g tissue, could be distinguished from living rats that did not calcify. Sensitivity of in vivo micro-CT was was high enough to detect calcifications in 70% of the animals. After sacrifice, in vitro micro-CT analysis accurately quantified aortic calcifications (with almost 95% reliability), providing a complete analysis of both degree and distribution of the calcification.

In conclusion, micro-CT proves to be a promising non-invasive imaging technique allowing quantification of ectopic calcification both in living animals and in extracted tissues.

[1] Persy, V., et al. Arteriosclerosis Thrombosis and Vascular Biology, 2006, 26 : 2110.

Conflict of Interest: None declared

Su-P065

EFFECT OF STOPPING ANASTROZOLE ON BONE MINERAL DENSITY: SEVEN-YEAR RESULTS FROM THE ATAC TRIAL

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Purpose: The 'Arimidex', Tamoxifen, Alone or in Combination (ATAC) trial (median follow up 100 months) has shown that adjuvant anastrozole given for breast cancer is associated with higher fracture rates than tamoxifen. On stopping therapy, there is a rapid decline in fracture rates in the anastrozole group to levels similar to rates in the tamoxifen group. Examining the changes in bone mineral density (BMD) following the end of the treatment period provides additional important information on the skeletal health effects of study treatment withdrawal. Patients and methods: This prospective sub-study of the ATAC trial assessed BMD changes in postmenopausal women with invasive primary breast cancer receiving anastrozole (1 mg/day) or tamoxifen (20 mg/day) as adjuvant therapy for five years. Lumbar spine and total hip BMD were assessed at baseline and after 1, 2, 5, 6 and 7 years. The table 1 below shows the percentage change in BMD (anastrozole vs tamoxifen, $^{**}p < 0.01$, $^{***}p < 0.001$). Conclusions: The increased bone loss seen with anastrozole compared with tamoxifen during the five-year treatment period did not continue into the off-treatment follow-up. A recovery of BMD at the lumbar spine and a slowing down in loss at the hip was seen in the anastrozole group.

Table 1

Treatment	Anastrozole 0–5 yrs N = 57	Tamoxifen 0–5 yrs N = 51	Anastrozole 5–7 yrs N = 24	Tamoxifen 5–7 yrs N = 29
Lumbar spine, %	−6.1***	+2.8	+4.0**	−0.2
Total hip, %	−7.2***	0.7	−0.9	−2.4

Conflict of Interest: R. Eastell, AstraZeneca, Grant Research, Consultant, Speakers Bureau
R.A. Hannon, AstraZeneca, Grant Research
J. Mackey, AstraZeneca, Consultant, Speakers Bureau
J. Mackey, Sanofi, Consultant, Speakers Bureau
J. Mackey, Pfizer, Consultant, Speakers Bureau
J. Mackey, Eli Lilly, Consultant, Speakers Bureau
J. Mackey, Amgen, Consultant, Speakers Bureau
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A. Howell, Novartis, Consultant, Speakers Bureau
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R. Coleman, AstraZeneca, Speakers Bureau
G. Clack, AstraZeneca, Shareholder, Other (employee)

Su-P066

COLLAGEN AND PROTEOGLYCAN PERTURBATIONS IN CARTILAGE OF PATIENTS WITH MORQUIO A DISEASE

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Morquio A disease, mucopolysaccharidosis type IVA, is a lysosomal storage disease caused by a deficiency of N-acetylgalactosamine-6-sulfate sulfatase (GALNS; E.C. 3.1.6.4). This lysosomal enzyme is involved in the digestion of keratan sulfate and chondroitin-6-sulfate and its deficiency gives rise to a lysosomal accumulation of partially digested glycosaminoglycans (GAGs); a phenomenon seen particularly in bone and cartilage. Although Morquio disease is a disorder of connective tissue no reports have appeared on effects of the extracellular matrix. In the present study we analyzed the biochemical properties of collagen of both bone and cartilage of Morquio A patients and the ultrastructure of the cartilage matrix.

Bone and cartilage samples were obtained from two non-related Morquio A patients. The biochemical analyses included (i) the cross-links hydroxylysylpyridinoline (HP) and lysylpyridinoline (LP), and (ii) hydroxylysine (Hyl) and hydroxyproline (Hyp). Bone and cartilage samples of normal individuals (n = 41 and 34, respectively) served as controls. For microscopy tissues were fixed in 1% glutaraldehyde and 4% formaldehyde, embedded in plastic and further processed.

Biochemical analyses revealed that in bone samples hydroxylation and cross-linking of collagen did not differ from the controls. Analyses of the cartilage, however, showed remarkable differences. The amount of Hyl residues in the triple helix of cartilage collagen was decreased by approximately 50% and the ratio of HP/LP was strongly increased due to a decreased level of HP and a concomitant increase in LP. Electron microscopy showed, in addition to a high number of vacuoles in the chondrocytes, a wide rim of extremely well ordered layers of proteoglycans surrounding the cells. In this pericellular area collagen fibrils were absent. The fibrils found interchondrally proved to be relatively thin. Our data demonstrate that in Morquio patients the hydroxylation and cross-linking of cartilage collagen, but not of bone collagen, is severely disturbed. In addition we show that the extracellular arrangement of proteoglycans is affected. So it seems that deficiency of GALNS not only disturbs intracellular digestion of GAGs but also affects processing of various extracellular matrix components.

Conflict of Interest: None declared

Su-P067

INTRAVENOUS ZOLEDRONIC ACID (ZOL) COMPARED TO IV PAMIDRONATE (PAM) IN CHILDREN WITH SEVERE OSTEOGENESIS IMPERFECTA (OI)

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OI is a heritable disorder characterized by fragile bones and recurrent fractures. IV PAM increases bone mineral density (BMD) and reduces fractures in children and adolescents with OI. In an international, multicenter, randomized, open-label trial, the efficacy and safety of IV ZOL and IV PAM in 150 patients (1 to 17 yrs, mean 8.5 yrs) with severe OI (type III or IV; or type I with $\geq$3 minimal trauma fractures or deformities requiring surgery) were evaluated. ZOL infusions were given over 30 to 45 min at 0.025 mg/kg body weight and 0.05 mg/kg for children $\leq$3 yrs and 3–17 yrs, respectively. PAM infusions occurred over 3 days (dose per cycle: 3 mg/kg). PAM and ZOL were administered every 3 mos for 1 yr except for PAM patients <2 yrs of age (every 2 mos). The primary efficacy variable was the percentage change in lumbar spine (LS) areal BMD at 12 mos vs baseline. Compared to PAM, ZOL patients had a significantly greater increase in LS BMD (42.7% vs 34.7%; P = 0.013) and significantly greater reductions in serum biomarkers of bone resorption and formation at 12 mos. ZOL patients had greater increases in LS Z-score, compared to PAM (mean change +1.7 vs +1.4; P = 0.088). The proportion of patients with clinical fractures was similar between groups. Both treatments significantly reduced clinical fractures. In the ZOL group, the number of fractures per patient decreased from a median of 3.0 (range 0 to 20) in the 12 mos before baseline to a median of 0 (range 0 to 24) in the 12 mos of study. In the PAM group, the number of fractures per patient decreased from a median of 2.0 (range 0 to 7) to a median of 0 (range 0 to 7). Adverse events, primarily transient post-infusion symptoms, were comparable between treatment groups. Hypocalcemia occurred in 22% of ZOL patients and 9% of PAM patients, mostly after the first infusion and asymptomatic. In patients with OI types III and IV the incidence of femur fractures was similar between treatment groups, but among OI type I patients was higher in the ZOL group. ZOL patients with femur fractures had more severe disease at baseline than PAM cases, as indicated by: median LS BMD (0.28 g/cm^2 vs 0.32 g/cm^2), median number of fractures over the lifetime (14.0 vs 9.5), and median number of fractures in the 12 mos preceding first study drug infusion (3.0 vs 1.5). We conclude that ZOL is effective therapy for children with severe OI. Evaluation of long-term safety and efficacy of ZOL in patients with severe OI is ongoing.

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FH Glorieux, Novartis, Consultant
J-P Devogelaer, Novartis, Consultant

Mo-P068

PRIMARY HYPERPARATHYROIDISM - A PROTEIFORM DISEASE

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Primary hyperparathyroidism (HPP) is a common endocrine disease in those countries where hypercalcemia is readily detected and the prevalence and incidence of disease were found to be much higher than previous estimates. Today, the clinical profile had shifted from a

symptomatic disorder with hypercalcemic symptoms, kidney stones, overt bone disease, or a specific neuromuscular dysfunction, toward a more asymptomatic state.

The diagnosis of hyperparathyroidism is revealed by persistent hypercalcemia in the presence of inapropriate elevated levels of PTH.

Background:We have evaluated clinically, biochimically,and from the hormonal and evolutive perspective, a number of 65 patients with primary hyperparathyroidism (in the last 3 years)who had presented in the osteoporosis center from National Institute of endocrinology. Methods: The patients were biochemically evaluated at 6 months intervals with serum levels of calcium, phosphorus, alcaline phosphatase, bone turnover markers (crosslaps and osteocalcine), urinary calcium/24 hours; we have measured at baseline and anually serum levels of PTH and 25 hydroxy vitamin D. Bone mineral density was measured anually by dual X-ray absorbtiometry. Results: There were 15 patients (23.7%) with asimptomatic HPP and 50 patients (86.93%) with simptomatic HPP. Mean value of PTH was 255 $\pm$ 135,8 pg/mL.

In the study, 31 patients underwent parathyroidectomy and 19 patients were followed without surgery and they received antiresorbtive therapy.Parathyroidectomy in patients with both types of HPP (symptomatic and asymptomatic) led to normalization of serum calcium concentrations, and a mean increase in lumbar-spine bone mineral density of 11.76% ($p = 0.02$) after 1 year. The asymptomatic patients who did not undergo surgery had no significally changes in serum calcium concentration, urinary calcium excretion or bone mineral density. Majority of the patients (97%) were associate deficite or insufficiency of 25OH vitamin D. Conclusions:In patients with HPP parathyroidectomy results in the normalization of biochemical values and increase of bone mineral density. Asymptomatic patients who did not undergo surgery did not have progresion of disease. Esential characteristic of Romanian patients with HPP is the association with secondary hyperparathyroidism through defficiency or insuficiency of vitamin D.

Conflict of Interest: None declared

Mo-P069

NOVEL INTERACTION BETWEEN SQSTM1 AND ALFY IN HUMAN OSTEOCLASTS AND EFFECT OF SQSTM1 MUTATIONS

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Paget's disease of bone (PDB) is a late-onset disorder characterised by focal areas of increased bone turnover. Within Pagetic lesions, osteoclasts are increased in size, number, nuclearity and activity; osteoblasts are also overactive, depositing bone that is disorganised and prone to fracture. Mutations in the ubiquitin-associated (UBA) domain of the gene for Sequestosome-1/p62 (*SQSTM1*) have been identified as a cause of PDB. To date, fourteen different variants have been reported in individuals affected with PDB, all of which cluster in and around the UBA domain of the protein and affect the ability of SQSTM1 to bind ubiquitin. We sought to determine whether novel protein interactions with SQSTM1 in osteoclasts might account for the cell-specific effects of *SQSTM1* mutations.

Human osteoclast-like (hOCL) cells were generated from M-CSF-dependent peripheral blood mononuclear cells treated with RANKL. Once multinucleated hOCLs had formed, cells were lysed. SQSTM1 in complex with interacting proteins was co-immunoprecipitated from the hOCL lysate using GP62N antibody (Progen). Interacting proteins were separated by SDS-PAGE and visualised using GelCode Blue Protein Stain (Pierce). Bands were excised from the gel and subjected to proteomic analysis using tandem mass spectrometry.

Database searching identified Autophagy-Linked FYVE protein (ALFY) as a component of one of the bands. In non-resorbing hOCL, endogenous SQSTM1 was present throughout the nucleus and endogenous ALFY was located at the nuclear membrane. Under conditions of cell stress (starvation), SQSTM1 and ALFY both relocalised to the cytoplasm. Overexpression of wild-type SQSTM1 in HEK293 cells resulted in colocalisation of SQSTM1 and ALFY in cytoplasmic aggregates. When PDB variants were overexpressed, missense variants of SQSTM1 (392L and 425R) colocalised with ALFY in much larger cytoplasmic aggregates, whereas deletion of the UBA domain (396X) lead to a redistribution of ALFY but prevented the formation of cytoplasmic aggregates.

In summary, we have identified a novel interaction between SQSTM1 and ALFY in human osteoclasts. The interaction with SQSTM1 is regulated by cell stress and is affected by mutations associated with PDB. It is possible that mutations in the UBA domain of SQSTM1 affect autophagic clearance of ubiquitinated protein aggregates within osteoclasts. However, the role of ALFY in osteoclasts and its contribution to the Pagetic phenotype remains to be clarified.

Conflict of Interest: None declared

Mo-P070

VALUE OF HIGH RESOLUTION COMPUTED TOMOGRAPHY IN DIAGNOSTIC OF DEGENERATIVE CHANGES OF LUMBAR FACET JOINTS IN PATIENTS WITH LOWER BACK PAIN

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Degenerative changes of lumbar facet joints are important ethiopathogenetic factors contributing to lower back pain. Considering the fact that diagnostic of degenerative changes of intervertebral discs has been widely reported in studies, the diagnostic criteria for CT (computed tomography) and MR (magnetic resonance) evaluation have been elaborated. The aim of the study was to determine value of high resolution computed tomography for diagnostic of degenerative changes within lumbar facet joints pertaining to articular cartilage and articular processes. The degree of articular cartilage degeneration and articular process sclerosis was scored using conventional anatomical criteria for CT evaluation and graded between I° and III°. CT examination of lumbar spine was performed using spiral CT technique on 3 mm/pich thick cross-sectional slices; they were then reconstructed as 1.5 mm thick images of high resolution algorithm. The examination was performed within the L1–S1 segments of the spine. 50 patients suffering from lower back pain have undergone evaluation—25 each of males and females aged between 18–78, mean age 51.14; in all, 500 facet joints were evaluated. For each facet joint, degree of degenerative changes and sclerosis of the processes were evaluated separately. Altogether, degenerative changes of the aricular cartilage were found in 238 facet joints (I°-137, II°-81, III°-20), while degenerative changes of articular processes were found in 332 facet joints (I°-231, II°-40, III°-61). In conclusion, high resolution CT technique has been recognized as a valuable diagnostic tool for evaluation of degenerative changes of the articular cartilage and articular processes in patients suffering from lower back pain. Furthermore, this technique provides possibility for optimal grading of degenerative changes of the articular cartilage and articular processes.

Conflict of Interest: None declared

Mo-P071

BONE MARKERS IN CHRONIC INFLAMMATORY BOWEL DISEASES IN CHILDREN AND ADOLESCENTS

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Chronic inflammatory bowel diseases in children may cause impairment of bone metabolism and increased risk of osteoporosis. The aim of this study was assessment of bone metabolism in 47 patients (19 boys, 28 girls, age 13.72 +/−2.77, range 7–20 years) with Crohn's disease (30), ulcerative colitis (11) and non-determined colitis (6). Measurements comprised 25-OH D, bone markers - osteocalcin, procollagen 1 propeptide (P1CP), collagen 1 telopeptide also in serum and urine (crosslaps); and lumbar spine densitometry. Follow-up in 27 patients included measurement of bone markers every 6 months and densitometry after 12 months. No difference existed between sexes, diagnoses or z-scores. Bone markers were on average increased as compared to adult values. In girls, greater variation with higher values at age 10–12 and decrement therafter was observed. In boys higher values were found at 12–14 years and decrease at age 16 years. Initial 25-OH D was decreased in most patients (<50 nmol/L in 67%, <80 nmol/L in 88%). Z-scores were mostly greater than -2.5, except in 3 patients. At the start of the study negative correlation with age was found for 25-OH D ($p < 0.03$), telopeptide in serum ($p < 0.004$) and urine ($p < 0.001$). Positive correlation existed between bone markers. In the follow-up only 25-OH D showed significant increment ($n = 21$, $p < 0.009$) which coincided with sampling in Spring and Summer. Patient monitoring by densitometry and bone markers did not reveal a trend related to clinical outcome (improvemet, unchanged, deterioration) or was affected by age. These results show that in children with chronic inflammatory bowel disease vitamin D deficiency is the most prominent finding. Measurement of bone markers demonstrated skeletal growth and high bone turnover at puberty, with later cessation toward adult values. Skeletal integrity as assessed by densitometry and bone markers was not compromised. Continuation of patient monitoring might reveal bone metabolism variations related to disease and treatment.
Conflict of Interest: None declared

Mo-P072

CELLULAR MECHANISM OF DECREASED BONE FORMATION IN BRTL MOUSE: INCREASED OSTEOCLASTS ARE INDEPENDENT OF DECREASED OSTEOBLAST FUNCTION AND RANKL/OPG RATIO

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Introduction: The Brtl mouse, a knock-in model for moderately severe osteogenesis imperfecta (OI), has a glycine substitution (G349C) in half of type I collagen alpha1(I) chains. Brtl femoral geometric properties are reduced throughout life, although fracture load normalizes after puberty.

Materials and Methods: Brtl static and dynamic histomorphometry, immunohistochemistry of TRAP, RANKL, OPG, CFU assays, urinary DPD crosslinks and real-time RT-PCR.

Results: Brtl cortical and trabecular bone are reduced before and after puberty, with BV/TV reduced 40–45%. Brtl ObS/BS is comparable to Wt, and Brtl and Wt marrow can replenish osteoblasts equally, as shown by equivalent numbers of CFU at both ages. However, OcS/BS is increased in Brtl at both ages (36–45%), as are the number of TRAP positive cells (57–47%). After puberty, Brtl ObS/BS decreases comparably to wild-type mice, but MAR falls to half of wild-type values. In contrast, Brtl OcS falls only moderately ($\sim 16\%$) and Brtl TRAP staining remains significantly elevated. As a result, Brtl BFR declines from a normal level at 2 months to half of wild-type values at 6 months. Immunohistochemistry and real time RT-PCR reveal increased RANKL and OPG levels in Brtl, but the RANKL/OPG ratio is comparable to wild-type, suggesting that this signaling pathway is not responsible for the cellular asynchrony. Also, Brtl urinary DPD crosslink levels were normal, as in many OI patients. This may reflect Brtl matrix insufficiency or resistance of abnormal matrix to resorption.

Conclusions: Brtl OcS and TRAP staining are elevated compared to wild-type despite normal Brtl ObS and declining matrix apposition. The cellular imbalance results in declining BFR as Brtl ages, consistent with the reduced Brtl femoral geometry. The disparity in cellular number and function is independent of RANKL/OPG ratio and may result from other signaling pathways or increased precursors.
Conflict of Interest: None declared

Mo-P073

BONE TURNOVER AFTER LIVER TRANSPLANTATION:RELATIVE CONTRIBUTION OF BONE MARKERS AND OSTEOPROTEGERIN/ RANK-LIGAND SYSTEM

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High bone turnover osteoporosis has been described in liver transplantation patients, but the relative contribution of osteoprotegerin (OPG)/RANK-Ligand system has not been elucidated yet.

Our aim was to investigate the usefulness of serum OPG and RANK-L in the assessment of osteoporosis associated with liver transplantation (LTx), as well as its relationship with bone mass and bone turnover markers.

Methods: 113 LTx patients (27 females, 86 males; mean age 55.1 ± 10.7 years) were cross-sectionally evaluated. Of them, 73 patients were studied in the first month after LTx, whereas the rest of patients ($n = 40$) were studied 11.3 ± 5.6 months after transplantation (range 2–24 months). BMD was measured in all patients with DXA (Hologic QDR 4500) at lumbar spine (L1–L4) and hip. Fasting serum samples were obtained for β-CTX and P1NP in 100 patients (Elecsys 1010, Roche Diagnostics), and for OPG and RANK-L ($n = 58$; ELISA, Biomedica). Urinary Deoxipyridinoline (D-Pyr) was measured in second morning void ($n = 103$;Immulite 2000 DPC). None of them was taking any antiosteoporotic drug at the entry in the study.

Results: 29% of patients had osteoporosis (lumbar and/or hip T-score <−2.5). In the first month after LTx, β-CTX was increased in 45% of patients, and D-Pyr in 58% of patients, whereas more than 6 months after transplantation, only 12.5% had increased β-CTX.

Serum levels of β-CTX were inversely correlated with lumbar spine BMD ($r = -0.37$, $p < 0.01$), total hip BMD ($r = -0.39$, $p < 0.01$), femoral neck BMD($r = -0.35$, $p < 0.01$), trocanteric and intertrocanteric BMD($r = -0.36$ and $r = -0.37$; $p < 0.01$). No significant differences were found between osteoporotic and no-osteoporotic patients in serum OPG (6.4 ± 4.4 vs 6.2 ± 2.7 pmol/L) or RANK-L (0.246 ± 0.251 vs 0.188 ± 0.194 pmol/L). Serum OPG and RANK-L were not correlated with BMD nor with bone turnover markers. Time elapsed after LTx had a significant positive correlation with BMD at hip ($r = 0.31$, $p = 0.001$) and lumbar spine ($r = 0.34$, $p, < 0.001$) whereas β-CTX levels had a negative correlation with time elapsed since transplantation ($r = -0.39$, $p < 0.001$).

In conclusion, osteoporosis with high bone resorption is common soon after liver transplantation. In the long term high bone turnover declines at the same time that BMD improves. In these patients, measurement of serum OPG and RANK-L levels doesńt add relevant clinical information.

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Conflict of Interest: None declared

Mo-P074

RECEPTOR ACTIVATOR OF NUCLEAR FACTOR KAPPA B LIGAND (RANKL) DIRECTLY MODULATES GENE EXPRESSION PROFILE OF RANK-POSITIVE SAOS-2 HUMAN OSTEOSARCOMA CELLS

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RANK/RANKL/osteoprotegerin (OPG) are the key regulators of bone metabolism. Recent finding clearly suggested a pivotal role of RANK in bone-associated tumors by presenting RANKL-triggered RANK-positive tumor cell migration. Furthermore, a positive correlation has been reported between constant expressions of RANK with decreased/absent expression of RANKL and a high metastatic phenotype in breast carcinoma. Moreover, we have recently demonstrated functional RANK expression both in a mouse and several human osteosarcoma cell lines. RANK expression in bone-associated tumors is therefore very hot spot of tumor-bone biology; however biological effects of RANKL on RANK-positive osteosarcoma cells remain to be determined. In this study, the RANKL effects on RANK-positive Saos-2 human osteosarcoma cells were determined in terms of cell viability, cell migration and gene modulation. A Mann-Whitney's U test was used for statistical analyses. Neither XTT assay nor manual cell counting could demonstrate any significant difference of Saos-2 cell proliferation after RANKL treatment. Inconsistent with the previous report in other RANK-positive tumor cells, RANKL treatment did not modulate Saos-2 osteosarcoma cell migration in the experimental conditions used. Interestingly, cDNA microarray demonstrated that 69 genes out of 6,864 cancer-related genes analyzed were significantly modulated by RANKL treatment in Saos-2 cells compared to the control group; 48 were down-regulated whereas the remaining 21 were up-regulated. Ten percents of these RANKL-modulated genes were followed by quantitative real-time RT-PCR and demonstrated same tendency. These RANKL-modulated genes included genes that were implicated in protein metabolism, nucleic acid metabolism, intracellular transport, cytoskeleton organization and biogenesis, apoptosis and signaling cascade. Our results strengthen the involvement of the RANK/RANKL/OPG axis in osteosarcoma biology. Whether RANKL induce pro- or anti-tumor activity in RANK-positive osteosarcoma by gene modulation should be determined; however our finding clearly demonstrated a capability to device novel therapeutic approaches targeting RANK-positive osteosarcomas.

Conflict of Interest: None declared

Mo-P075

TERIPARATIDE IN THE TREATMENT OF ADULT OSTEOGENESIS IMPERFECTA - A PROSPECTIVE OBSERVATIONAL TRIAL

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Brittle bones and a susceptibility to fracture from normal impacts of daily living are the characteristics of the genetic disorder Osteogenesis imperfecta (OI). An antiresorptive therapy with bisphosphonates has been shown to have beneficial effects on the clinical outcome of OI, especially in children. There is however a lack of data on the use of osteoanabolic drugs in this disabilitating disorder.

The aim of the "Teriparatide in Osteogenesis Imperfecta" trial (TOI-trial) is to study the efficacy of an anabolic treatment with teriparatide (rhPTH-1-34) in adults with clinically symptomatic disease. In this 18-month prospective, observational, single center trial we included 10 patients (6 men, 4 women) above the age of 30 years (mean age 44.9) with a T-score < -3.0 at the lumbar spine and < -2.0 at the total hip, and at least one prevalent vertebral and one non-vertebral fracture. Bisphosphonates, alfacalcidol or fluoride had been used previously over different intervals, but during the last 6 months only calcium and vitamin D. During the study, all patients received daily subcutaneous injections of 20 μg teriparatide plus oral supplements of 1200 mg calcium and 800 IU vitamin D per day.

Baseline characteristics of patients included a mean height of 154.1 cm, mean weight 66.3 kg, average T-scores of -4.03 at the lumbar spine and -3.24 at the hip, and a mean number of 1.5 new vertebral and 0.8 non-vertebral fractures per patient during the last year before intervention.

Measurement of BMD at 6-month intervals showed highly significant increases at both sites and amounted to an average gain of 10.8% at the lumbar spine and 8.4% at the total hip after 18 months. During the 18 months we observed only one new vertebral and two non-vertebral fractures. This is remarkable in relation to the respective average fracture rates during the last year before starting teriparatide injections. There was a significant decrease in back pain. Moderate, transient adverse events occurred in 5 of 10 patients.

All patients will be followed during a subsequent antiresorptive therapy up to month 36. These very positive preliminary results should encourage further, controlled trials with an anabolic therapy in osteogenesis imperfecta.

Conflict of Interest: JD Ringe, Lilly, Grant Research Support, Speakers Bureau
H Faber, None declared
P Farahmand, None declared
T Nickelsen, Lilly full-time employee

Mo-P076

INVOLVEMENT OF THE CHONDRO-OSSEOUS JUNCTION IN CANINE EXPERIMENTAL OSTEOARTHRITIS. EFFECT OF CALCITONIN

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Aim: To assess the thickness and density of the chondro-osseous junction (COJ) in a model of experimental canine osteoarthritis (OA) and to determine to what extent calcitonin (CT) can change these parameters.

Methods: After anterior cruciate ligament transection (ACLT) in the right knee, 12 dogs received a daily nasal spray delivering either 400 U of CT ($n = 6$) or a placebo ($n = 6$). The animals were killed 84 days after surgery. Proximal tibiae of each dog were stained with basic fuchsin and embedded in methacrylate. Bone mineral density (BMD) of the medial and lateral COJ was measured in each plateau by pQCT. Undecalcified 80-μm-thick coronal sections through the tibiae were microradiographed. The thicknesses of calcified cartilage and subchondral bone plate were measured in the microradiographs and the sections. The quantitative data were analysed with two-tailed t-tests. A p value < 0.05 was considered statistically significant.

Results: All operated knees presented macroscopic signs of OA, though less extensive in the CT group than in the placebo one. In both groups, BMD of the medial COJ was significantly lower in the operated tibiae than in the non-operated ones and was not affected by CT treatment. Neither ACLT nor CT treatment had any effect on the thickness of both calcified cartilage and subchondral bone plate.

Conclusions: Our study is the first to show a decrease of BMD of the COJ in early stages of canine OA. This decrease was not counteracted by CT, that had been shown previously to attenuate the OA cartilage lesions in this model. In contrast to similar studies, we did not find any OA-related increase in COJ thickness. Changes in COJ do not seem to play an important role in the development of early OA cartilage lesions.

Conflict of Interest: None declared

Mo-P077

SEVEN YEARS OF PAMIDRONATE TREATMENT IN YOUNG ADULTS WITH POLYOSTOTIC FIBROUS DYSPLASIA OF BONE: CLINICAL, BIOCHEMICAL AND DENSITOMETRIC OUTCOME

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In the last 10 years it has been reported that pamidronate (APD) treatment of Fibrous Dysplasia (FD) of bone can reduce bone pain and markers of bone turnover, improve radiological appearance of lesions and increase BMD of affected areas. However, there is limited information about long-term bisphosphonates treatment in adults. We analyzed clinical, biochemical and densitometric response to long-term APD treatment, in a homogenous group of young adults with polyostotic FD. Five women and 2 men with polyostotic FD, mean age of 31.0 ± 7.2 y ($X \pm SD$)(r:22–43y), where treated with APD for an average of 6.9 y(median:7.1 y; r:3.7–10.9 y). APD was administered every 6 m(180 mg, 60 mg/d for 3 d), for 2 y. There after, APD was indicated, according to bone markers levels, reducing or increasing intervals between APD cycles. Final accumulated dose of APD was 1709 ± 821 mg. All patients received calcium and vitamin D supplements. Clinical symptoms, serum bone alkaline phosphatase(BALP), urinary C-terminal cross-linking telopeptide of type I collagen(uCTX) were assessed regularly. DXA BMD of FD areas(FDa) was performed at baseline, 12 and 24 m; and was compared with the counter lateral healthy side(CL) using the region of interest program. X-rays were performed annually.

APD reduced bone pain in all patients, and none reported a new fracture. After the whole treatment period, BALP diminished in all patients, from 749 ± 1240 IU/l, to 276 ± 338 UI/l($p < 0.02$); 3 patients reached the normal range(31–95 UI/l). Levels of uCTX, measured in 3 patients, decreased an average of −56%(ns). BMD absolute values increased in 9/13 of FDa analyzed. Average BMD of FDa increased 5.9% at 12 m($p < 0.05$), and 7.3% at 24 m($p = 0.08$). Average BMD of FDa was −15.3% compared with CL at baseline; this difference was reduced with APD treatment to −10.8% at 12 m, and −9.3% after 24 m($p < 0.05$). Radiological evaluation showed no progression in almost all lesions, although, refilling of osteolytic areas was not clearly evident. Two patients reported transient flue like symptoms after the first APD cycle.

APD showed a persistent effect on bone pain and bone turnover reduction, after an average of seven years of treatment. BMD increase in FDa was rapidly evident, even though not in all lesions. Simultaneous determination of markers of bone turnover and BMD of FDa was useful to evaluate the response of treatment and to define intervals between APD cycles. Our results suggest the safety of long-term APD treatment in FD patients.

Conflict of Interest: None declared

Mo-P078

BALLOON-KYPHOPLASTY IN THE TREATMENT OF OSTEOLYTIC VERTEBRAL FRACTURES CAUSED BY MULTIPLE MYELOMA - 3 YEARS PROSPECTIVE FOLLOW-UP

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Purpose: Vertebral body fractures secondary to a malignant osteolysis like multiple myeloma are an increasingly common problem. The purpose of this study was to assess functional outcomes and radiographic results in the long term.

Materials and methods: The 59 (40 male, 19 female) patients prospectively included in our study had progressive and painful osteolytic fractures as a result of multiple myeloma. 117 thoracic and lumbar vertebral fractures were treated with Balloon-Kyphoplasty, 49 patients (36 male, 13 female) with 98 vertebral fractures could be followed-up over the period of 36 months. Preoperatively conventional radiographs in lateral and a.p. view, CT and / or MRI were preformed. Pre- and postoperatively the clinical parameters VAS (Visual Analogue Scale) and the Oswestry score were evaluated. Radiographic scans were performed pre- and postoperatively and after 3, 6, 12, 24 and 36 months. The vertebral height and endplate angles were measured.

Results: The median pain scores (VAS) decreased from pre- to post-treatment significantly ($p < 0.001$) as well as the Oswestry score ($p < 0.001$). Balloon-Kyphoplasty led to a significant and sustained reduction of pain resulting in a significant functional improvement of the patients. A significantly restoration of vertebral height and reduction of the kyphotic angle could be achieved with the balloon technique ($p < 0.05$). Further, the minimal-invasive procedure was

able to stabilize the spine also over a longer period of 36 months. A radiation therapy and / or chemotherapy could be performed without loss of time.

Conclusion: Balloon-Kyphoplasty provided a safe and effective treatment for pain and disability in patients with osteolytic vertebral fractures secondary to multiple myeloma. In addition, Balloon-Kyphoplasty was able to restore vertebral height and stabilize the fractured vertebrae in the long-term and was able to prevent an increase of kyphotic deformity.

Conflict of Interest: None declared

Mo-P079

OSTEOPROTEGERIN, BONE MARKER AND PARATHYROID HORMONE LEVELS IN ELDERLY POSTMENOPAUSAL WOMEN WITH COELIAC DISEASE

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Premenopausal women with coeliac disease (CD) have elevated levels of OPG, which may as a decoy receptor for RANKL, help to counter bone loss in CD. We examined whether postmenopausal women with CD have elevated levels of OPG and other bone markers and investigated intercorrelations between markers.

Serum and urine samples were collected from postmenopausal women > 65 yrs with CD attending a metabolic clinic for routine management. Patients were divided into 2 groups: those diagnosed for <1 yr (New CD) and those diagnosed for > 3 yrs (Old CD). All claimed to be taking a gluten free diet. Samples were collected from nominally healthy community-dwelling postmenopausal women (Controls). Subjects on bisphosphonates were excluded. OPG (IDS Ltd), Ca, ALP and intact PTH were measured in serum and NTx/Cr in urine.

Data from 23 CD patients aged 73.6 ± 4.7 yrs were reviewed, including 14 New CD patients and 9 Old CD patients (diagnosed for an average of 3.9 ± 1.1 yrs). Controls comprised 23 women aged 74 ± 4.9 years. In all CD patients PTH levels were higher compared to the control group (median 5.7 pm/l vs 1.5; $p < 0.0001$) while serum Ca levels were reduced (2.32 ± 0.11 mm/l vs 2.4 ± 0.08; $p < 0.05$). The PTH/Ca ratio was also increased in the CD subjects (median 2.43 vs 0.57, $p < 0.001$). OPG levels were not significantly different in the 2 groups (Median 4.6 pm/l CD vs 4.2 control). Levels of the bone markers ALP and NTx/Cr also showed no significant differences. In the new CD group PTH levels were increased compared to controls (median 6.8 vs 1.5; $p < 0.0001$). The PTH/Ca ratio was also increased in this group versus controls (median 3.02 vs 0.57; $p < 0.001$) but no other differences were demonstrated. In the Old CD group PTH values were higher than in controls (median 5.2 vs 1.5; $p < 0.005$) while serum Ca was lower (2.31 ± 0.06 vs 2.4 ± 0.08; $p < 0.05$). PTH/Ca ratio was increased in the Old CD group versus controls (median 2.27 vs 0.57; $p < 0.001$). OPG negatively correlated with ALP in the Old CD group ($r = -0.856$, $p < 0.01$) but not with NTx/Cr. There were no significant differences between the New CD and Old CD group.

In elderly postmenopausal women with CD levels of the bone markers OPG, ALP and NTx/Cr were not altered. There were significant differences in serum PTH, Ca and PTH/Ca ratio in patients with CD. These findings remained more than 3 years after diagnosis suggesting that aberration of calcium metabolism continues long term after diagnosis in elderly women.

Conflict of Interest: None declared

Mo-P080

PROTEOGLYCANS ARE POTENTIAL REGULATORS OF OSTEOPROTEGERIN ANTITUMORAL AND ANTI-BONE RESORPTION ACTIVITIES IN OSTEOSARCOMA

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Osteosarcoma is the most frequent primary bone tumor that develops mainly in the young, the median age of diagnosis being 18 years. Despite recent improvements in chemotherapy and surgery, survival rate is around 50% after 5 years. To break the vicious cycle that takes place between tumor cell proliferation and bone resorption in bone tumors, the use of anti-resorptive agents is a promising approach. Osteoprotegerin (OPG) is a potent inhibitor of osteoclast differentiation and activation but its use as therapeutic agent in cancer remains controversial due to its ability to bind TNF-Related Apoptosis Inducing-Ligand (TRAIL) and to inhibit its apoptotic effect on tumor cells. In order to overcome these limitations, the therapeutic effects of two forms of OPG (full length 1–401 and 1–194, lacking the heparin-binding domain) delivered by gene transfer were compared in a murine model of osteosarcoma.

In vivo, a transplantable model of osteosarcoma in C3H/He mice was used, leading to the development of a primary osteolytic tumor and pulmonary metastases dissemination. A synthetic biopolymer vector (Lutrol®) was used as a carrier for DNA delivery. The Lutrol®/DNA formulations were injected into the skeletal muscle once a week, beginning at 7 days before tumor implantation up to 21 days post-implantation.

The two forms of OPG were differentially active on tumor growth: tumor incidence and tumoral progression were significantly diminished in the OPG 1–194 group as compared to the OPG 1–401 and control groups, leading to significant increase in animal survival in the OPG 1–194 group. The efficacy of OPG as anti-resorptive agent was confirmed by evaluating the prevention of tumor-associated bone degradation. BiaCORE analyses showed that OPG 1–401 and 1–194 are both able to bind TRAIL with the same affinity, inhibiting TRAIL-induced apoptosis. These results revealed that TRAIL binding is not involved in the different biological activity observed between the two forms of OPG in vivo. Other candidates of OPG 1–401 bio-activity regulation are Proteoglycans (PG) that can bind OPG via its heparin binding domain. The presence of several PG members (biglycan, syndecans-1, -2 and -4) has been demonstrated in bone tumor environment, and BiaCORE experiments revealed that glycosaminoglycans are able to inhibit OPG 1–401 binding to TRAIL and to RANKL. These results suggest that PG may modulate OPG availability and biological activity in bone tumor microenvironment.

Conflict of Interest: None declared

Tu-P081

THERAPEUTIC EFFICACY OF SOLUBLE RECEPTOR ACTIVATOR OF NF-KAPPAB DELIVERED BY NON VIRAL GENE TRANSFER IN A MOUSE MODEL OF OSTEOLYTIC OSTEOSARCOMA

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Osteosarcoma is the most frequent primary bone tumor that develops mainly in the young, the median age of diagnosis being 18 years. Despite improvement in osteosarcoma treatment, survival rate is only 30% at 5 years for patients with pulmonary metastases at diagnosis. This warrants exploration of new therapeutic options and among them the anti-bone resorption molecule Receptor Activator of NF-kB (RANK), a naturally occurring protein is very promising in blocking the vicious cycle between bone resorption and tumor proliferation that takes place during tumor development in bone site.

The cDNA encoding murine RANK-Fc was administered by gene transfer using an amphiphilic polymer in the osteolytic POS-1 mouse model of osteosarcoma. Mice were treated with 100 microgr of the DNA/synthetic vector complex once a week, beginning 7 days before tumor implantation, and during 4 weeks of tumor development.

RANK-Fc gene transfer was effective in preventing the formation of osteolytic lesions associated with osteosarcoma development, in reducing the tumor incidence and the local tumor growth, in decreasing lung metastases dissemination leading to a 3.9-fold augmentation of mice survival 28 days post-implantation. On the contrary, RANK-Fc did not prevent the development of pulmonary metastasis alone, suggesting that bone environment is necessary for RANK-Fc therapeutic efficacy. As RANK-Fc has no direct activity on osteosarcoma cells in vitro as assessed for cell proliferation, apoptosis or cell cycle distribution, we demonstrate that RANK-Fc exerts indirect inhibitory effect on tumor progression through inhibition of bone resorption.

Conflict of Interest: None declared

Tu-P082

EFFECTS OF BONE-TARGETED NANOPARTICLES ON BONE METASTASIS

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Bone is the third most common site of incidence of metastasis from different primary tumors, and bone cancer is associated with a high mortality rate. The current therapeutic strategies are unfortunately ineffective on the progression of the tumor, and are moreover associated with a range of systemic side effects.

Biocompatible and biodegradable nanocarriers are being investigated as a new therapeutic tool that is able to carry antineoplastic drugs to specific targets meanwhile reducing the systemic toxicity associated with a free drug distribution through the body. The aim of this study was to test the effectiveness of nanoparticles (NPs) engineered to feature a high affinity to bone and loaded with doxorubicin (DXR).

NPs were obtained by binding sodium alendronate to poly(D,L-lactide-co-glycolide) (PLGA), characterized for their chemical-physical properties and biocompatibility, and then loaded with DXR. The in vitro study was performed on six human cell lines, considered as models of bone tumors as osteosarcoma, renal and breast adenocarcinoma, and neuroblastoma. To analyze the uptake of the NPs, the cells were observed by confocal microscopy after incubation with the free or loaded DXR. For the analysis of the antineoplastic effects, the percentage of growth inhibition was determined after incubation with the free or loaded DXR. To evaluate the in vivo activity of NPs, a preliminary study was performed on a bone metastasis model. Nude mice were used for intratibial inoculation of a human breast cancer cell line. The free or loaded DXR was given by intraperitoneal administration for 4 weeks and the progression of the tumor was monitored by X-rays. The radiographs were analyzed for lesion number.

All cell lines were able to selectively incorporate the DXR loaded NPs, as free DXR. Selective nuclear uptake of the drug, typical of sensitive cells, was observed after 30' of exposure. All cell lines were sensitive to free DXR, as confirmed by a significant growth inhibition after exposition to the free DXR. NPs-loaded DXR was effective at the equivalent concentration as free DXR. The in vivo activity on metastasis was also demonstrated. In fact, the incidence of metastasis in NPs-treated mice was lower in comparison to the untreated mice, as well as in comparison to DXR-treated mice.

In summary, NPs loaded with DXR were able to inhibit cell proliferation in vitro at the same conditions as free DXR, and to reduce the incidence of the skeletal metastasis in vivo.

Conflict of Interest: None declared

Tu-P083

MATRIX EXTRACELLULAR PHOSPHOGLYCOPROTEIN (MEPE) REGULATES PHOSPHATE HOMEOSTASIS IN AN EXPERIMENTAL MODEL OF CHRONIC KIDNEY DISEASE

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Secondary hyperparathyroidism caused by chronic kidney disease disturbs phosphate, PTH, calcium and creatinine homeostasis. These imbalances disturb the dynamic processes of bone formation and resorption resulting in renal osteodystrophy and cardiovascular diseases by enhanced vascular and increased myocardial calcification. Phosphate homeostasis is determined by renal excretion, intestinal absorption and internal bone absorption and excretion. Currently existing phosphate binders show a decrease in hyperphosphatemia and can cause hypercalcemia and potential side effects such as metabolic acidosis. Here we report the effects of a novel phosphatonin, matrix extracellular phosphoglycoprotein (MEPE) and its activity in phosphate metabolism in a model of renal insufficiency. It has been shown previously that the phosphatonin MEPE inhibits renal phosphate absorption in vitro and in acute in situ experiments. In this study we investigated the effects of MEPE under chronic kidney disease (CKD) conditions in a 5/6 nephrectomy model. Following a full kidney removal at week 1, 2/3 of the second kidney was removed at week 2. The animals stayed on a normal diet for 2 weeks. Subsequently, the animals received a high phosphate (1.2%), low calcium (0.4%) diet for an additional 6 weeks. Urine and serum were analyzed during disease progression at weeks 4 and 8. Administration of MEPE began at week 9 and the diet was changed to low phosphate (0.3%), low calcium (0.4%) during the treatment period. MEPE was dosed intravenously (vehicle, 0.2 mg/kg, 1.0 mg/kg and 5.0 mg/kg) daily for 14 days. Serum and urine parameters were analyzed directly before dosing at time 0 and at 2 hours and day 14 of MEPE dosing. Soft and hard tissue was histopathologically analyzed following the 2 weeks of MEPE administration. The results of this study indicate that MEPE regulates the phosphate metabolism dose dependently with the most active dose at 1 mg/kg after a 14 day treatment. At 1 mg/kg MEPE significantly decreased serum phosphorus and PTH when compared to time 0. Serum vitamin D increased with a 14 day MEPE treatment. Serum CaxPO4 product and creatinine did not

change significantly. These novel findings indicate that MEPE might be a potent agent in regulating phosphate homeostasis in chronic kidney disease patients.
Conflict of Interest: None declared

Tu-P084

RESIDUAL (GHOST) SOCKETS IN BISPHOSPHONATE USE

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Background: Bisphosphonates are used to slow osteoclastic and osteoblastic activity in patients with metastatic disease (or the potential of same), myeloma, Paget disease, hypercalcemia and osteoporosis. Numerous recent examples have documented extremely poor healing of alveolar bone after relatively minor trauma (e.g. extraction), perhaps resulting in chronically exposed bone. It would seem logical that, even in cases with soft tissue healing, extraction sockets may remain radiographically visible for an extended period of time after surgery. However, only one such case has been reported to date. Objective: To report a case involving numerous residual or ghost sockets, with lamina dura outlines visible radiographically, in a patient on chronic zoledronic acid (Zometa) use for metastatic cancer.

Case Report: A 73 year old woman with a 3 year history of metastatic breast adenocarcinoma had her 14 remaining teeth removed for full denture construction. Her metastatic disease was being controlled by monthly IV infusions of 0.5 mg zoledronic acid and pamidronate (Aredia), although the infusions were stopped 1 year prior to her current dental visit. The extractions were uneventful but she developed pain several months after and at 9 months several regions of exposed bone and sequestra were seen in her maxilla and mandible. One year after the extractions only three sockets demonstrated radiographically obvious healing or remodeling; all others appeared as if they were new extraction sockets. Uninflamed mucosa covered all alveolar sites except the two largest of the original sites of exposed bone.

Conclusions: Firstly, residual sockets provide additional evidence of poor bone healing or very slow bone turnover in patients using bisphosphonates. Secondly: the presence of such a radiographic sign may alert the surgeon to potential healing problems in subsequent surgery or trauma of alveolar bone. Thirdly, it is clearly possible for adequate soft tissue healing to occur above ghost sockets. Final note: residual sockets are also seen in chronic ischemic jawbone disease without bisphosphonate. It is not known what the relative influence of the bisphosphonates is compared to local ischemia
Conflict of Interest: None declared

Tu-P085

HYPERCALCEMIA IN KIDNEY TRANSPLANT RECIPIENTS - RELATIONSHIP TO BONE TURNOVER

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Bone metabolism disorders and hypercalcaemia occur often in kidney transplant recipients. In order to investigate the relationship between hypercalcemia and bone metabolism disorders in 78 (41 M, 37 F, aged 24–70 years, creatinine clearance > 60 ml/min./1.73 m^2) kidney recipients the following serum parameters were estimated: iPTH, bone formation markers; total alkaline phosphatase (tALP), bone alkaline phosphatase (bALP), procollagen I C- terminal propeptide (PICP), osteocalcin (OC), bone degradation marker; crosslaps (cs), 25(OH)D3, total Ca, Ca++, Pi, creatinine. Results: Ca, median 2.535 (2.05–3.18) mmol/L (reference range 2.14–2.53). Two patients were hypocalcemic, 37 normocalcemic, and 39 hypercalcemic. Ca++, median 1.325 (1.04–1.72) mmol/L (reference range 1.12–1.23). In 3/78 patients serum Ca++ values were below, and in 68/78 above the reference range. Serum Ca and Ca++ correlated significantly, $p < 0.00001$. Serum calcium correlated significantly positively with serum iPTH, tALP, bALP, OC, cs, dialysis duration prior to transplantation and negatively with post-transplant period duration and Pi. Comparison of normocalcemic and hypercalcemic patients (according to total serum calcium levels): dialysis duration was shorter, serum iPTH, OC, cs were lower, and Pi higher in normocalcemic than hypercalcemic patients. No significant difference was found for tALP, bALP, PICP. In conclusion: Hypercalcemia is very frequent among kidney transplant recipients. Hyperparathyroidism and increased bone degradation, not accompanied by adequate bone formation increment are risk factors for hypercalcemia. Longer dialysis duration prior to transplantation is also a risk factor for hypercalcemia after transplantation. Serum calcium levels are higher in earlier than in the later post-tranplant period.
Conflict of Interest: None declared

Tu-P086

AVASCLUAR NECROSIS AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION: AN OBSERVATIONAL STUDY ON THE EFFECTS OF ZOLEDRONIC ACID

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An increased prevalence of avascular necrosis (AVN) has been described after allogeneic hematopoietic stem cell transplantation (AHSCT). This is an observational study describing effects of zoledronic acid administration for osteoporosis treatment in patients who had undergone AHSCT due to hematological malignancies.

AVN was diagnosed by RMN and triphasic scintigraphy in 11 patients (5F) with pain and functional limitation, 3–114 months after AHSCT. Median age of the patients at AHSCT was 35 yrs (range, 19–46). A total of 24 joints were involved (19 femoral heads, 2 knees and 3 humeral heads); femoral heads were affected in all of the patients and most of them had multiple joint involvement. As risk factors were identified: recent exacerbation of the chronic graft-vs-host disease, corticosteroids dose and reduction of in vitro growth of osteoblast precursors (obtained from bone marrow biopsies) by a colony-forming unit (CFU-F) assay.

Five AVN of femoral heads were of grade 3–4 and required total hip arthroplasty. Three femoral head AVNs were treated with decompression. The remaining AVNs were at stage 2, at 16 joints in 7 patients. All of these patients received 3 monthly i.v. infusions of zoledronic acid because of concomitant osteoporosis or rapid bone loss. All of them reported improvement of pain and joint function that lasted up to one year after the treatment beginning. Improvement in clinical symptoms was associated with improved RMN picture of 8 joints (5 femoral heads, 1 knee and 2 humerous), while the remaining 8 joints resulted stable 12 months after the treatment beginning. Patients who had evidence of radiological improvement had higher CFU-F colony numbers than those with subjective improvement only.

In conclusion, we observed a stabilization or improvement of AVN at large joints during a short term zoledronic acid treatment, in

patients after AHSCT. This is an important observation, since the patients were young with a good likehood for long term survivor after being cured from malignancies. No osteonecrosis of the jaw (ONJ) occurred in any of 30 AHSCT patients treated by zoledronic acid. Absence of ONJ in our cohort confirms the role of age, history of dental procedures, trauma or infection as important risk factors. Finally, the differences in bone microenvironment between large bones and jaw and the presence of potentially pathogenic micro-organisms in oral cavity may explain the different behaviour of the jaw and epiphyseal large bones during bisphosphonate administration.
Conflict of Interest: None declared

Tu-P087

HIGH RESOLUTION ELECTRON MICROSCOPY IDENTIFIES DISTINCTIVE BINDING OF OCHRONOTIC PIGMENT TO COLLAGEN FIBRES IN ALKAPTONURIA

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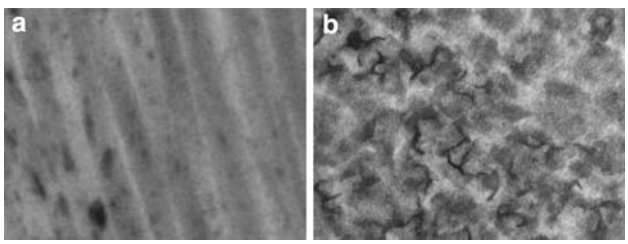
Alkaptonuria (AKU) is a genetic disorder of tyrosine metabolism and was the first disease shown by Garrod 100 years ago to conform to Mendelian laws of autosomal recessive inheritance. The condition is characterised by excessive accumulation of homogentisic acid (HGA) due to lack of homogentisate dioxygenase (HGO). HGA is deposited as ochronotic pigment in connective tissue, especially cartilage and leads to severe arthopathies. However, the mechanism underlying the deposition of pigment in AKU has not been elucidated. Light (LM) and electron microscopy (EM) were used to identify the binding of ochronotic pigment to collagen in joint tissues. Using Schmorl's stain and LM we noted a significant variation in the amount of pigment deposition within tissues and between individuals. This indicates other factors in addition to HGA contribute to pigment deposition. EM examination of capsule from an AKU femoral head, revealed a distinctive binding of ochronotic pigment to the surface of collagen fibres when viewed in L.S. (A). The shard-like deposits were abundant in some regions yet a few microns away there were non-pigmented regions. The initial stages of pigment deposition appear to be associated with cross banding of the collagen fibres. When viewed in T.S. (B), the pigment appeared to penetrate the fibres, indicating deposition during collagen assembly or subsequent invasion after fibre formation. In parallel studies, we developed an in vitro model of ochronosis in which pigment was deposited in cultures of osteoblasts and chondrocytes incubated in medium containing HGA. We are investigating if the deposition in these cultures is identical to the distinctive binding in ochronosis in vivo. Identification of the mechanism of pigment deposition is the initial step in developing new therapeutic strategies to prevent ochronosis and subsequent arthropathy in AKU.



Conflict of Interest: None declared

Tu-P088

MARKERS OF BONE METABOLISM IN THE FIRST 3 WEEKS OF LIFE OF PRETERM INFANTS: TRANSIENT DECREASE AFTER ANTENATAL BETAMETHASONE

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Background and aims: Both intrauterine growth retardation resulting in small for gestational age infants (SGA), and use of antenatal betamethasone may affect calcium metabolism/bone strength during the first weeks of life. Changes in serum markers of bone formation (osteocalcin [OC], aminoterminal propeptide typeI procollagen [PINP]), and of bone resorption (serum C-telopeptide [sCTx] and urinary helical peptide [HP]) are early indicators of effects on bone. The aim was to study the relationship between the serum markers and gestational age (weeks 26–31), with and without antenatal betamethasone, during the first 3 weeks of life in preterm infants.

Methods: All infants < 32 weeks gestational age without congenital malformations were eligible for inclusion. Serum/urinary markers were measured at birth and days 7 and 21. Respiratory distress syndrome, bronchopulmonary dysplasia, birth weight and time of antenatal betamethasone before birth were noted.
Results: 128 preterm infants were eligible, 123(13 no betamethasone) were included, mean(+SD) birth weight $1210 + 343$ grams and gestational age $29.4 + 1.5$ wks. At birth 1–2 d after betamethasone, OC, sCTx and HP were significantly lower than in infants without betamethasone ($p < 0.05$), but not after intervals > 3 d and on days 7 and 21. The serum markers of all infants correlated positively with birth weight at the three time points measured, and positively with gestational age at day 7 and 21. While, at birth, significance was only reached if the infants with betamethasone interval < 3 d were excluded. Helical peptide measured in urine showed no correlation with gestational age.

Conclusion: The serum bone markers osteocalcine, PINP, and sCTx increase with gestational age and during the first 21 postnatal days of the preterm infants, and are positive correlated with birth weight. Markers of bone metabolism show transient decrease after betamethasone.

This work was supported by a grant of Friesland Foods.
Helical peptide kits were a gift of Quidel Corp.
Conflict of Interest: None declared

Tu-P089

INFLIXIMAB IN INFLAMMATORY BOWEL DISEASE: 'CAN WE HEAL TWO DISEASES WITH ONE DRUG?'

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Background:Osteoporosis is common among Inflammatory Bowel Disease (IBD) patients. To date, there is a wealth of data regarding the

use of infliximab in treatment of IBD patients, however fewer reports exist regarding its role on bone metabolism.

Aims: To elucidate the effect of infliximab maintenance therapy on bone metabolism in IBD patients.

Methords: Sera from 20 IBD patients (16 Crohn's disease and 4 Ulcerative Colitis) and 20 healthy subjects were isolated. Parathyroid hormone (PTH), Vitamin D (VD), bone formation markers (P1NP-pro collagen type 1 N propeptide and OC-osteocalcin), bone resorption marker (sCTx-serum carboxyterminal cross linking telopeptide of bone collagen), and transforming growth factor (TGFβ1) were measured at baseline, week 6, 30 and 54 post infliximab therapy. The effects of different concentrations of infliximab on viability, differentiation and mineralization of the osteoblast cell line hFOB 1.19 were studied. Cell viability was measured by Alamar Blue assay, cell differentiation by Alkaline Phosphatase (ALP) activity and cell mineralization by Von Kossa staining.

Results: Sera from (20 patients at baseline; 20 patients at 6 weeks, 10 patients at 30 weeks and 3 patients at 54 weeks) were available for analysis. P1NP levels rapidly increased by 63%, $p = 0.014$ at week 6 and remained increased at week 54, $p = 0.003$. OC also increased, but at a slower rate reaching a significant level at week 30, $p = 0.018$ and continued to remain increased at week 54, $p = 0.0022$. VD levels increased over time and reached a significant level at week 54, $p = 0.0056$. TGF β1 decreased at all time points, p = not significant. No significant changes were seen with sCTx and PTH over time. The effect of infliximab (0.1% to 50%) on the viability of osteoblast cells in culture over time showed that the cells continue to proliferate rapidly through 10 days culture, with proliferation decreased at higher infliximab concentrations. The increase noted was not significant. Maximum ALP activity was achieved with physiological doses of infliximab (0.1% to 1%), which was comparable to normal media over the 10 days culture. Preliminary results also indicate that strongest mineralization was seen with 5% infliximab.

Conclusion: This first long term evaluation of infliximab therapy has shown a significant beneficial effect on bone metabolism in IBD patients and indicates infliximab has a possible role as a bone protector in IBD patients.

Conflict of Interest: None declared

Tu-P090

IMPACT OF ADALIMUMAB THERAPY ON BONE METABOLISM IN CROHN'S DISEASE PATIENTS: A 3 MONTHS FOLLOW UP STUDY

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Background: Osteoporosis is a recognized complication of Crohn's disease (CD). Elevated pro-inflammatory cytokines have been implicated in this process. Recently, infliximab, a chimeric monoclonal antibody against tumour necrosis factor (TNF) α have been shown to have beneficial effects on bone metabolism in Crohn's disease patients although the exact mechanisms of action are not fully elucidated yet.

Aims: To evaluate the impact of the recently licensed CD therapy; Adalimumab, a fully human antibody against TNFα on bone metabolism using a combined clinical and in vitro model.

Methods: Serum from 12 CD patients was isolated. Parathyroid hormone (PTH), Vitamin D (VD), bone formation markers (P1NP-pro collagen type 1 N propeptide and OC-osteocalcin), bone resorption marker (sCTx-serum carboxyterminal cross linking telopeptide of bone collagen), and transforming growth factor (TGFβ1) were measured at baseline, 1 month and 3 months post adalimumab. Dual energy X-ray absorptiometry (DEXA) scan was also performed at baseline. Human osteoblasts (hFOB 1.19) were treated with serum from CD patient's pre, 1 month and 3 month post adalimumab therapy up till 14 days. Cell viability and cell differentiation was measured using Alamar Blue assay and Alkaline phosphatase (ALP) activity respectively.

Results: The median T score value on the DEXA scan was -1.5 (0 to -2.8). At 3 months post treatment, TGFβ1 levels significantly decreased; $p = 0.023$. PTH, VD, OC and P1NP increased at 3 months (28%, $p = 0.14$; 24%, $p = 0.57$; 40%, $p = 0.068$ and 49%, $p = 0.18$) respectively. Interestingly viability of osteoblasts exposed to 10% serum was high pre treatment and this decreased at 3 months post treatment, $p = 0.017$(day 4) and $p = 0.024$ (day 10). There was an increase in the amount of early osteoblast differentiation marker ALP secreted by the osteoblasts at 3 months post treatment (92.9%, Day 4 and 95.1%, Day 10).

Conclusion: This first study evaluating the role of Adalimumab as a possible bone protector in CD patients has shown promising beneficial effects on bone metabolism both in vivo and in vitro.

Conflict of Interest: None declared

Tu-P091

INTAKE OF VITAMIN D AND RISK OF BREAST CANCER—A META-ANALYSIS

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Background: Vitamin D has been shown to be associated with a number of non skeletal conditions including diabetes, multiple sclerosis and the overall risk of cancer. Several studies exist on the intake of vitamin D and breast cancer. We aimed at studying the association between vitamin D intake and risk of breast cancer in a meta-analysis.

Material and methods: We performed a meta-analysis (Search date July 1, 2007) by searching Pubmed, Embase, and Web of Science using the MESH terms "vitamin D" and "breast cancer". The outcome variable had to be breast cancer or not, and the exposure total daily intake of vitamin D from diet or supplements in IU. All study types (cohort, case-control, and cross sectional) were included.

Results: A total of 1,731 studies were identified, but only six studies contained original data on the association between intake of vitamin D and risk of breast cancer. Overall there was no association between amount of vitamin D and risk of breast cancer (RR = 0.98, 95% CI: 0.93–1.03, test for heterogeneity $p < 0.01$, six studies). However, most studies reported on very low intakes of vitamin D (typically in the range 100–400 IU/day). Restricting the analyses to intakes $\geq$400 IU/day yielded a more homogenous result with a trend towards less breast cancer with $\geq$400 IU/day vs. the lowest intake (typically <50 to 150 IU/day), RR = 0.92, 95% CI: 0.87–0.97, p for heterogeneity 0.14, three studies.

Conclusion: There may be a trend towards fewer cases of breast cancer with higher intakes of vitamin D ($\geq$400 IU/day). However, more research is needed, preferably in the form of randomized controlled trials with high doses of vitamin D (e.g. $\geq$ 800 IU/day).

Conflict of Interest: None declared

Tu-P092

PREVALENCE AND RISK FACTORS FOR RADIOGRAPHIC OSTEOARTHRITIS OF THE KNEE AND LUMBAR SPINE IN JAPAN: THE RESEARCH ON OSTEOARTHRITIS AGAINST DISABILITY (ROAD) STUDY

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Backgrounds/aims: Although osteoarthritis (OA) of the knee and lumbar spine is a major cause of disability in the elderly, few epidemiologic studies have been performed. We established a large-scale nationwide prospective study called Research on Osteoarthritis Against Disability (ROAD) in 2005, and created a comprehensive and systemic database including clinical and genetic information in 3 cohorts of urban, mountainous and seacoast populations. To clarify prevalence and risk factors for the prevention of OA, we analyzed the baseline database of the ROAD study.

Methods: We recruited 3040 participants in total, from which 1492 subjects $> = 50$ years old in mountainous and seacoast areas (537 men, 955 women; mean age 68.4 years) were enrolled. Radiographic severity of OA was determined according to Kellgren/Lawrence (KL) grade (0–4) at femoral-tibial joints of bilateral knees and at intervertebral spaces from L1/2 to L5/S1 of the lumbar spine. Risk factors for analysis were selected from the baseline questionnaire regarding the occupational activity of the main job of each participant.

Results: Prevalence of radiographic knee OA (KL grade ≥ 2) in either joint was 45.6% in men and 61.4% in women, while that of radiographic lumbar spondylosis in either intervertebral space was 79.3% in men and 58.0% in women. Risk factors for radiographic knee OA were age, body-mass index (BMI) (women), occupational activities of standing (> 2 h/day), squatting (> 1 h/day) (women), walking (> 3 km/day), climbing (> 30 steps/day), and lifting heavy weights (> 10 kg more than once/week). Risk factors for radiographic lumbar spondylosis were age, BMI and occupational activities of climbing and lifting weights in women. Occupational activity of sitting (> 2 h/day) was associated with reduced risk of knee OA and lumbar spondylosis in men. Neither smoking nor alcohol drinking was associated with knee OA or lumbar spondylosis.

Conclusion: The investigation of baseline data from the ROAD study revealed a high prevalence of radiographic OA in knee and lumbar spine among elderly people. This present study also clarified that occupational activities were significantly associated with radiographic knee OA and lumbar spondylosis. Further progress of the ROAD study will elucidate underlying environmental and genetic backgrounds of the two disorders.

Conflict of Interest: None declared

Tu-P093

THE EFFECT OF ZD4054 ON BONE METASTASIS IN PATIENTS WITH M1 HORMONE-RESISTANT PROSTATE CANCER

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Background: The specific endothelin A receptor antagonist ZD4054 has demonstrated clinical activity in patients with M1 hormone-resistant prostate cancer (HRPC). As an important component of the action of endothelin receptor antagonists in HRPC is believed to be via osteoblasts, effects on bone metastasis might be expected. The aim of this study was to assess the effect of ZD4054 on bone metastasis in patients with M1 HRPC.

Methods: A standardized protocol was developed for quantitative and semi-quantitative estimation of metastatic disease progression. Patients were randomized to receive once-daily ZD4054 (15 or 10 mg/day po), or placebo in a double-blind Phase II multicentre trial. Bone scans were collected at baseline and at discontinuation for all patients, and were reviewed centrally by an independent panel of radiologists using pre-specified reading guidelines to minimize inter-reader variability. The number of bone metastases and the bone scan index were recorded for each scan.

Results: Of 312 patients randomized (intention-to-treat population), 277 had evaluable bone scans at baseline (ZD4054 15 mg, $n = 85$; 10 mg, $n = 97$; placebo, $n = 95$). The median number of bone metastases at baseline was 17, 7 and 11 in the ZD4054 15 mg, 10 mg and placebo groups, respectively. The overall number of bone metastases increased with time for all treatment groups, and exhibited high variability. At discontinuation of study treatment (last available post-baseline bone scan) there was a 14% decrease in the rate of rise in the number of bone metastases for ZD4054 15 mg compared with placebo (treatment ratio 0.86, 80% CI 0.70, 1.06; $P = 0.349$), and an 11% reduction for ZD4054 10 mg compared with placebo (treatment ratio 0.89, 80% CI 0.74, 1.07; $P = 0.416$). Both results were statistically non-significant.

Conclusion: Interpretation of the results is complicated by the variability in number of bone metastases at baseline. Nevertheless, the results of this study suggest that ZD4054 may have an effect on tumour biology in the skeleton, leading to reductions in bone metastases on bone scintigraphy.

Conflict of Interest: P. Beuzeboc, None declared
M. Borre, AstraZeneca, Grant Research Support, Consultant
S. Culine, None declared
N. James, Astrazeneca, Grant Research Support, Consultant
T. Morris, AstraZeneca, Full Time Employee
H. Payne, AstraZeneca, Consultant, Speakers Bureau
D. Phung, AstraZeneca, Full Time Employee
B. Zonnenberg, AstraZeneca, Grant Research Support, Consultant

Su-P094

INTRATRABECULAR TUNNELING INCREASES TRABECULAR NUMBER THROUGHOUT THE SKELETON OF OVARIECTOMIZED RHESUS MONKEYS TREATED WITH PARATHYROID HORMONE (1–84)

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We have previously reported that daily treatment of osteopenic ovariectomized (OVX) rhesus monkeys with recombinant human parathyroid hormone 1–84 (PTH) (5, 10, or 25 μg/kg) for 16 months increased trabecular bone volume (BV/TV) and number (Tb.N) at lumbar vertebra-3 (L3). We proposed that the increased Tb.N was achieved by stimulation of intratrabecular tunneling, a remodeling event orientated parallel to the long axis of a trabecula at a non-nodal location. Bone formation followed resorption maintaining normal trabecular thickness (Tb.Th). Tb.N and connectivity which are important determinants of bone strength. We have now quantified intratrabecular tunneling at L3 of these animals and extended it to investigate the effects of PTH treatment on trabecular bone at distal radius, proximal femur, and iliac crest. At L3, tunneling events were rare in control sham and OVX animals ($\sim$0.05/mm2) but increased significantly in a dose-dependent manner in PTH-treated animals (0.27, 0.49 and 0.95/mm2 with the 5, 10, and 25 μg/kg doses, respectively). Very similar values were observed at the other 3 skeletal sites. Iliac crest biopsies were also collected at baseline and after 6 months of treatment and showed significant time- and dose-related increases in tunnels. For example, in the 10 μg/kg PTH dose group the number of tunneling events was 0.03, 0.36, and 0.64/mm2 at baseline, month 6 and month 16, respectively. Although the pattern and magnitude of response varied slightly from site-to-site, the PTH-induced intratrabecular tunneling significantly increased Tb.N as well as BV/TV and BFR at all sites. A modest, but statistically significant increase in Tb.Th occurred only at the iliac crest. In summary, intratrabecular tunneling was rare in control monkeys but increased substantially with PTH 1–84 treatment. This phenomenon provides a plausible explanation for the PTH-induced increase in Tb.N observed at all trabecular bone locations of OVX rhesus monkeys.

Conflict of Interest: M.A.Miller, Own stock in NPS pharmaceuticals

Su-P095

IDIOPATHIC MULTIPLE LUMBAR INTEVERTEBRAL DISC CALCIFICATIONS

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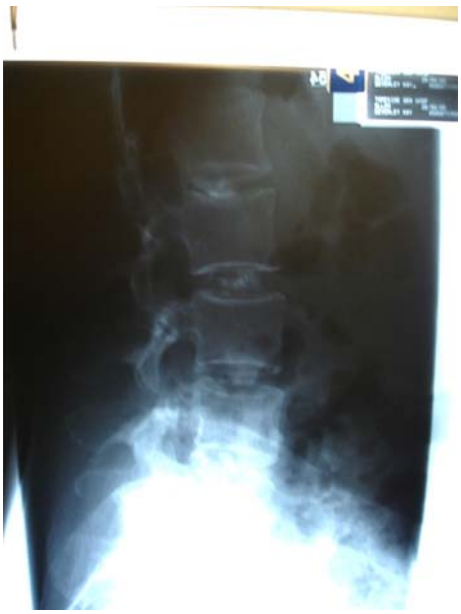

Intervertebral disc calcification is a rare disorder that occur mainly in the lumbar and thoracic spine. In the literature, its incidence is quoted as 5–6% of the general adult population as seen on chest and abdominal X-ray films. Intervertebral disc calcification can occur secondary to either biochemical alteration of the involved tissues e.g. homogentisic acids, calcium pyrophosphate, amyloid; or to immobilisation e.g. poliomyelitis, fusion of spine. We describe a very rare case with idiopathic multiple disc calcifications in the lumbar spine with no obvious cause. Idiopathic multiple lumbar intervertebral disc calcifications is a diagnosis of exclusion. Causes of disc calcification should be excluded before treating this condition conservatively. This condition does not cause back pain as it affects the nucleus pulposus which lacks sensory fibres and terminals.

Conflict of Interest: None declared

Su-P096

PROTEIN EXPRESSION ANALYSIS OF A HUMAN OSTEOBLAST DIFFERENTIATION MODEL

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Bone is a very dynamic tissue, being continuously resorbed by osteoclasts and rebuild by osteoblasts. In vitro, glucocorticoids promote stepwise changes in human osteoblast morphology, differentiation with extracellular matrix production and ultimately mineral deposition. Over the last years proteomics technology has made tremendous progress. Nowadays mass spectrometry-based proteomics tools can be applied not only to generate qualitative information but also quantitatively, to gain a more holistic view of biological systems.

The aim of this study is to investigate protein expression during the time-course of dexamethasone-induced osteoblast differentiation using mass spectrometry-based proteomics tools.

Human pre-osteoblasts (SV-HFO) were cultured for 19 days either in the presence or absence of dexamethasone. For both conditions, proteins (and RNA for gene expression profile) were isolated by the Trizol method at three different time-points during culture, precipitated and enzymatically digested into peptides. The peptide masses were measured by Matrix-Assisted Laser Desorption/Ionization (MALDI)-Fourier Transform Mass Spectrometry (FTMS) and the correspondent intensities used to semi-quantitatively determine their abundances. Data analysis was done using a home-made software (Titulaer et al., 2006). Peptides with p-values (Wilcoxon test) < 0.001 and $p < 0.01$ showing absolute difference were considered for subsequent measurements and respective protein identification by tandem mass spectrometry (MS/MS). To this end, a nano-LC-LTQ was used and spectra searched against the Human International Protein Index database using SEQUEST.

Currently, 25 different proteins were found to be differentially expressed when comparing different conditions either in a time-course and treatment dependent manner. These include proteins whose function in osteoblast differentiation is not completely acknowledged (Lamin-A) as well as proteins known to be involved in this process, such as Annexin A2 and Fibronectin 1. As an example, Fibronectin 1, found to be up-regulated at all time-points in differentiating cultures is reported in literature to be important for osteoblast differentiation and their survival once mature.

With this approach, combining high accurate FTMS measurements and MS/MS for protein identification, we were able to identify proteins differentially expressed during osteoblast differentiation as well as within mineralizing and non-mineralizing conditions.

Conflict of Interest: None declared

Su-P097

OSTEOBLAST FIBRONECTIN AFFECTS THEIR BEHAVIOR IN VIVO

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Fibronectin is produced by osteoblasts and incorporated in the extracellular matrix of bone. In vitro studies have shown that fibronectin is required for the formation of a collagen network, which comprises more than 90% of the extracellular matrix in the bone. We therefore examined the effect of the deletion of fibronectin production by the osteoblasts on bone.

Fibronectin floxed mice were mated with mice carrying the cre gene under the control of the collagen α1(I) (2.3 kb) promoter in order to delete fibronectin production by the osteoblasts. Osteoblast cultures were performed, and bones were examined by immunohistochemistry, Western blotting, bone mineral density measurements, infrared spectroscopy, and histomorphometry.

Even though deletion of fibronectin in the osteoblasts examined in vitro was successful, no significant decrease in the amount of fibronectin in the matrix was seen by using immunohistochemistry or Western blotting. Nevertheless there was a significant decrease in trabecular BMD measurements of the distal femur in both 6-week old mice (cKO = 227.0 ± 41.2 vs. CT = 252.4 ± 55.3, n = 25 and 26, $p < 0.05$) and 4 months old mice (cKO = 233.1 ± 19.5 vs. 247.0 ± 15.4, n = 26 vs. 28, $p < 0.05$). Similarly the total BMD was also significantly diminished in the knockouts as compared to the controls in 6-week old mice (466.2 ± 50.5 vs. 496.3 ± 54.5, $p < 0.05$) and in the 4-month old mice (cKO = 557.3 ± 42.6 vs. 583.6 ± 36.6, $p < 0.05$). Infrared spectroscopy failed to show a significant decrease in mineralization when adjusted to matrix content. Dynamic histomorphometry failed to show any difference (mineralizing surface and bone formation rate were similar between conditional knockout mice and controls). However, static histomorphometry revealed an increase in the number of osteoblasts (41%, $p < 0.01$), osteoid surface (27%, $p < 0.05$), and time to mineralization (58%, $p < 0.01$), as well as a 34% decrease in the bone formation rate at the basic multicellular unit (Adjusted apposition rate: Aj.AR, $p < 0.01$).

The deletion of fibronectin in the differentiating osteoblasts affects the number and function of the osteoblasts and leads to a decrease in bone formation at the basic multicellular unit, but not at the whole bone level. This ultimately affects bone mineral density without any effect on the end mineralization of bone when adjusted to bone matrix. Thus, fibronectin produced by the osteoblasts seems to be part of a feedback loop that affects both osteoblast numbers and function.

Conflict of Interest: None declared

Su-P098

CIRCULATING PLASMA FIBRONECTIN IS NEEDED FOR A NORMAL BONE DENSITY

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The liver produces a number of proteins that are able to infiltrate the bone such as albumin and alpha2-HS-glycoprotein. These proteins appear to affect bone matrix composition. Our aim was to examine the effect of circulating fibronectin originating from the liver on bone, because deletion of fibronectin in the osteoblasts had no effect on the total content of fibronectin in bone.

Plasma fibronectin was labeled using Oyster-500 and injected in mice on three consecutive days. Before the first injection patients received alizarin complexone (30 mg/kg), and on the fifth day mice were euthanized and examined. Fibronectin floxed mice were mated with mice carrying the Cre gene under the control of the Mx or the albumin promoters in order to delete fibronectin production by the hepatocytes. Bones were characterized using immunohistochemistry, bone mineral density measurements, microcomputer tomography, and histomorphometry. Fibronectin levels were determined using ELISA.

Labeled fibronectin was able to infiltrate the bone, despite its large size (480 kD). This was not limited to areas of new bone formation, since the infiltration extended beyond the areas of alizarin complexone staining into the bone. More than 90% decrease in the amount of circulating fibronectin was achieved using the two strains of genetically manipulated mice. This was associated with a clear decrease in the amount of fibronectin in bone matrix as evidenced by immunohistochemistry. The deletion of fibronectin in the liver was also associated with up to 13% decrease in bone mineral density in the Mx-cre harboring line (cKO: 177.9 ± 5.4 vs. CT: 205.4 ± 6.2 mg/cm^3, n = 24 cKO and 34 littermate controls, $p < 0.005$). This was also associated with a 23% decrease in bone volume over tissue volume (cKO: 6.3 ± 0.4 vs. CT: 8.2 ± 0.7%, n = 26 and 33, $p < 0.05$), 40% decrease in connectivity density, which reflects a decrease in the number of connections of the trabeculae with their environment in the cKO mice (cKO: 43 ± 4 vs. CT: 72 ± 8/mm^3, $p < 0.01$), and a 10% decrease in trabecular number (3.6 ± 0.1 vs. 4.0 ± 0.1/mm, $p < 0.01$) ($p < 0.05$) by microcomputer tomography. There were no clear changes on bone histomorphometry, however.

Circulating fibronectin originating from the hepatocytes in the liver infiltrates the bone matrix, where it affects the bone mineral density, bone volume and trabecular number. These effects are independent of an effect on the bone forming or bone resorbing cells. Thus fibronectin originating from the liver is needed for the achievement of normal bone.

Conflict of Interest: None declared

Su-P099

STRONTIUM RANELATE-INDUCED INCREASES IN OPG ARE MEDIATED BY THE OSTEOBLASTIC CALCIUM-SENSING RECEPTOR

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Strontium ranelate reduces vertebral and non-vertebral fractures in post-menopausal women. Previous studies have shown that strontium ranelate increases bone formation and decreases bone resorption. Moreover, strontium is an agonist of the calcium-sensing receptor (CaSR), a receptor involved in the strontium ranelate-induced increase in osteoblast replication and osteoclast apoptosis. We previously showed that strontium ranelate induced replication and differentiation, as well as increasing the survival of primary human osteoblasts (HOBs) subject to stress. In the current study, we investigated the effects of strontium ranelate on osteoblast-derived osteoclastogenic signals and hypothesized that these effects could be mediated by the CaSR. After treatments as short as 24 h, strontium

ranelate dose-dependently increased OPG mRNA expression (qRT-PCR), up to 1.9-fold with 2 mM strontium ranelate ($p < 0.001$). This effect was confirmed by a strong up-regulation of the secretion of OPG (ELISA) with 1 and 2 mM strontium ranelate ($p < 0.001$). In parallel, RANKL mRNA expression decreased by 75% after treatment with strontium ranelate (0.1, 1 and 2 mM, $p < 0.01$, $p < 0.01$ and $p < 0.001$, respectively). The expression of RANKL at the HOB surface was also strongly down-regulated after 48 h treatment with 1 mM strontium ranelate, as shown by immunoprecipitation followed by western blotting. HOBs were transfected with siRNA directed at the CaSR, scrambled sequence, or no siRNA. Transfection occurred over 24 h and the extent of the knockdown of the CaSR in HOBs was confirmed by western blot analysis. Knocking down the CaSR had no significant effect on OPG mRNA expression in vehicle treated cells, but diminished the stimulatory effects of strontium ranelate up to 46 % (2 mM, $p < 0.001$). In conclusion, strontium ranelate increases the production of OPG, while decreasing the production of RANKL at the osteoblast surface, thus supporting an indirect inhibitory effect on osteoclastogenesis. Previous studies have shown that the CaSR is involved in both the increase in osteoblast replication and osteoclast apoptosis induced by strontium ranelate. Our results show that the CaSR at the surface of the human osteoblasts is involved in the strontium ranelate-induced increase in OPG. This observation strengthens the proposal that the CaSR plays a key role in the dissociating effect of strontium ranelate on bone formation and bone resorption.

Conflict of Interest: None declared

Su-P100

THE OSTEOPOROTIC EFFECTS OF ROSIGLITAZONE ARE NOT CAUSED BY A DIRECT NEGATIVE ACTION ON HUMAN OSTEOBLAST DIFFERENTIATION

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The peroxisome proliferator-activated receptor gamma (PPARG) agonist rosiglitazone (rosi) is widely prescribed to patients with insulin resistance. Recently, rosi was shown to exert detrimental skeletal effects by inhibiting bone formation (1), though its molecular mechanism remains to be identified. However, it has been suggested that PPARG stimulates adipocyte on the expense of osteoblast differentiation from a common precursor, the mesenchymal stem cell, leading to bone marrow adipogenesis and osteoporosis. The PPARG gene gives rise to two distinct proteins: PPARg-1 and PPARg-2. Expression of PPARg-2 is restricted to adipocytes, whereas PPARg-1 is ubiquitously expressed. Both protein variants only differ in their N-terminal domain, but contain similar ligand-binding domains. Our aim was to assess the presence and function of PPARg-1 signalling in human osteoblasts. The human fetal osteoblastic cell line SV-HFO, and human bone marrow-derived mesenchymal stem cells (hMSC) were differentiated towards mature osteoblasts in the presence of dexamethasone and b-glycerophosphate in a three-week period. Realtime-PCR analysis and quantitative western blotting revealed a significant up-regulation of PPARg-1 expression during differentiation, whereas PPARg-2 expression remained virtually absent. Incubation with rosi directly induced endogenous expression of a confirmed PPAR target gene, ANGPTL4, suggesting that endogenous PPARg-1 signalling is functional in human osteoblasts. In order to elucidate the role of PPARg-1 signalling, we cultured SV-HFO and hMSC under the continuous presence of rosi. Both cell models showed significantly higher mineralization and alkaline phosphatase activity when cultured in the presence of rosi compared to their controls. Furthermore, expression levels of other osteoblast-specific markers, e.g runx2, osteopontin and collagen type 1, were significantly elevated in rosi-treated cultures. In conclusion, PPARg-1 signalling plays an important role in osteoblast differentiation. Rosiglitazone alone is not sufficient to induce adipogenesis from human mesenchymal stem cells, but stimulates differentiation of both osteoblastic and adipocytic cell lineages in vitro. Therefore, the molecular mechanisms of PPARG signalling in bone marrow adipogenesis resulting in osteoporosis are not mediated by a direct suppression of osteoblast differentiation.

1. Grey A, et al. JCEM 2007;1305–10

Conflict of Interest: None declared

Su-P101

SEVERE OSTEOPENIA IN PERK-KNOCKOUT MICE IS DUE TO IMPAIRED OSTEOBLAST DIFFERENTIATION AND PROLIFERATION, AND ER RETENTION OF PROCOLLAGEN-I

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PERK deficiency in humans (Walcott-Rallison Syndrome) and mice causes multiple skeletal dysplasias, severe osteopenia, exocrine pancreas atrophy, and permanent neonatal diabetes. As revealed by Micro-CT analysis, PERK deficient mice show a remarkable reduction in trabecular bone mineralization and cortical bone thickness as early as postnatal day 2. The expression of osteoblast markers (Alkaline phosphatase, Osteocalcin and Type I Collagen) was found to be significantly down-regulated in Perk KO mice by $\sim$60–70% compared to wild-type littermates, and osteoclast markers were also reduced. To aid in the in situ identification of mature osteoblasts, the Col2.3GFP transgene was introduced into Perk KO mice. We found that the reduction in the expression of the osteoblast markers in Perk KO mice was the result of fewer mature osteoblasts and lower expression of osteoblast-specific genes in each osteoblast cell. Perk KO osteoblasts exhibit reduced proliferation but do not exhibit increased cell death. A reduction of osteoblast markers was also seen in osteoblast-specific Col2.3 Perk KO mice, suggesting that PERK intrinsically regulates osteoblast differentiation. In addition, to reduced proliferation and differentiation, Perk KO osteoblast exhibit abnormal retention of procollagen-I in the ER which can be reversed by treatment of osteoblast in culture with a chemical chaperone. The differentiation, proliferation, and ER defects seen in Perk KO osteoblasts are similar to defects in the insulin secreting beta-cells in these mice suggesting that PERK has the same function in these two important secretory cells. Supported by NIH AR49816 (D.R.C.).

Conflict of Interest: None declared

Su-P102

PRIMARY MURINE OSTEOBLAST CULTURES CONTAIN MACROPHAGES THAT ENHANCE OSTEOBLAST MINERALISATION

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Delineation of the phenotype and functional capacity of osteoblasts (OBs) has been widely studied using primary OBs harvested via

enzymatic digestion of neonatal rodent calvaria (calvarial OBs). Previous studies have suggested heterogeneity within this cell preparation and reported immune functions that aOre not traditionally performed by mesenchymal cells. These immune functions have been attributed to the OB, however, the heterogeneous nature of the culture has not been considered. We have demonstrated that macrophages are a significant population in standard calvarial OBs using cells isolated from MacGreen mice (macrophages express an eGFP transgene driven by a myeloid restricted promoter). Microarray analysis of differentiating calvarial OB cultures (day 5, 14 and 21) confirmed a large number of macrophage-associated genes at all time points. Clustering analysis using microarray datasets representing nearly all tissues and cell lineages (symatlas.gnf.org) linked the gene expression profiles of calvarial OBs to macrophages and osteoclasts. Immunocytochemistry and flow cytometry confirmed that calvarial OB preparations co-isolated a population of F4/80+ macrophages that persist and expand during OB differentiation in vitro. Multiple passaging did not eliminate macrophages from these cultures. Bone explant cultures were also examined as an alternative approach to generating primary OBs and were similarly shown to contain F4/80+ macrophages. Given these observations, and our recent data demonstrating that macrophages are intercalated within bone lining tissues, we hypothesised that macrophages and OBs cooperate in the control of bone metabolism. To delineate the cooperative and distinct functional roles of macrophages and OBs, we used magnetic-assisted cell sorting to generate a population of highly enriched calvarial OBs by removing haematopoietic cells. The majority of cells removed via this method expressed the F4/80 macrophage marker. Strikingly, macrophage removal significantly decreased both osteocalcin mRNA expression levels and in vitro mineralisation (von Kossa) in enriched OB differentiation cultures. The presence of a persistent population of macrophages within primary OB cultures raises the possibility that our existing understanding of in vitro OB biology has been influenced by the contribution of this cell population. Our data provide evidence that macrophages regulate OB function and specifically enhance mineralisation in vitro.

Conflict of Interest: None declared

Su-P103

INVOLVEMENT OF GAS7 ALONG THE ERK 1/2 MAP KINASE AND SOX9 PATHWAY IN CHONDROGENESIS OF HUMAN MARROW DERIVED MESENCHYMAL STEM CELLS

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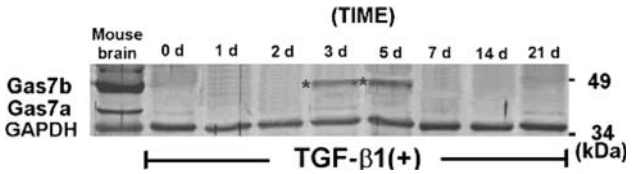
Objective: The growth-arrest-specific protein, Gas7, has been shown to be involved in reorganization of the cytoskeleton and for inducing changes in cell shape during cell differentiation. The goals of this study were to investigate the novel role of human Gas7 (hGas7) in chondrogenic differentiation of human mesenchymal stem cells (hMSCs) and to identify the relationship between hGas7, extracellular signal-regulated kinase (ERK1/2) and SOX9 in the chondrogenic pathway.

Methods: Bone marrow-derived hMSCs were induced to undergo chondrogenic differentiation with transforming growth factor-betaβ1 (TGF-b1) in an aggregate culture system. The expression of hGas7 and SOX9 and phosphorylation of ERK1/2 at multiple time points were investigated. Chondrogenic capacity was evaluated by the size of aggregates, by glycosaminoglycan content, and by type II collagen and proteoglycan deposition after interfering with expression of hGas7, ERK1/2 or SOX9.

Results: Treatment of hMSCs with TGF-b1 (resulted in a transient up-regulation of hGas7b, one of the two isoforms of hGas7 (day 3 to day 5) (Fig.1A), a transient phosphorylation of ERK1/2 (0.5 h to 4 h) and an up-regulation of SOX9 (2 h to day 14). Interference with hGas7b production by hGas7b-specific antisense oligonucleotide (hGas7b-As)or inhibition of p-ERK with PD98059, a specific inhibitor of ERK signaling pathway, or interference with SOX9 production by SOX9 siRNA all caused adverse effects of chondrogenic differentiation of hMSCs. Meanwhile, inhibition of p-ERK or SOX9 both blocked the expression of hGas7b. However, the p-ERK and SOX9 pathway were not affected by inhibition of hGas7b.

Conclusion: These results provide evidence that the transient expression of hGas7b, regulated by activation of ERK1/2 and SOX9 pathway, is essential for chondrogenic differentiation of hMSCs.



Conflict of Interest: This work was supported by the Chang Gung Memorial Hospital Research Grand

Su-P104

(-)-EPIGALLOCATECHIN-3-GALLATE (EGCG) INCREASES OPG/RANKL EXPRESSION IN OSTEOCLAST FEEDER CELL, ST2

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Introduction: Green tea is one of the most popular beverages in the world. Among the catechins, (-)-epigalloncatechin -3-gallate (EGCG) has received by far the most attention. Recent surveys have reported to reduce the risk of having a hip fracture with higher bone mineral density by habitual tea drinkers. Osteoprotegerin (OPG) and receptor activator of nuclear factor-kB ligand (RANKL), regulate the proliferation and differentiation of osteoclast. OPG/RANKL/ receptor activator of nuclear factor-kB (RANK) has been set in the current model to study preosteoblastic/stromal cell regulation of osteoclastogenesis. In this study, we study the effect of EGCG on the expression of OPG and RANKL in the feeder cells of osteoclast, ST2.

Methods: The feeder cells of osteoclast, ST2, were maintained in MEM-α with 10% fetal bovine serum. For the experiment, cells were plate at a density of $1* 10^4$ cells per cm^2 in culture medium. The medium was changed 24 hours after adding the cells and every 48 hours thereafter. Cells were treated by EGCG with concentrations of 0.1, 1 and 10 μmol/L. Gene expression of OPG and RANKL were analyzed by real-time PCR after EGCG treatment for 2 days.

Results: EGCG increases the mRNA expression of OPG and decreases taht of RANKL in ST2 cells at the concentration of 0.1, 1 and 10 μmol/L. The mRNA expressions of OPG and RANKL in the EGCG treated cells were compared with those of the control cells. After EGCG 0.1, 1 and 10 μmol/L treatments, OPG mRNA expression was increased by 10% ($P < 0.05$), 16% ($P < 0.05$) and 18% ($P < 0.05$), respectively. The mRNA expression of RANKL was decreased by 23% ($P < 0.05$), 45% ($P < 0.05$) and 61% ($P < 0.05$), respectively. The ration of OPG/RANKL increased to 162% ($P < 0.05$), 257% ($P < 0.05$) and 300% ($P < 0.05$), respectively, when compared with the control treatment.

Conclusion: Our results illustrated that the effective concentration of EGCG is at the range of 0.1 to 10 μmol/L. Previous report

indicated that one cup of green tea drinking could accumulate the circulating level of EGCG to 1 μmol/L. Our results indicated that EGCG acts on bone marrow mesenchymal cells by modulating OPG/RANKL/RANK system through increasing the mRNA expression of OPG and decreasing the mRNA expression of RANKL. These results suggest that osteoclastogenesis may be decreased through the mechanism of intensified OPG/RANKL by EGCG.

Conflict of Interest: None declared

Su-P105

EFFECTS OF HL39 ON BONE REMODELLING: A ROLE FOR THE PROLIFERATION AND DIFFERENTIATION

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Background: Bone is a living organ that undergoes remodeling throughout life. Bone remodeling involves the removal of mineralized bone by osteoclasts followed by the formation of bone matrix through the osteoblast that subsequently become mineralized. The osteoporosis is a systemic disease of multicausal etiopathogenesis. We screened over 100 natural extracts, which known for traditional bone drugs in Asia. Among them HL39 induced osteoblasts differentiation, and reduced osteoclasts differentiation.

Methods: We studied effects of HL39 in osteoblasts (C3H10T1/2 and MC3T3-E1 cell) and osteoclasts (Primary bone marrow stromal cell). Osteoblastic proliferation and differentiation were evaluated with MTT, alkaline phosphatase (ALP), and mineralization nodule assays. Osteoclastic differentiation was determined with osteoprotegerin (OPG) assay and tartrate resistant acid phosphatase (TRAP) staining. Also, we investigated that the effects of HL39 in bone marker mRNA levels.

Results: We found that HL39-Ac and HL39-H increased ALP activity as 5–7-fold of control in osteoblast. Dexamethasone (Dex) decreased ALP activity, but HL39-Ac and -H increased as 2–10-fold of Dex. HL39-Ac and HL39-H were increased mineral nodule formation in osteoblast. OPG secretion was increased as 2.5-fold of control. TRAP-positive cells were decreased in HL39-Ac and HL39-H treated osteoclast. Finally, we found that HL39-Ac and -H are also able to influence bone marker mRNA levels.

Conclusion: These finding indicated that HL39-Ac and HL39-H promoted osteoblast proliferation and differentiation, and suppressed osteoclast differentiation.

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Conflict of Interest: Y Chung, HL Genomics, Grant Research Support

Su-P106

EFFECTS OF ARACHIDONIC ACID AND DOCOSAHEXAENOIC ACID ON DIFFERENTIATION OF AND MINERALIZATION BY MC3T3-E1 OSTEOBLAST-LIKE CELLS

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Osteoblasts in culture can differentiate into mature mineralizing osteoblasts when stimulated with osteogenic agents. Clinical trials and in vivo animal studies suggest that specific polyunsaturated fatty acids (PUFAs) might benefit bone health. The aim of this study was to investigate whether PUFAs affect osteogenesis in osteoblasts.

MC3T3-E1 osteoblast-like cells were exposed to the n-6 PUFA arachidonic acid (AA) and the n-3 PUFA docosahexaenoic acid (DHA) at 2.5 to 20 microg/mL for periods of 2 to 14 days. The differentiation of these cells into functional osteoblasts as indicated by alkaline phosphatase (ALP) activity was investigated, while von Kossa staining was used to visualize bone nodule formation. Since fatty acids and their metabolites are ligands for members of the PPAR family, and are in part responsible for adipocyte differentiation, visualization of adipocyte formation was included in this study. ALP activity versus Oil Red O staining was used as criteria to determine osteoblastic versus adipocytic differentiation. Three separate experiments were conducted ($n = 4$).

Results from this study show that in the osteogenic supplemented model, long-term exposure (14 days) to AA inhibited ALP activity in the MC3T3-E1 cell line, which might be prostaglandin E2 -mediated. DHA exposure also inhibited ALP activity in these cells, which was evident after both short- (2 days) and long-term (14 days) exposures. The mechanism whereby DHA inhibits ALP activity is not clear and needs to be investigated. Although exposure to PUFAs inhibited ALP activity, the mineralizing properties of these cells were not compromised as evidenced by von Kossa staining, and adipocyte-like features were not induced in these cells. More research is indicated to elucidate the cellular mechanisms of action of PUFAs on bone.

This work was supported by the National Research Foundation (South Africa).

Conflict of Interest: None declared

Su-P107

EFFECTS OF CYCLOSPORINE, TACROLIMUS AND RAPAMYCIN ON OPG, RANKL AND IL6 EXPRESSION, AND CELL SURVIVAL IN HUMAN OSTEOBLASTS

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Osteoporosis has been consistently reported in patients treated with immunosuppressants. However, the possible direct effects of these drugs on bone cells are not fully known. During the last years new immunosuppressants were developed with less general adverse effects than classical cyclosporine. The aim of this work was to study the effects produced by cyclosporine (CsA), tacrolimus (FK506) and rapamycin (RAPA) on the expression of osteoprotegerin (OPG) and the receptor activator of nuclear factor KappaB ligand (RANKL), main keys of osteoclastogenesis and bone remodelling, and interleukin 6 (IL6), a known activator of bone resorption, in human osteoblasts. Osteoblast apoptosis after 24 and 48 hours of incubation with these drugs was also studied. The concentration of immunosuppressants used in this work were those considered as acceptable or high in serum patients under a clinical point of view.

Human primary osteoblast like cells from three men undergoing orthopaedic surgery were used. Cells were cultured in DMEM supplemented with 20% FBS and stimulated with CsA (250 and 1000 ng/ml), FK506 (5 and 20 ng/ml) or RAPA (12 and 50 ng/ml) for 1 h, 3 h, 6 h and 16 h and mRNA expression of OPG, RANKL and IL6 was measured by quantitative RT-PCR. Apoptotic cell death after 24 or 48 h with the same concentrations of immunosuppressants was quantified by flow cytometry of DNA content in permeabilized, propidium iodide-stained cells.

CsA (250 ng/ml), FK506 (5 ng/ml) and RAPA (12 ng/ml) produced a significant increase in mRNA expression of OPG and

RANKL, suggesting and increase of bone remodelling produced by these drugs. It is interesting to note that the same serum levels of these drugs are considered as acceptable in patients who had undergone solid-organ transplantation.

The three studied immunosuppressants at both concentrations produced an increase in the expression of IL6 mRNA, being that increase in the case of FK506 at 20 ng/ml significantly higher than the increase produced by the other studied drugs.

Both FK506 and RAPA, at the studied concentrations, increase significantly the degree of apoptosis of human osteoblasts after 24 and 48 h of incubation. CsA did not exert any effect on osteoblasts apoptosis.

The above results suggests that CsA, FK506 and RAPA produced direct effects on osteoblasts metabolism and survival that could explain, at least partially, their deleterious effect on bone mass. (Grant: FIS PI060025)

Conflict of Interest: None declared

Su-P108

CATABOLIC FUNCTIONS OF ENDOSTEAL BONE LINING CELLS ANALYZED BY LASER-ASSISTED MICRODISSECTION AND RT-PCR

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Background: Wether endosteal bone lining cells (EBLC) have anabolic, catabolic of protective functions to preserve bone mass is still unclear. This might be due to the difficulty to study their properties within their complex microenvironment. Therefore we aimed to establish laser-assisted microdissection for isolation of endosteal lining cells from native human bone. Subsequently we considered the mRNA expression of matrix metallo proteinases (MMP) and their inhibitors (TIMP) in order to elucidate catabolic abilities of this particular cell type covering almost 80% of normal endosteal surfaces in the adult skeleton.

Methods: Fresh human bone from femoral heads was cut into small cubes. Consecutively kryosectioning was performed followed by staining with Nuclear Fast Red for 60 s. Laser-assisted microdissection was performed using PALM and Leica systems. 4 to 8 pooled single cells c-DNA was synthesized and introduced to a qualitative single-cell PCR. Furthermore, immunofluorescence studies were performed on iliac crest biopsies.

Results: We established a protocol for laser-assisted microdissection to isolate single cells from native human bone tissue with special focus on cells of the endosteal lineage. Both, PALM LPC and Leica LMD turned out to allow a fast and precise isolation of EBLC. Cell specific m-RNA expression was proved by identification of Cbfa1 (Run-X2) mRNA expression in the isolated cells. Furthermore, we investigated mRNA expression of MMP13 and TIMP-1 in EBLC.

Conclusions: By qualitative RT-PCR Cbfa1 could be demonstrated as house keeping gene of the osteoblastic lineage in isolated EBLC. The expression of mRNA for MMP13 and TIMP-1 indicate catabolic properties of EBLC. Both enzymes play a pivotal role for matrix degradation (MMP13) and for bone cell activity by their anti-apoptotic effect (TIMP-1). Therefore EBLC may take part in the process of slow bone loss in aging man by acting synergistically with osteoclastic bone resorption. The adaptation of laser-assisted microdissection to bone tissue presents a new promising approach to study different bone cells in their specific complex microenvironment.

Conflict of Interest: None declared

Su-P109

THE INFLUENCE OF CASEINPHOSPHOPEPTIDES ON INTRACELLULAR CALCIUM CHANGES IN PRIMARY HUMAN OSTEOBLASTS: A NUTRIENT DEPENDENT MODULATION OF BONE CELL METABOLISM

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Caseinphosphopeptides (CPPs) are a family of peptides originating from in vivo and in vitro hydrolysis of casein. They possess a sequence of three phosphorylated serines followed by two glutamic acids, the acidic motif, able to bind minerals such as calcium. These nutritional compounds display the ability to increase calcium solubility in the digestive tract. Thus, CPPs were hypothesized to increase the calcium absorption and retention in vivo, with potential effects on bone mineralization. Notwithstanding, there are controversial reports on CPP action. The methodological approach used by different laboratories to study calcium absorption and bone mineralization resulted unable to out light whether the peptides have a specific effect on bone metabolism besides the enhancement of calcium availability. We have therefore designed the following study to evaluate a possible direct role of CPPs in bone cell metabolism. Primary human osteoblasts were established in culture using trabecular bone samples obtained from waste materials during orthopedic surgery of patients without metabolic or malignant bone disease. Cytosolic calcium changes were measured by video-microscopy using the fura-2 method on single cells. A mixture of CPPs of commercial origin as well as pure synthetic CPPs were used. The administration of CPPs to human osteoblasts caused an immediate but transient intracellular calcium change in a dose dependent manner. This CPP-induced effect, analogous to that reported for human intestinal cells, is not cytotoxic and is triggered by an influx of the extracellular ions through the cell plasma membrane. The osteoblast pre-treatment with the active form of vitamin D, known to differentiate human osteoblast, does not affect the cell responsiveness to CPP administration. The 24 hours cell incubation with CPPs induced the increase of the activity of alkaline phosphatase, a marker of osteoblast differentiation, reaching a level similar to that produced by vitamin D. The same CPP treatment caused a small but significative reduction in cell rate proliferation and a slight increase in apoptosis activity. Taken together these results indicate that CPPs are endowed of a bone specific effect which underlying mechanism requires further evaluation. CPPs may act not only as a mere carrier for improving calcium absorption and utilization, but also as a trophyc compound for bone health by enhancing osteoblast differentiation and activity.

Conflict of Interest: None declared

Su-P110

PERIOSTIN IS A PARATHYROID HORMONE REGULATED OSTEOBLAST DERIVED BONE MATRIX PROTEIN

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Background: Periostin is a 90 kDa secreted protein originally identified in osteoblast-like MC3T3-E1 cells. The protein is highly expressed in periosteum and periodontal ligaments and its distribution is restricted to collagen-rich tissues and several tumors.

Methods: We have studied the co-localization of Periostin and Collagen 1a2 using RNA in situ hybridization in mouse embryos (ED 16.5) and immunohistochemistry. Periostin mRNA and protein expression were also studied in cell cultures of mouse primary osteoblasts, human fetal osteoblasts and some human osteosarcoma cell lines by Northern blot, RT real-time PCR and Western blot analysis and correlated to their ability to mineralize. A possible regulation of Periostin was also investigated by the treatment of mouse primary osteoblasts with human parathyroid hormone (1–84).

Results: Mouse primary osteoblasts, the human fetal osteoblasts (hFOB 1.19) cell line and three human osteosarcoma cell lines (MHM, KPDXM and Eggen) express Periostin. The mRNAs of Periostin and Collagen 1a2 co-localize in cells giving rise to the anlagen of intervertebral discs and ribs, and in tissues surrounding cartilage from ED 16.5 mouse embryo. Periostin is present also in the basal lamina of the skin and in bone matrix possibly associated with Collagen type 1. The expression of Periostin is inversely related to the degree of differentiation and mineralization of bone cells and its mRNA is transiently up-regulated by PTH in cell culture. siRNA knock down of Periostin mRNA in differentiating hFOB 1.19 cells caused a 75% reduction in its mRNA together with down-regulation of Osteocalcin (50%) and PTHR1 (50%) mRNAs.

Conclusion: Our data demonstrate that Periostin is synthesized in osteoblasts expressing Collagen 1a2 and secreted as a matrix protein possibly in association with Collagen type 1. Periostin is expressed in early stages of mouse bone and skin development. Moreover, Periostin is regulated by PTH, and knock down of Periostin mRNA is followed by a reduction in Osteocalcin and PTHR1 mRNAs indicating a role in OB regulation.

Conflict of Interest: None declared

Su-P111

EXPRESSION OF TWO-PORE DOMAIN POTASSIUM (K2P) CHANNELS IN OSTEOBLASTIC CELLS IS RELATED TO DIFFERENTIATION

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Twin pore domain potassium (K2P) channels are widely expressed throughout the body, and have a variety of roles including setting the resting membrane potential, responses to hypoxia and pH changes, and in mechanotransduction. Recently, one member of the K2P family, TREK-1, has been reported to be present in human osteoblasts. However, the presence and role of other K2P channels in bone cells remains unknown.

Primers were designed to target all functional K2P channels. Preliminary studies in rat osteoblasts and UMR-106 cells identified mRNA encoding for TASK-1, TRAAK and TWIK-2. Screening moved onto the human cell lines TE-85, MG-63 and SaOS-2, which represent increasing stages of differentiation according to their expression of osteoblastic markers. Finally, primary human osteoblasts were investigated. The data from human cell lines, MG-63 and SaOS-2 were similar to those observed in rat osteoblasts, with expression of TASK-1, TRAAK and TWIK-2. However, TE-85 cells and primary human osteoblasts addtionally expressed TASK-2 & -5, TWIK-1 & TREK-1.

The TE-85 cell line represents a model of less-differentiated osteoblasts. The large number of channels observed in this cell line could point to a role for K2P channels in early osteoblast function and differentiation. Primary human osteoblasts in culture may be expected to contain cells at differing stages of maturity. TASK-1 has been shown to be highly sensitive to small fluctuations in physiological pH. The TRAAK channel produces an outwardly rectifying current in response to unsaturated fatty acids and stretch. TWIK-2 is a weak outward rectifier thought to be important in setting membrane potential. Possible roles for these channels in bone include involvement in pH-sensitive and mechanosensitive bone remodelling. Further work aimed at clarifying the significance of the RT-PCR results using immunocytochemistry and QRT-PCR is on-going. We have already identified some channels using commercially available antibodies. QRT-PCR will enable us to directly compare the level of expression between cell lines, and permit functional studies to measure transcriptional changes in response to modulators of bone formation.

Conflict of Interest: None declared.

Su-P112

REGULATION OF PROTEASE-ACTIVATED RECEPTOR-2 EXPRESSION IN MOUSE PRIMARY OSTEOBLASTS BY HORMONES, GROWTH FACTORS AND CYTOKINES

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Protease-activated receptor-2 (PAR-2) is a G-protein coupled receptor activated by trypsin, tryptase and various other serine proteases. Activation of the PARs occurs through proteolytic cleavage of the extracellular domain, resulting in generation of a new N-terminal "tethered" ligand. PAR-2 is expressed by osteoblasts, and activation of PAR-2 in mouse bone marrow cultures leads to inhibition of osteoclast differentiation induced by parathyroid hormone (PTH), interleukin-11 (IL-11) or 1,25 dihydroxy vitamin D3. The objective of our study was to investigate the modulation of expression of PAR-2 in mouse primary osteoblasts by hormones, growth factors and cytokines that are important in bone cell function. Confluent mouse primary osteoblast cultures were treated with various factors in serum-free medium for 24 hours and subsequently the mRNA was analysed using quantitative reverse transcriptase-polymerase chain reaction (qPCR). Expression of PAR-2 mRNA was upregulated by PTH or fibroblast growth factor-2. PAR-2 mRNA was downregulated significantly when cells were treated with 1,25 dihydroxy vitamin D3, tumour necrosis factor-α or a combination of IL-6 and soluble IL-6 receptor. The other factors studied, including insulin-like growth factor-1, transforming growth factor-β, bone morphogenic protein-2, IL-1β and IL-11, had no effect on the expression of PAR-2 mRNA. The results suggest that PTH may partially limit its own pro-osteoclastogenic effect by inducing PAR-2 expression, while other pro-osteoclastogenic factors may enhance their effects by suppressing PAR-2 expression.

Conflict of Interest: None declared

Su-P113

BMP-4 IS DOWN-REGULATED TOGETHER WITH RUNX2 IN THE PERIPHERAL BLOOD OF PATIENTS WITH RHEUMATOID ARTHRITIS AND OSTEOARTHRITIS BUT NOT ANKYLOSING SPONDYLITIS

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Background/aims. Three major forms of chronic joint diseases are classified in clinical practice: osteoarthritis (OA), rheumatoid arthritis (RA) and spondyloarthritis (SpA) that includes several forms (ankylosing spondylitis (AS), psoriatic arthritis (PA), etc.). They differ in the pathophysiological mechanisms and the intensity of cartilage and bone destruction, with RA as a prototype of "destructive" arthritis, OA "steady-state" arthritis and SpA "remodeling" arthritis. The aim of our study was to test changes in the expression of selected bone morphogenetic proteins (BMPs), known for their osteoinductive action, in the peripheral blood mononuclear cells (PBMC) of patients with RA, OA and SpA, and analyze them in relation to the expression of Runx2, an essential transcriptional factor for osteoblast differentiation.

Methods. Blood samples were collected from healthy controls (Ctrl; $n = 31$, age range 24–61) and RA patients ($n = 52$, age range 27–57), OA patients ($n = 15$, age range 45–79) and SpA patients, either with AS ($n = 26$, age range 32–46) or PA ($n = 20$, age range 34–52), after the informed consent. RNA was extracted from PBMCs, converted to cDNA, amplified by quantitative PCR using TaqMan assays for BMP-2, -4 and Runx2, and expressed as the relative amounts of RNA (mean $\pm$ SD) for target genes normalized to GPDH.

Results. BMP-2 expression was not significantly different among groups, whereas BMP-4 expression was significantly down-regulated in RA and OA patients compared to SpA patients and control subjects (RA 2.35 ± 1.55 and OA 2.36 ± 1.80 vs. AS 5.86 ± 4.67, PA 4.17 ± 2.44 and Ctrl 5.94 ± 4.20, $p < 0.001$, ANOVA). In addition, Runx2 was also significantly down-regulated in RA and OA patients compared to other groups (RA 3.01 ± 1.42 and OA 4.47 ± 4.55 vs. AS 8.95 ± 3.78, PA 6.17 ± 1.77 and Ctrl 7.89 ± 2.63, $p < 0.001$, ANOVA).

Conclusions. Our results indicate that RA and OA have decreased expression of BMP-4 and Runx2 in contrast to SpA, which reflects insufficient osteogenesis and joint reparation in those types of arthritis. Our further investigation will try to identify more bone-regulatory factors changed systemically in arthritic disease and their effects on bone cell survival, differentiation and activity, which may be helpful for novel therapeutic strategies aimed to restore joint homeostasis.

Conflict of Interest: None declared

Su-P114

A 90KDA BONE-SPECIFIC ANTIGEN LOCALISED TO THE GOLGI APPARATUS OF OSTEOBLASTS

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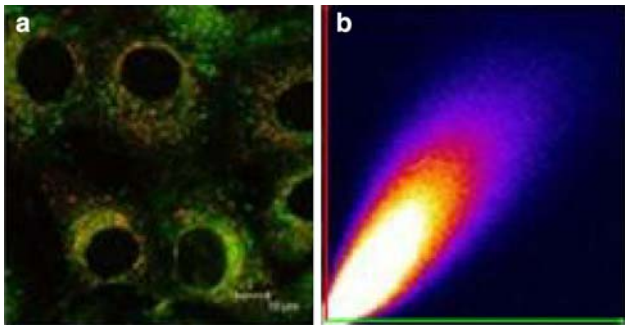
OCA-1 is a bone-specific antigen highly expressed in osteoclasts and at lower levels in osteoblasts and osteoblastic cell lines TE85, MG63 and SaOS-2. The specific localisation in bone indicates that it may play an important role in regulating bone cell function. Our aim was to further characterise OCA-1. We utilised osteosarcoma cells for live cell imaging and to produce enough antigen for characterisation. Antibody was labelled with Alexa 488 fluorescent dye, (Molecular Probes), and Chariot, (Vector Laboratories), was used as a vector to mediate uptake into the cell. Live imaging was performed over 8 days until the cells reached confluence. Antibody against collagen VI and Chariot minus antibody were run as controls. Western blots were performed on osteosarcoma-cell lysed with 1% Triton X plus protease inhibitors. Immunoprecipitation was performed using magnetic protein G-coated Dynal beads, (Invitrogen). Antibody was pre-bound to the beads prior to incubation with cell lysate taken from osteosarcoma cells. Samples were then run in two SDS-PAGE gels;- one for Coomassie staining and one for Western blotting. OCA-1 co-localised with the golgi apparatus,Fig. 1A. The scatterplot, Fig. 1B, compares the red pixelation of the antibody signal with the green of the golgi, ($r = 0.94$). Live imaging showed that OCA-1 remained in its perinuclear position from 30% through to full confluence, suggesting no effect of cell proliferation on expression. At 100% confluence 70% of cells appear to express OCA-1. Western blotting and immunoprecipitation indicated the molecular weight of OCA-1 to be 90 kDa. OCA-1 was detected in the cell culture medium of the least differentiated cell line, MG63, but restricted to an intracellular location in the other cell lines, suggesting its function varies dependent on stage of differentiation and that it may have a role in extracellular signalling in bone.



Conflict of Interest: None declared

Su-P115

ERK1/2 AND FRA-1 MEDIATE PHOSPHATE-DEPENDENT STIMULATION OF MATRIX GLA PROTEIN EXPRESSION IN OSTEOBLASTS

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Inorganic phosphate (Pi) and the mineral-binding protein MGP (Matrix Gla Protein) are key regulators of mineralization in bone-forming cells. Recently, it has been demonstrated that Pi stimulates MGP expression through the ERK1/2 signaling pathway in growth plate chondrocytes (1). In addition, mice lacking the AP-1 transcription factor Fra-1 are osteopenic and exhibit a strong reduction in skeletal MGP expression (2). In this context, we sought to determine whether Pi could regulate MGP expression in osteoblasts and decipher the role of Fra-1 in this regulation.

Osteoblastic MC3T3-E1 (MC) cells and primary calvaria-derived osteoblasts (OB) from wild type or Fra-1 knockout mice were used. MGP and Fra-1 expressions were examined by real-time PCR and Western Blot. The activation and role of signaling pathways were determined by Western Blot and use of specific inhibitors. Chromatin Immunoprecipitation (ChIP) assay was used to examine the in vivo binding of Fra-1 to the MGP promoter. In MC, MGP was expressed at the highest level after the early phase of cellular differentiation. At this stage of differentiation, Pi increased MGP expression at both the mRNA and protein levels in MC cells. Pi was also found to trigger a significant increase in MGP expression in OB. The expression pattern of Fra-1 in MC cells paralleled that of MGP and Pi induced a marked increase in Fra-1 expression. Investigation of the involved intracellular signaling pathways revealed that Pi activated ERK1/2 in MC cells and OB. UO126, an inhibitor of the ERK pathway, blocked Pi-stimulated Fra-1 and MGP expression, indicating that ERK1/2 mediates Pi effects in both cell models. Finally, among the putative AP-1 binding sequences identified in the MGP promoter, our ChIP assays indicate that Pi significantly increased the recruitment of Fra-1 in positions -3402 and -3079/3058, indicating that this transcription factor could regulate MGP transcription in response to Pi. Finally using osteoblasts isolated from Fra-1 knockout mice, we demonstrated that in cells deleted for Fra-1, Pi failed to stimulate the expression of MGP.

Taken together, our data established for the first time that Pi regulates MGP expression in osteoblasts via the ERK1/2-Fra-1 pathway. Whether these data may be of relevance for the control of skeletal and ectopic calcification would be paid further attention.

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Conflict of Interest: None declared

Su-P116

CALCIUM IS REQUIRED FOR PHOSPHATE-DEPENDENT STIMULATION OF MGP AND OPN EXPRESSION IN OSTEOBLASTS

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Inorganic phosphate (Pi) acts as a signalling molecule in bone-forming cells, affecting cell functions and gene expressions (1). Particularly, Pi stimulates the expression of mineralization-associated genes such as OPN and MGP through the ERK1/2 pathway (2,3). With respect to the concomitant presence of elevated extracellular calcium and phosphate levels during bone remodelling, we questioned whether calcium may play a role in the phosphate-dependent effects in osteoblasts. As evidenced by real-time PCR and Western blot, we first confirmed that ion pair (10 mM Pi; 1.8 mM calcium) stimulates MGP and OPN expression through ERK1/2 phosphorylation in MC3T3-E1 osteoblastic cells. ERK1/2 phosphorylation secondary to ion pair stimulation was biphasic, displaying an early acute (30 min) and a late sustained (8 h) phase. Our data indicate that the ERK 1/2 phosphorylation acute phase was not necessary for the sustained phase to occur. In addition, both ERK1/2 phosphorylation peaks required the concomitant presence of calcium and phosphate in the culture medium to occur. The use of increasing phosphate and calcium concentrations showed that at least 1.8 mM calcium are required for Pi-dependent ERK1/2 phosphorylation and MGP/OPN up-regulation to take place. In addition, ion pair-dependent cellular effects were blocked using foscarnet or EDTA, a Pi transporter inhibitor and a calcium chelating agent, respectively. Because the exact mechanisms by which Ca and Pi act on osteoblasts remain unclear, we then questioned whether ion pair-dependent cellular effects may be mediated through the formation of calcium phosphate crystals. By transmission electron microscopy and elemental microanalysis (EDX), we demonstrated that adding Pi (10 mM) in the culture medium containing 1.8 mM calcium led to the formation of apatitic crystals. Interestingly, phosphocitrate, an inhibitor of crystal formation, inhibited ion pair-induced cellular effects. Our data strongly suggest that calcium is required for Pi-dependent ERK1/2 phosphorylation as well as regulation of mineralization-associated genes in osteoblasts. Whether the cellular effects of calcium and phosphate are mediated by specific sensing receptors, ion transporters or by crystal-mediated mechanisms would be paid further investigation.

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2. Beck GR, Jr., Knecht N 2003. J Biol Chem 278(43):41921–9.
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Conflict of Interest: None declared

Su-P117

GROWTH HORMONE STIMULATES PROLIFERATION AND DIFFERENTIATION OF M2H4 ODONTOBLASTIC CELL LINE

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Growth hormone (GH) is a peptide secreted by somatotropic cells in the anterior lobe of the pituitary gland. Many reports have described the key role of GH on bone metabolism. GH plays an important function in the regulation of longitudinal bone growth and stimulates both bone formation and resorption. Regardless of the similarities between bone and dental tissues, few studies describe the role of GH in the craniofacial growth and in the dental development.

The biological effects of GH are mediated by a specific receptor (GHR) located on the surface of target cells. The presence of GHR was described on dental papilla in the rat and dentin matrix dimensions measured in giant (GH-Excess) mice and dwarf (GH-Antagonist and GH-Receptor-Knockout) mice revealed that GH status regulates the dentin thickness. However, no information is available on the specific effect of GH on odontoblasts, the cells responsible for dentinogenesis.

The objectives of this work were to study in the rat M2H4 odontoblastic cell line (i) the effects of GH on odontoblast proliferation (cell counting) and differentiation (real time PCR) and (ii) to identify the signalling pathways involved in the cellular effects of GH. Our results show for the first time that M2H4 expressed STAT-activating functional GH receptors. In addition, GH increased the cellular proliferation in a dose-dependant manner at least through the ERK1/2 signalling pathway. Finally, we observed that GH up-regulates the cellular differentiation of M2H4 as evidenced by the increased expression of IGF-1, Runx2, BMP2, BMP4 as well as one of the major odontoblastic markers, DMP1.

In conclusion, our data revealed that growth hormone stimulated both proliferation and differentiation of odontoblasts and suggest that the GH morphogenetic effects on dentin could be mediated by the induction of both bone morphogenetic proteins and insulin-like growth factor-1 expression.

Conflict of Interest: None declared

Su-P118

INDEPENDENT OF THEIR RESORPTIVE ACTIVITY, OSTEOCLASTS SECRETE AN ACTIVITY INDUCING BONE FORMATION AND CANONICAL WNT SIGNALLING IN OSTEOBLASTIC CELLS

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Some osteopetrotic mutations lead to low bone resorption, increased numbers of osteoclasts and increased bone formation, whereas other osteopetrotic mutations lead to low resorption, low numbers of osteoclasts and decreased bone formation. These findings support the hypothesis that the osteoclasts independent of their resorptive activity are sources of anabolic signals for the osteoblasts.

The aim of the current study was to investigate whether osteoclasts secrete bone anabolic signals, and to elucidate the type of factor involved.

Conditioned media from mature human osteoclasts cultured on either bone slices or plastic were collected. Measuring TRACP and CTX-I validated osteoclast maturity and resorption. Conditioned media were applied to cultures of MC3T3-E1 preosteoblasts, followed by bone formation assessment by Alizarin red and Von Kossa staining after 20 days' culture. We assessed key osteoblast regulatory pathways by using UMR106.01 cells transiently transfected with several reporter constructs. These were the TOPFlash vector, which contains 8 TCF/LEF response elements, the osteocalcin promoter with x6 tandem OSE repeats (6 × OSE), NFAT, AP-1 and NFkB.

Conditioned media from osteoclasts cultured on either bone or plastic stimulated nodule formation by the MC3T3-E1 cells to levels comparable to stimulation with 10 ng/mL BMP-2. Conditioned media from osteoclasts cultured on both bone and plastic specifically induced activation of the TCF/LEF response system at a level comparable to induction by 20 ng/mL of Wnt3A. The Wnt 3a and conditioned medium signals were inhibited, albeit to different extents, by addition of either 100 ng/mL of DKK1 or 1 mg/mL of sclerostin, consistent with activation of the canonical Wnt signaling pathway. No activation of the OSE, NFAT, NFkB, or AP-1 reporters was detected, suggesting specific wnt activity.

We present evidence that osteoclasts, independent of their resorptive activity, secrete an activity that stimulates osteoblastic bone formation. The same media contains an activity that signals through the wnt activation cascade, indicating either that the osteoclasts secrete a wnt, or that the factor from the osteoclasts induces wnt production in the osteoblasts.

Conflict of Interest: None declared

Su-P119

LOCALIZATION OF RUNX2, OSTERIX AND OSTEOPONTIN IN CEMENTOGENESIS IN RAT MOLARS

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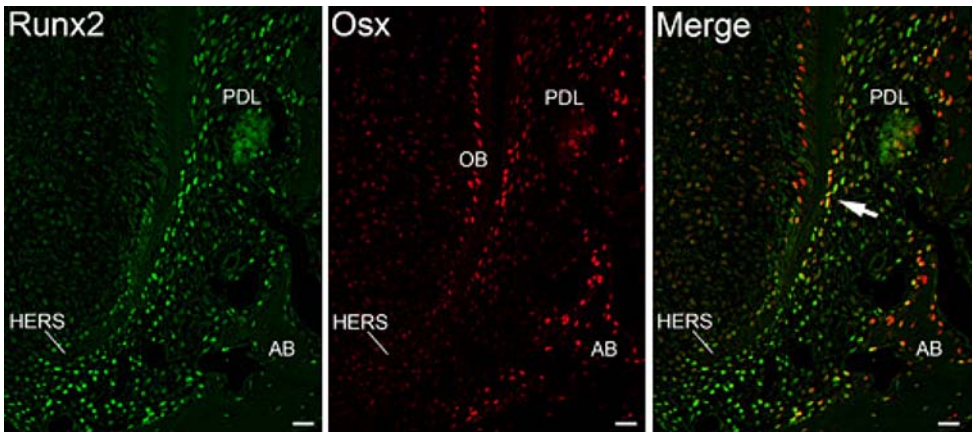
Background/aims: Cementogenesis starts with the differentiation of dental follicular cells into cementoblasts. Though cementum is structurally similar to bone, the exact mechanism of cementogenesis remains unclear. In this study, we determined the immunolocalization patterns of Runx2 and Osterix, which are transcriptional factors of osteoblasts, and osteopontin, one of the bone matrix proteins, during tooth root formation. The aim of this study was to clarify their roles in cementoblast differentiation and to determine the origin of cementoblasts.

Methods: We evaluated the immunoreactivity for Runx2, Osterix and Osteopontin in the mandibular first molar of 2-week old rat. We also examined the localization of BrdU and alpha smooth muscle actin to identify proliferating cells and undifferentiated mesenchymal cells, respectively.

Results: Both acellular and cellular cementum were present on the root dentin. Hertwig's epithelial root sheath (HERS) was observed at the apical end of tooth root with loss of continuity. BrdU-labeled cells were seen in the dental follicle near HERS. Periodontal ligament cells as well as odontoblasts and osteoblasts located on alveolar bone surface showed immunoreactivity for Runx2. Co-localization of Runx2 and Osterix was detected in cementoblasts which had penetrated through the ruptured HERS and attached to root dentin. However, cells adjacent to cementoblasts showed only Runx2-positive reactivity. Co-localization of Runx2 and Osteopontin was observed in cementoblasts facing the root surface. Neither Runx2 nor Osterix was seen in cementocytes.

Conclusion: These results suggest that Runx2-positive cells in dental follicle and periodontal ligament proliferate. Additionally, both Runx2 and Osterix are essential to differentiate into cementoblasts.

Conflict of Interest: None declared



Su-P120

PIOGLITAZONE AND DEXAMETHASONE INDUCE ADIPOGENESIS IN D1 BONE MARROW STROMAL CELL LINE, BUT NOT THROUGH THE PPARΓ PATHWAY

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Osteoblasts and adiopocytes share a common progenitor from bone marrow. Peroxisome proliferator-activated receptor-γ plays a critical role in adipogenesis. Using a mouse pluripotent mesenchymal cell, D1, as a model, several reports have demonstrated that dexamethasone, a glucocorticoid, can induce adipogenesis. We first examined whether adipogenesis induction in D1 cells is initiated by activation of peroxisome proliferator-activated receptor-γ. The results revealed that pioglitazone induces adipogenesis in D1 cells in dose-dependent manner and decreases alkaline phosphatase activity in D1 cells. Interestingly, this adipogenesis was not blocked by bisphenol A diglycidyl ether, a peroxisome proliferator-activated receptor-γ antagonist. A peroxisome proliferator-activated receptor-γ-mediated reporter gene assay showed no response to pioglitazone. We then asked whether dexamethasone-induced adipogenesis can be repressed by mifepristone (RU486), an antagonist of glucocorticoid receptor. The results disclosed that mifepristone cannot counteract dexamethasone-induced adipogenesis, and mifepristone itself induced adipogenesis in D1 cells. Moreover, glucocorticoid receptor-mediated reporter gene assay was not responsive to dexamethasone or mifepristone. We concluded that the adipogenesis induced by pioglitazone and dexamethasone in D1 cells may not occur via a peroxisome proliferator-activated receptor-γ and glucocorticoid receptor pathway. Finally, we analyzed the gene expression profile of D1 by cDNA microarray after treatment with dexamethasone. We found that the expressions of several adipogenesis-related genes are highly provoked by this agent.

Conflict of Interest: None declared

Mo-P121

BONE LOSS IN PATIENTS WITH PRIMARY BILIARY CIRRHOSIS IS CAUSED BY A FIBRONECTIN ISOFORM

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Osteoporosis is a major cause of morbidity and decreased quality of life in patients with chronic cholestatic liver disease. This osteoporosis results from decreased bone formation, but the mechanisms for the interaction between liver and bone remain elusive. The aim was to test the hypothesis that an increase in the production of cellular fibronectins during liver disease may result in decreased osteoblast-mediated mineralization, and thus explain the decrease in bone formation.

We performed a prospective cross-sectional study in patients with primary biliary cirrhosis and matched controls, followed by experiments in vitro on human and mouse osteoblasts as well as injections in mice in vivo. The methods used were: protein purification, ELISA, staining, protein labeling, bone mineral density measurements and bone histomorphometry.

In patients with primary biliary cirrhosis the increase in a fibronectin isoform containing the oncofetal domain correlated significantly with the decrease in osteocalcin, a marker of bone formation ($r = -0.57$, $p < 0.05$). In vitro, amniotic-fluid fibronectin (aFN) containing mainly the oncofetal-domain and EIIIA-domain resulted in decreased osteoblast-mediated mineralization in human osteoblasts (69% decrease; $p < 0.01$) and mouse osteoblasts (71% decrease; $p < 0.05$). Removing the EIIIA-domain from aFN similarly suppressed mineralization by osteoblasts (78% decrease; $p < 0.05$). Injection of aFN in mice (1 mg/day) over 10 days resulted in circulating levels of oFN that were 40% higher than controls ($p < 0.01$) similar to the increase seen in patients with primary biliary cirrhosis compared to controls. Bone mineral density decreased by 17% ($p < 0.05$), and histomophometry showed a decrease in mineralizing surface ($MS/BS = 45 \pm 3$ in controls vs. 31 ± 2 % in injected mice, $p < 0.005$), and a decrease in the number of osteoblasts ($Ob.N/BS = 18 \pm 1$ in controls and 10 ± 1/mm in injected mice, $p < 0.05$).

Increased production of a fibronectin isoform containing the oncofetal domain and its release in the circulation in patients with primary biliary cirrhosis is at least partially responsible for the decrease in bone formation seen in these patients.

Conflict of Interest: Non declared

Mo-P122

EFFECT OF BARIUM ON DIFFERENTIATION OF HUMAN MESENCHYMAL-STEM-CELLS INTO OSTEOBLASTS IN VITRO

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Effect of barium on differentiation of human mesenchymal-stem-cells into osteoblasts in vitro.

Introduction: Calcium and strontium are known their beneficial effects on bone metabolism. Here we focus upon a trace element, barium, because it is chemically similar to calcium and strontium. This study examined the effect of barium on gene expression in differentiation of human mesenchymal-stem-cells into osteoblasts in vitro.

Materials and Methods: Human bone marrow stem cells were cultured in 6 well culture dishes at an initial density of 25,000 cells/well. Cells were cultured for $0 \sim 14$ days in osteogenic differentiation medium and added strontium chloride and barium chloride to the wells at final concentrations of 0.1 mM, 0.3 mM, 1 mM. Alkaline phosphatase (ALP) activity staining was used as a measure of osteoblast differentiation. Total RNA was extracted after 1,3,7 and 14 days, and the analysis of Runx2, BMP-2, Cbfa1 and BSP gene expression was done by real time RT-PCR.

Results: We found that both strontium and barium had better enhancing effect on cell proliferation as compared to those cultured in media without strontium or barium. Barium (BaCl2) produced a $\sim$2-fold increase of Runx2 expression at 14 days. Strontium (0.1–0.3 mM SrCl2) produced a $\sim$2-fold increase in the expression of Runx2 at 14 days. There was little change at $1 \sim 7$ day culture period. We found that barium produced $\sim$1.5-fold increase of BMP-2 expression at 1 or 3 days. Barium (BaCl2) produced a 1.6-fold increase of Cbfa1 expression after 1-day culture and a 0.1-fold decrease of Cbfa1 expression after 7 or 14-days culture. Strontium also produced a 1.3-fold increase of Cbfa1 expression at 3 days and a 0.1-fold decrease at 7 or 14 days. We found barium produced a $1.5 \sim 1.7$ fold increase in the expression of BSP at 1 day and strontium produced a $\sim$2-fold increase in the expression of BSP at 14 days.

Conclusion: We demonstrate that barium is potent stimulator of osteogenetic gene expression during osteoblast differentiation. We recommend that barium is one of the important factors for inducing

mesenchymal stem cells to differentiate into osteoblasts with further enhancement on bone formation. These possibilities merit further testing.
Conflict of Interest: None declared

Mo-P123

OSTEOBLAST MATURITY DICTATES RESPONSE TO VASCULAR ENDOTHELIAL GROWTH FACTOR

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Vascular endothelial growth factor (VEGF) has a multifaceted role in bone cell activity. It is implicated in early stages of human osteoblastogenesis, aids bone repair by promoting angiogenesis and also acts as a chemotactic factor promoting migration of several cell types needed for bone formation. Consequently, VEGF is a good candidate for therapeutic strategies in bone healing and tissue engineering. The aim of this study was to characterise osteoblast responsiveness to VEGF during osteogenesis. Osteoblasts were isolated from rat calvariae and treated with recombinant VEGF165. Cellular responses to VEGF including proliferation, differentiation and extra cellular matrix mineralization were assessed using an MTT assay, by measuring Alkaline Phosphatase activity and Von Kossa staining. Short term responses to VEGF treatment were investigated by Western blotting with an antibody against phospho-p44/42. RT-PCR with primers for VEGFA, B, C, D, VEGF Receptors 1, 2, 3 and Osterix, Runx2, Alk. Phos., OC, OP, and BMP2 were used for transcript profiling. Treatment of primary rat osteoblasts with VEGF under osteogenic conditions for 14 days did not demonstrate any affect on alkaline phosphatase activity levels observed in each group. However in longer term experiments cultures treated with VEGF (1 ng/ml–10 ng/ml) exhibited significantly increased mineral deposition and enhanced bone nodule formation compared to controls (ANOVA, $P = 0.042$). To characterise how VEGF influenced bone formation, its activity was assessed on immature bone cells. In proliferation assays VEGF had no effect on early osteoblasts though some evidence of signal transduction was observed with MAPK activation. To further clarify if VEGF responsiveness in our culture system was differentiation dependent, experiments were performed to analyse VEGF-related transcripts at 0, 7, 14, 21 and 28 days. RT-PCR identified transcripts for VEGF isoforms and receptors with apparent changes in mRNA abundance over time. VEGFR3 transcripts declined as osteoblast maturation progressed while VEGFR2 mRNA levels were upregulated. VEGFR1 mRNA levels remained unchanged. Furthermore transcripts for differentiation factors Runx2 and Osterix were upregulated at 24 h–48 h when osteoblasts matured for 21 days were treated with VEGF whilst immature cells were unresponsive. We hypothesise that osteoblast maturity dictates the ability to respond to VEGF and this is driven by differential VEGF receptor expression.
Conflict of Interest: None declared

Mo-P124

THE REGULATORY ROLES OF INORGANIC PHOSPHATE IN GROWTH PLATE CHONDROCYTE APOPTOSIS

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Programmed cell death (apoptosis) is the final fate of growth plate chondrocytes. Impaired apoptosis of growth plate chondrocytes results in impaired endochondral ossification. Recent findings suggested that inorganic phosphate (Pi) regulates apoptotic events in growth plate chondrocytes (Adams et al. 2001 J Biol Chem 276:20316–20322). Hypophosphatemic mouse models have elongated hypertrophic zones and decreased apoptotic rates in the growth plate resulting in the formation of rickets (Sabbagh et al. 2005 Proc Natl Acad Sci USA 102:9637–9642). Apoptosis may also play an important role during cartilage pathology. However, little is known about the mechanisms regulating apoptotic events in chondrocytes. The purpose of this study was to determine the mechanism of how Pi regulates apoptosis of growth plate chondrocytes. Primary growth plate chondrocytes isolated from d19 embryonic chick tibia growth plate cartilage were cultured in the absence or presence of 5% fetal calf serum (FCS), retinoic acid (RA), various concentrations of Pi (1 mM, 2.5 mM, 4 mM, and 8 mM), and phosphoformic acid (PFA). The rate of apoptosis in the cultures was determined by caspase-3 activity, TUNEL labeling, and bcl2 (anti-apoptotic) and bax (pro-apoptotic) expression using real time PCR analysis. When growth plate chondrocytes were cultured under serum-free conditions increasing amounts of Pi were protective against apoptosis in the presence of RA. In the absence of RA caspase-3 activity was low under serum free conditions independent of the Pi concentration. TUNEL labeling and flow cytometric analysis revealed similar results. In addition, the bax/bcl-2 expression ratio was the highest in the presence of RA and 1 mM Pi and decreased in the presence of higher amounts of Pi in a dose dependent manner. In the presence of PFA, an inhibitor of phosphate transport proteins, caspase-3 activity increased in cultures treated with RA and 8 mM Pi compared to caspase-3 activity of cultures treated with 8 mM Pi and RA. When growth plate chondrocytes were maintained in medium containing 5% FCS and treated for 6 days with RA and various concentrations of Pi caspase-3 activity and percentage of TUNEL positive cells increased with increasing concentrations of Pi. Under these conditions, terminal differentiation was stimulated first followed by apoptosis. In conclusion, our findings suggest that Pi is protective against apoptosis in less mature chondrocytes (hypertrophic) but stimulates apoptosis in more mature cells (terminally differentiated).
Conflict of Interest: This study was supported by grants from NIH/NIAMS.

Mo-P125

THE EFFECT OF FLUID SHEAR STRESS ON RECRUITMENT OF OSTEOCLAST PRECURSORS INDUCED BY OSTEOCYTES

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Bone is a tissue that responds to a mechanical load by changing its internal architecture. Ostecytes are the predominant bone cells and it is currently believed that osteocyte which responds to mechanical strain may send signal to other cells. Healthy or apoptotic osteocytes can send signals to other bone surface cells like osteoblasts, osteoclasts, osteoclast precursors and bone lining cells through their networking in canaliculi. Therefore, we hypothesized that osteocytes stimulated by mechanical strain could modulate the other bone cell recruitment and proliferation. We used the MLO-Y4 cells as in vitro model for osteocytes, RAW 264.7 cells as osteoclast precursor, and 2T3 cells as osteoblasts. MLO-Y4 cells conditioned medium (Y4-CM) was collected after 24 h culture. For fluid flow experiments, MLO-Y4 cells were exposed to 2 hrs of pulsatile fluid flow (PFF) at 2, 4, 8, 16 $\pm$ 0.6 dynes/cm^2 using Flexcell Streamer^{TM} system. We did proliferation assay of RAW 264.7 and 2T3 cells with control media or 10% Y4-CM at specific time. The migration of RAW 264.7 and 2T3 cells was assayed using transwells with control media or 10, 20, 50, 100% Y4-CM. MLO-Y4-CM increased osteoclast precursor proliferation and migration. Y4-CM decreased 2T3 cell proliferation and migration. After MLO-Y4 cells were exposed to PFF, Y4-CM decreased RAW 267.4 cell proliferation, migration and 2T3 migration compared to

control CM (Y4-CM without strain). However Y4-CM exposed PFF had no effect on 2T3 osteoblastic cell proliferation. These results suggest that osteocytes can regulate the bone remodeling by communication with osteoclast precursors and osteoblasts and that fluid flow shear stress may inhibit bone resorption which is induced by osteocytes. This work was supported by the Korean Research Foundation Grant funded by the Korean Government. (R04-2004-000-10146-0)
Conflict of Interest: None declared

Mo-P126

TIBIA AND HUMERUS BOWING IN A MOUSE MODEL FOR NEUROFIBROMATOSIS TYPE 1

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Neurofibromatosis type 1 (NF1; von Recklinghausen disease) is one of the most common genetic disorders. Beside the typical neurofibroma app. 50% of patients develop skeletal symptoms. These include sphenoid wing dysplasia, kyphoscoliosis, reduced bone mineral density, mild short stature, tibial bowing, and pseudarthrosis. As the most mouse models of Nf1 do not reproduce the skeletal aspects of the human disease, we ablated Nf1-flox conditional allele during skull and early limb development by Prx1 induced Cre-recombinase excision (Kolanczyk M., Human Molecular Genetics 2007).

Resulting Nf1Prx1 mutant mice show reduced long bone length, defective skull mineralization, and joint fusions of variable degree. Due to fusion of hip joints Nf1Prx1 mice are unable to move their hind limbs. We observed bowing of the mechanically unloaded tibia as well as of the loaded humerus during very early postnatal development. Such bowing indicates a mechanically instability of affected long bones. Material properties of Nf1Prx mutant cortical bone were weakened due to increased porosity, reduced calcium content, and diminished hardness. Stability of cortical bone was further impaired by increased osteoid and diminished mineralized bone width. Moreover, at the chondro-osseous junction doubling of osteoclast number induces a premature resorption of the growth plate at 6 weeks of age. Accelerated loss of growth plate cartilage leads to decrease of primary spongiosa trabecular bone density from 34.8 % ($\pm$ 7.6) in control humeri to 25.3 % ($\pm$ 3.1) in Nf1Prx1 mutant humeri at P14. Likely due to defective joint function Nf1Prx1 humeri expose unilateral switching from periostal to endostal bone formation.

Nf1Prx1 mice recapitulate different aspects of the human NF1 bone phenotype such as short stature, hypomineralization, and tibia bowing. Long bone bowing is caused by reduced bone mineral content, premature growth plate resorption, decreased trabecular bone volume, thinning of cortical bone, increased osteoid thickness, and unproper mechanical loading due to misformed joints. Defective Nf1Prx1 mice long bone formation will help to unravel the mechanisms of postnatal bone development. Furthermore, it will help to develop strategies to prevent tibia bowing and pseudarthrosis in NF1 patients.
Conflict of Interest: None declared

Mo-P127

CRANIOMETAPHYSEAL DYSPLASIA IS CAUSED BY REDUCED PLASMA MEMBRANE ACCESSIBILITY AND ALTERED SUBECELLULAR LOCALIZATION OF MUTANT ANK

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Heterozygous mutations in the ANKH gene cause craniometaphyseal dysplasia (CMD), characterised by sclerosis of the skull and reduced modelling of the long bones. ANK, the protein encoded by ANKH, is believed to transport the mineralisation inhibitor pyrophosphate (PPi) from the cytosol to the extracellular space. Although it is crucial for the proposed transport function that ANK resides in the plasma membrane a detailed analysis of the subcellular distribution has not been performed.

In subcellular fractionation experiments using sucrose gradient centrifugation of liver lysates ANK clearly co-localised with marker proteins for endosomes and the trans-Golgi. This Golgi-localization was corroborated by immunostaining either of ANK overexpressed in different cell lines or of endogenous ANK in wildtype fibroblasts and chondocytes. Signals were absent in fibroblasts from ank/ank mice. While the Golgi-localisation was preserved in the overexpressed ANK mutants C331R and G389R, the mutation F377del lead to a dispersal of the protein throughout the cytoplasm.

In immunostainings a significant plasma membrane localisation was only observed when ANK was overexpressed in HEK293 cells. These findings were mirrored by surface biotinylation experiments revealing that only app. 10% of ANK is present in the plasma membrane in wildtype cells and after mild retroviral overexpression of wildtype ANK. The plasma membrane access was further reduced if ANK harbouring CMD mutations was retrovirally expressed.

Our data reveal subcelluar enrichment of ANK in the Golgi-apparatus. In contrast, only a minor fraction of ANK resides at the plasma membrane, indicating a cycling within the late secretory pathway. Reduced plasma membrane access of ANK harbouring CMD mutations could explain alterations of extracellular PPi levels. Our data point to a potential role of the Golgi-apparatus in PPi metabolism, which has to be further investigated.
Conflict of Interest: None declared

Mo-P128

EFFECTS OF SIMULATED SPACE RADIATION ON MURINE BONE CELL DIFFERENTIATION AND GENE EXPRESSION

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The most common alteration observed in astronauts performing long-term space flights is bone loss. Weightlessness may result in bone demineralization, especially in load bearing bones, at a rate up to one to two percent per month. This osteopenia is explained by the mechanical unloading of bone in weightlessness and by nutritional deficiency. However, the detailed mechanism of the bone remodelling process is still a mystery. Currently, a contribution of space radiation exposure to bone resorption can not be excluded. A radiation-induced cell cycle arrest could enhance or accelerate the osteoblastic differentiation process. To assess the bone forming capacity of the investigated bone cells, the cell lines were cultured either in standard culture medium or in osteoinductive medium containing beta-glycerophosphate and ascorbic acid. During the process of osteoblastic cell differentiation, the expression of the bone specific marker genes osteocalcin (OCN) and osteopontin (OPN) were recorded. Compared with the control culture conditions, the marker genes were highly expressed during the osteogenic differentiation process. Radiation-induced premature differentiation was investigated with regards to the biosynthesis of specific osteogenic marker molecules and cell cycle regulatory proteins after X-irradiation, using quantitative real time reverse transcription PCR (qRT-PCR). The distribution of cells in the

cell cycle phases G1, S and G2 was determined by propidium iodide (PI) staining and flow cytometric analysis. Regarding cell cycle alterations, X-rays provoked a dose dependent arrest in G2M phase of the cell cycle. This was accompanied by a dose dependent regulation of the cyclin kinase inhibitor CDKN1A (p21/WAF) and transforming growth factor beta 1 (TGF-beta1). TGF-beta1 is known to affect osteoblast differentiation, matrix formation and mineralization. Modulation of its expression could influence the expression of main osteogenic transcription factors. The cell cycle delay after X-ray exposure could act synergistically with the TGF-beta1 expression on osteoblastic differentiation. The presented bone cell model offers a new tool to investigate and understand bone cell differentiation in combination with different radiation qualities. The interrelationship of differentiation status and radiation exposure may play an important role in bone formation and bone maintenance, especially for astronauts planning long-term space missions.

Conflict of Interest: None declared

Mo-P129

EFFECTS OF PREGNANCY IN PRIMARY BONE CELLS EXPOSED TO OSCILLATORY FLUID FLOW

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During pregnancy, the calcium level of the mother adapts to demands of the fetus and as a result, bone diseases are important health concerns. Imbalance between bone formation and bone resorption is the main cause of osteoporosis and other bone fracture problems. Mechanical loading is an important regulator of bone remodeling and many studies have investigated the response of bone cells to mechanical loading. However, the relationship between pregnancy and loading-induced bone remodeling has not been well studied. Therefore, the aim of this study was to investigate whether bone during pregnancy responded differently to mechanical loading compared to bone during non-pregnant conditions.

Bone cells were isolated from 11 week old ICR mice. The mice were either non-pregnant, 10-day pregnant, or 18-day pregnant. The long bones were harvested and removed of bone marrow and surface cells. The bones were cut in small pieces and bone cells were allowed to migrate out of the bone pieces into a culture dish. 48 hours prior to mechanical loading, cells were seeded on glass slides and treated with vitamin D3 for expression of receptor activator of NF-kB ligand (RANKL). Oscillatory fluid flow-induced shear stress was applied to cells at 1 Pa for 1 hr. Control cells received no flow. Immediately after end of loading mRNA was extracted. Real-time RT-PCR was performed to quantify the following genes: core binding factor a1 (cbfa1), osteopontin (OPN), collagen I, osteoprotegerin (OPG), and RANKL.

Results for 18-day pregnant mice only have been quantified thus far. For genes related to bone formation, loading increased OPN mRNA expression by 10% compared to non-loaded control group. Also, loading had a dramatic effect on cbfa1 (5-fold increase) and collagen I (3-fold increase) in 18-day pregnant mice. For genes related to bone resorption, RANKL and OPG expression both significantly increased but the resulting ratio of RANKL/OPG was significantly decreased by 40% with loading.

Comparing these results to previous studies suggest that pregnancy does not impair the effect of mechanical loading on bone remodeling. Similar to studies performed using non-pregnant condition cells, genes that enhance osteoblastic activity such as cbfa1, OPN, and collagen I are upregulated with loading in bone cells from pregnant mice. Also, RANKL/OPG ratio is significantly decreased suggesting that loading may decrease osteoclast formation and activity with loading during pregnancy.

Conflict of Interest: None declared

Mo-P130

CELL ATTACHMENT AND PROLIFERATION BEHAVIOR OF OSTEOBLAST-LIKE E1 CELLS AND GINGIVAL FIBROBLASTS GROOVED SURFACE OF ZIRCONIA CERAMICS

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Surface properties, including topography and chemistry, are important in establishing the response of tissues to implant materials. Surface topography, especially that of grooved surfaces, play a crucial role for permucosal oral implants in providing a biological seal to inhibit epithelial downgrowth and bacterial invasion. Protective barrier of attached gingiva around the transmucosal abutment requires a material that is non-toxic and favors the attachment and growth for the surrounding tissues. Implant abutments of zirconia ceramics, which provides not only mechanical properties and esthetics but also good biocompatibility with soft tissue have been developed.

This study was performed to define attachment and growth behavior of osteoblast-like cells and gingival fibroblasts cultured on grooved surfaces of zirconium oxide and evaluate the genetic effect of grooves on zirconium oxide surfaces using the RT-PCR.

Osteoblast-like cells E1 and human gingival fibroblasts were cultured on (1)commercially pure titanium discs with smooth surface (T group), (2)yttrium-stabilized tetragonal zirconia polycrystal (Y-TZP) with smooth surface (ZS group), and (3)Y-TZP with 100μm grooves (ZG group). Cell morphology was examined by scanning electron microscopy (SEM) at 4 hrs, 24 hrs, cell proliferation activity was evaluated through MTT assay at 24 hrs. The mRNA expression of Runx2, alkaline phosphatase, osteocalcin, TGF-beta 1, IGF-1 in E1 cells were evaluated by reverse transcriptional-polymerase chain reaction analysis (RT-PCR). The mRNA expression of integrin α,β, Type I collagen, Type III collagen, laminin, fibronectin in gingival fibroblasts were evaluated by RT-PCR.

From the MTT assay, the mean optical density value showed no significant difference between 3 groups. Significantly more cells were observed to attach to the grooves and appeared to follow the direction of the grooves. At 48 hr cell incubation, no differences in mRNA expression of alkaline phosphatase, Runx2, G3PDH were noted between ZG group and other two groups. Osteocalcalcin, and TGF-beta 1, IGF-1 was highly expressed on the zirconia ceramic.

Zirconia ceramic and pure titanium showed similar biological responses of osteoblast-like cells and gingival fibroblasts during a short-time cell culture period. Grooves influence cell spreading and cause the cell to be aligned with surface grooves.

Conflict of Interest: None declared

Mo-P131

HEPARIN MODULATES THE EFFECT OF WNT3A ON OSTEOBLAST PROLIFERATION AND DIFFERENTIATION

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Wnts are growth factors with diverse roles during development. Wnts bind to a membrane receptor complex comprised of frizzled (FZD) G-protein-coupled receptors (GPCRs) and/or a low-density

lipoprotein (LDL) receptor-related protein (LRP) 5/6. The formation of this ligand-receptor complex initiates intracellular signaling cascades that includes the canonical/beta-catenin pathway, as well as several GPCR-related noncanonical pathways with a number of recent reports linking Wnt to the control of osteoblast differentiation and bone mass. Heparin, a hyper-sulfated heparan sulfate glycosaminoglycan (HS-GAG), binds to a number of growth factors, including Wnts, and cells mutant for HS-GAG biosynthesis are defective in Wnt-dependent FZD receptor activation and fail to initiate Wnt signaling, establishing that HS is required for FZD receptor function. In this study we investigated the mechanism by which heparin regulates the effect of Wnt3a on osteogenesis using a combination of Western blot, FACS, siRNA and ELISA-based assays. We show that Wnt3a mediates cell cycle progression of preosteoblasts (MC3T3-E1), with a significant increase in G1/S cells as well as viable cell number. Wnt3a also increased signaling through p44/42 mitogen-activated protein kinase (MAPK) and the expression of cyclin D1, c-Myc and $p21^{Cip1/WAF1}$, while decreasing the expression of $p27^{Kip1}$. Notably, inactivation of LRP 5/6 by Dickkopf (Dkk) -1 had no effect on the proliferative effect of Wnt3a, while knocking down the expression of beta-catenin or Lef1 only partially reduced Wnt3a stimulated cell growth, suggesting that the non-canonical Wnt pathway may be involved. We also observed that exogenous application of heparin opposed the proliferative effects of Wnt3a and required the presence of both N- and O-sulfation of the heparin. Furthermore, although heparin or Wnt3a minimally induced alkaline phosphatase (ALP) activity, their combination significantly increased ALP activity (4 fold). In conclusion, heparin abrogated Wnt3a proliferative effects and simultaneously facilitated Wnt3a-induced osteoblast differentiation. Thus heparin appears to convert Wnt3a from a mitogen to a differentiative factor in MC3T3E1 preosteoblasts.

Conflict of Interest: None declared

Mo-P132

OSTEOGENIC EFFECTS OF BOTH N- AND C-TERMINAL PARATHYROID HORMONE-RELATED PROTEIN FRAGMENTS IN AN EXPERIMENTAL MODEL OF REGENERATING BONE IN DIABETIC MICE

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Type 1 diabetes mellitus (DM) is associated with bone loss by undefined mechanisms. Parathyroid hormone-related protein (PTHrP)is a key modulator of bone formation whose expression decreases in age-related osteopenia. We examined the putative role of the N- and C-terminal domains of PTHrP in the altered osteoblast function in DM. In vivo, we used a marrow ablation model in diabetic mice by streptozotocin injection. Some mice were treated with PTHrP (1–36) or PTHrP (107–139) (100 μg/Kg/every other day) for 2 weeks before sacrifice. In vitro, mouse osteoblastic MC3T3-E1 cells were grown in differentiation medium (containing 50 μg/ml ascorbic acid and 10 mM beta-glycerolphosphate) up to 80% confluence, with or without high glucose (HG) (25 mM) (or mannitol, osmotic control), supplemented (or not) with either PTHrP peptide (100 nM). Gene expression was analyzed by real-time PCR after total RNA isolation from mouse tibiae and MC3T3-E1 cells. On day 6 after marrow ablation, DM induced weigth loss (15%), and a significant decrease (20–40%; $p < 0.05$) in the expression of the following genes: runx2, osterix, osteocalcin, PTHrP, the PTH/PTHrP type 1 receptor (PTH1R), vascular endothelial growth factor (VEGF), and its receptors 1 and 2 (VEGFR1 and VEGFR2, respectively). These changes were associated with an increase in peroxisome proliferator-activated receptor gamma and the RANKL/OPG ratio. DM mice showed an increased number of adipocytes (10-fold over control) and a decreased osteoblast number and osteoid surface (30%; $p < 0.05$) in the metaphysis of the regenerating tibia. These effects were reversed after treatment with either PTHrP peptide. In vitro, either HG- or mannitol-containing medium induced similar changes in gene expression of the aforementioned factors as observed in vivo, and also decreased collagen secretion; all which were reversed by either PTHrP peptide. Moreover, these osteoblastic effects of either medium were mirrored by adding a neutralizing anti-PTHrP antibody or the antagonist PTHrP (7–34) (1 μM) to basal medium in MC3T3-E1 cells. On the other hand, HG medium, in a similar manner to the PTHrP peptides, induced an increase of both alkaline phosphatase activity (ALP) and mineralization (alizarin red) in these cells. Conclusions: DM-related bone loss is associated with a deficit in PTHrP expression in osteoblasts. Our results also indicate that both PTHrP (1–36) and PTHrP (107–139) fragments can induce bone anabolic effects in this setting.

Conflict of Interest: None declared

Mo-P133

A NOVEL NUTRITIONAL MODEL FOR BONE QUALITY STUDIES

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Nutrition is a key factor in gaining peak bone mass during bone building years and maintain it as long as possible.

The purpose if this work is to set up a novel model to assess bone quality in pig. Pigs are favorable models for nutritional studies because they are omnivorous and their diet can be adjusted like human diet. The model is the result of a interdisciplinary approach of animal nutritionist, endocrinologists, pathologists and physicists to integrate each of their specialist areas of expertises and knowledge.

Methods: In this paper we present the pig osteoblast culture. The study was done on Landrace x Large white pigs. The cells were grown in alfa MEM+10% SFV + antibiotic/antimicotic+50 ug/ml ascorbic acid+10 mM ßglycerol phosphate. Specific markers were alkaline phosphatase assayed in cell lysates and histochemical specific staining for alkaline phosphatase and mineralization (von Kossa). The culture functionality was tested by treating the osteoblasts with dexamethasone (10-6-10-10 M).

Results: In vitro experiments were first done on osteoblasts culture. As cell source we tested several pig tissues: parietal bone, trabecular bone from femoral epiphysis and bone marrow. Parietal bone is not a suitable osteoblast source due to the low number of cells released from bone fragments and their reduced viability. Bone marrow has a moderate growth rate (average 2.5).

Trabecular bone has a high yield both by collagenization and outgrowing from bone explants. The cells had a high growth rate (5–6 for the first five passages). Calvarial cells highly expressed alkaline phosphatase, the culture was homogenous. Trabecular cells and bone marrow cells expressed alkaline phosphatase in 70% of cells in the first passage. The first mineralization nodules could be seen in the third passage. Dexamethasone inhibited alkaline phosphatase expression in osteoblasts.

Conclusion: Pig bone developed an osteogenic phenotype in culture with a high yield of dissociation and growth rate. In vitro

cultivated cells express the markers of early and late osteoblast differentiation.

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Conflict of Interest: None declared

Mo-P134

KERATIN 18 IS UPREGULATED IN CELLS FROM PAGETIC LESIONS AND AFFECTS GENE EXPRESSION IN HUMAN OSTEOBLASTS

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Paget's disease is a condition characterised by focal areas of accelerated bone turnover. The osteoclasts in the pagetic sites are grossly abnormal, and as a result have been the focus of most of the research on the cellular mechanisms of the condition. However, given that osteoblasts regulate the activity of osteoclasts, we hypothesise that osteoblasts also play an important role in the disease. We have collected RNA from osteoblasts and bone marrow grown from both pagetic and non-pagetic tissue. Microarray analysis identified a number of genes that were differentially regulated in the pagetic osteoblasts, suggesting that the osteoblasts within the pagetic lesion differ from those in normal bone. The intermediate filament keratin 18 was one of the most highly upregulated genes in osteoblasts from pagetic lesions (6.8-fold increase compared to non-pagetic controls, $p = 0.04$). Real time PCR in a larger set of samples (14 pagetic and 28 non-pagetic osteoblast samples, 14 pagetic and 21 non-pagetic bone marrow samples) confirmed that keratin 18 was upregulated more than 3-fold in pagetic osteoblasts and bone marrow. In order to investigate the effects of over-expression of keratin 18 in osteoblasts, we have transduced primary human osteoblasts with an adenoviral vector expressing keratin 18 and compared these to cells transduced with a control vector. While keratin 18 over-expression did not affect the proliferation of human osteoblasts, real-time RT-PCR analysis showed that the expression of several genes was altered. These changes included increased levels of alkaline phosphatase, FGF2 and the chemokine MCP1. Expression of these genes was also increased in SaOS2 cells over-expressing keratin 18. Interestingly, all these genes were also upregulated more than 1.5-fold in the pagetic osteoblasts tested on the microarrays. These results suggest that keratin 18 plays a role in osteoblast biology, and over-expression of this gene can reproduce some of the features of pagetic osteoblasts.

Conflict of Interest: None declared

Mo-P135

ERYTHROPOIETIN: A NOVEL DABA?

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Erythropoietin (EPO) is characterized by an elevated pleiotropism and in addition to regulates the production of erythrocytes, promotes cellular regeneration and angiogenesis in various other tissues. Recently Holstein et al, found EPO receptors also at level of bone callus showing implication of EPO in repairing process of fracture in rat. In the light of these observations, EPO could have a role in bone remodelling, but until now data in literature are poor and contrasting. Aim of our study was to evaluate the effect of EPO administration on bone density and structure in rat and moreover the influence on bone turnover markers. For this experiment we used 24 Sprague Dawley (Harlan, Italia) female rats (age: 8 weeks). Animals were divided in 4 groups:

- the 1st group: rats not ovariectomized (OVX) treated for 3 weeks only with saline (0,1 ml/rat sc);
- the 2nd group: OVX rats treated for 3 weeks only with saline (0,1 ml/rat, sc);
- the 3rd group: OVX rats treated for 3 weeks with rHuEPO (Globuren 1000 IU/ml, administered after dilution every day subcutaneously at dosage of 50 UI/rat);
- the 4th group: not OVX rats treated for 3 weeks with rHuEPO (at the same dosage).

After 3 weeks of treatment animals were sacrificed and their femura were collected and observed at electronic microscopy. Samples of urine and plasma were collected the day before the end of study to evaluate B-ALP e D-PYR.

All rats treated with EPO showed an increase in B-ALP and a decrease in D-PYR levels. At the histomorphometric exam, OVX rats treated with saline only presented a thinness of bone trabeculae, whereas this process was prevented by EPO administration. Moreover not OVX rats in active treatment showed an increment of trabecular number and thickness. Our study puts in evidence a double action, anabolic and antiresorpting of EPO on bone tissue of rat, with an increment of bone mass. This hormone would be, surprisingly able to protect bone tissue of rat from increase resorption due to ovariectomy and to preserve bone microarchitecture. In conclusion our results would underline further pleiotropism of this hormone and for the first time an effect on bone turnover and mass.

Conflict of Interest: None declared

Mo-P136

CALCITONIN GENE RELATED PEPTIDE (CGRP) MODULATES WNT SIGNALING IN HUMAN OSTEOBLAST-LIKE CELLS

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Backgrounds: Bone tissue contains CGRP-immunoreactive nerve fibers whose concentration increases during bone development and regeneration. CGRP could be a local factor involved in the regulation of bone remodeling by acting through a functional receptor expressed on human osteoblasts. CGRP stimulates osteoblast proliferation by activating PKC, and modulates osteoclastogenesis by inhibiting OPG production through PKA activation. The bone effects induced by CGRP could involve the Wnt/ß-catenin signaling that controls osteoblasts development and function. Activation of canonical Wnt signaling results in the inhibition of glycogen synthase-3-kinase beta (GSK3beta), which induces the degradation of ß-catenin. ß-catenin accumulates in the cytoplasm and migrates into the nucleus where it exerts a transcriptional control. We have therefore treated primary culture of human osteoblast-like cells (hOB) with CGRP 10^{-8} M and evaluated both beta-catenin expression, by real time PCR, and protein amount, by western blot.

Results: The results showed an increase (70% increase of treated vs untreated) of beta-catenin mRNA after treatment with CGRP for 4h. Pre-treatment with the receptor antagonist, $CGRP_{8-37}$, antagonized the enhancement of beta-catenin expression, thus indicating a receptor-specific effect of the neuropeptide.

A time course treatment with 10^{-8} M CGRP for 0-5-15-30-60-90 min showed that beta-catenin protein levels increased at 5 min after CGRP treatment and returned to basal levels after 90 min. This effect was inhibited by pretreatment with $CGRP_{8-37}$ at all time points. Forskolin, an activator of adenylate cyclase, was able to enhance time dependently beta-catenin production, the effect lasting up to 90 min treatment, and confirming that cAMP signaling pathway is involved in beta-catenin stabilization. Immunofluorescence experiments showed that treatment with CGRP 10^{-8} M for 30 min enhanced nuclear beta-catenin staining thus indicating that CGRP activated the nuclear translocation of beta-catenin, potentially leading to the activation of the transcriptional pathway controlled by Wnt.

Conclusions: These data suggest that the anabolic action of CGRP in bone is, at least in part, mediated by the activation of Wnt/beta-catenin signaling.

Conflict of Interest: None declared

Mo-P137

DISTURBED MESENCHYMAL STEM CELL DIFFERENTIATION AND OSTEOPOROTIC LIKE FEATURES IN AGEING MALE DNA REPAIR DEFICIENT TTD MICE

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Background: TTD mice, carrying mutations in the DNA repair gene XPD, show signs of accelerated ageing and can be used as a model system to study age related diseases like osteoporosis. Analyzing the bone architecture and the differentiation potential of bone marrow derived mesenchymal stem cells of these mice, we hoped to get further insight into the mechanisms behind the age related osteoporosis and adipose hypoplasia they display and the role of DNA repair in these mechanisms.

Methods: Tibiae from male TTD and WT mice (n = 4–6) of different ages (26,39,52, 65,78,91,104 weeks old) were scanned and analyzed for cortical and trabecular micro-CT parameters and tested for their strength. Bone marrow from male TTD and WT mice (n = 10) of different ages (26,39,45 and 85 weeks old) was cultured under osteogenic, adipogenic or osteoclastic conditions and analyzed for their differentiation and functional potential.

Results: Analysis of bone parameters displayed an overall accelerated ageing of male TTD bone. This was accompanied by a rapid 90% decrease in periosteal apposition at 78 weeks and an overall decline in bone strength of about 25% compared to male WT mice. Mesenchymal stem cells from male TTD mice turned out to have less differentiation potential towards the osteoblast lineage already early on in life. At 26 weeks a 27% decrease in the number of ALP positive osteoblast colonies can be observed in male TTD mice compared to WTs. Mineralization of ALP positive colonies was not affected. Osteoclast differentiation from hematopoietic stem cells and osteoclast resorption were not impaired. This leads to a decreased bone formation-bone resorption ratio at 26 weeks, which could be at the basis of the accelerated bone ageing observed later in life.

Conclusions: Our findings reveal the importance of genome stability and proper DNA repair for skeletal aging. The data show a predominant effect of impaired DNA repair at the osteoblast level. An early disruption in the bone formation-bone resorption balance, caused by a decrease in mesenchymal stem cell differentiation towards the osteoblast lineage, can be the basis of accelerated ageing of bone in TTD mice. The absence of periosteal apposition provides a tool for identification of new targets to control bone strength.

Conflict of Interest: None declared

Mo-P138

UPTAKE OF POSTPRANDIAL LIPOPROTEINS INTO OSTEOBLASTS IN MICE

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Dietary lipids and lipophilic vitamins, such as vitamin K, are transported by postprandial lipoproteins and are required for bone metabolism. Despite that, it remains unknown whether bone cells are involved in the uptake of circulating postprandial lipoproteins in vivo. The goal of the current study was to investigate the participation of bone in the systemic postprandial lipoprotein metabolism in mice, to identify involved cell type populations and to analyze whether lipoprotein uptake affects bone function in vivo.

Chylomicron remnants (CR) were injected intravenously into mice. Organ distribution and cellular uptake was analyzed by immunohistochemistry and electron microscopy. Next to the liver, bone appeared to contain the most active cells for the uptake of radioactive and fluorescent CR from the circulation in vivo. Complementary in vitro experiments showed uptake of CR by primary murine osteoblasts and hepatocytes within a similar range. Localization studies in bone in vivo showed strongest primary binding to sinusoidal endothelial cells, while particle uptake was also observed into macrophages and osteoblasts. Injection of CR containing vitamin K1 resulted in an increase of the carboxylation degree of the osteoblast-specific protein osteocalcin.

In conclusion, postprandial lipoproteins are taken up by bone in vivo. Osteoblasts are involved in the clearance of CR and this process has an impact on the secretory function of osteoblasts.

Conflict of Interest: None declared

Mo-P139

OSSEOINTEGRATION OF ORTHODONTIC TITANIUM MINIPLATES IN DOGS: MICRORADIOGRAPHIC AND HISTOLOGICAL ASSESSMENT

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Aims: This animal study aimed to evaluate the impact of orthodontic loading on osseointegration of screws supporting miniplates and to describe the histological components of the bone-screw interface.

Materials and methods: Eighty orthodontic miniplates were placed in the jaws of 10 dogs. Each miniplate was inserted with 2 titanium

miniscrews. After 2 weeks, a 125 g force was applied between the miniplates of one upper quadrant of each dog and between those of the controlateral lower quadrant. The other, nonloaded miniplates, were considered controls. Five dogs were sacrificed 7 weeks after implantation and the remaining 5 dogs after 29 weeks. Fluorochromes were injected at implantation and at sacrifice. Jaw quadrants were dissected, embedded, cut into undecalcified transverse sections through the screws and finally submitted to microradiographic analysis to allow assessment of bone-implant contact (BIC, %) and bone volume/total volume (BV/TV, %) around the screws. The sections were observed under ultraviolet light and stained in order to be examined under ordinary light.

Results: Osseointegration occurred around 90/160 screws and consisted mainly in limited repair and remodelling processes of lamellar bone, without inflammation. Wide variations were observed in BIC and BV/TV, but without any significant difference, neither between the loaded and the nonloaded screws, nor according to the direction of load. Both BIC and BV/TV were significantly higher after 29 than 7 weeks and in mandible than maxilla, although the success rate was significantly better in maxilla than mandible. Nonosseointegrated screws were surrounded by fibrous tissue. Osteoblastic activity, when present in front of these screws, was not sufficient to achieve stability.

Conclusions: Osseointegration underlying orthodontic anchorage was not affected by loading, but increased with time and varied according to implantation site. Particularly the tight-fitting screw insertion appeared crucial in determining the appropriate bone healing response.

Conflict of Interest: None declared

Mo-P140

EFFECT OF PROINFLAMMATORY CYTOKINES AND SERUM OF CROHN'S DISEASE PATIENTS ON PRIMARY HUMAN OSTEOBLAST PROLIFERATION

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Background: Patients with Crohn's disease are at increased risk of osteoporosis. Disease activity and circulating inflammatory factors are hypothesized to play a role in this process. We therefore evaluated the effect of proinflammatory cytokines and serum of patients with Crohn's disease on proliferation of primary human osteoblasts from both healthy controls and Crohn's disease patients. Methods: Iliac crest biopsies were obtained from 10 healthy donors who underwent a maxillary sinus floor elevation procedure and from 5 patients with quiescent Crohn's disease. Primary human bone cells were cultured in the presence of either 10 ng/ml IL-1alpha, IL-1beta, TNF-alpha or IL-6, or 10% serum of Crohn's disease patients. Cell proliferation was determined after 3, 5 and 7 days of culture using the XTT colorimetric assay (Roche). Results: Both IL-1alpha and IL-1beta time-dependently stimulated the proliferation of primary human osteoblasts from both healthy controls and Crohn's disease patients (up to 60% of untreated control condition). TNF-alpha did not affect proliferation. IL-6 had no effect after both 3 and 5 days of culture, but after 7 days it slightly increased proliferation by 30%. Preliminary data obtained from cells cultured in the presence of serum of Crohn's disease patients showed a time-dependent decrease in cell proliferation in 2 out of 5 patients (up to -25% of healthy control serum). Serum from 1 patient decreased proliferation only slightly by 10%, and serum from 2 patients did not affect cell proliferation. Conclusion: These data show that IL-1alpha, IL-1beta and IL-6 time-dependently increase the proliferation of osteoblasts from both healthy controls and Crohn's disease patients. Elevated concentrations of these cytokines are associated with Crohn's disease. Serum of these Crohn-patients, however, seems to decrease cell proliferation. This may be explained by a different effect of a combination of cytokines on osteoblast proliferation compared to the effect of a single proinflammatory cytokine. Therefore, the cytokine content of serum of patients with Crohn's disease needs to be elucidated.

Conflict of Interest: A.A. van Bodegraven, Aventis, Grant/Research Support

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Mo-P141

BISPHOSPHONATES INHIBIT OSTEOBLAST GROWTH AND BONE FORMATION

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Bisphosphonates (BPs) are analogues of the mineralisation inhibitor, pyrophosphate, and are widely recognised as potent inhibitors of osteoclastic resorption. Surprisingly, the direct actions of BPs on osteoblast (OB) function are not well documented. Using primary, bone-forming OB derived from neonatal rat calvariae, we found that 1 and 10 muM zoledronate (zol) reduced OB numbers by 61% and 90%, respectively, after 14 d. Pamidronate (pam) exerted a small inhibitory action and clodronate (clod) was without effect. Exposure to 10 muM zol was rapidly toxic, resulting in a 3-fold decrease in OB viability after 2 d; pam and clod were without effect. Treatment with 1 muM zol for 7–14 d inhibited soluble collagen production by up to 82%, whereas pam and clod had no effect at concentrations < 10 muM. In control cultures, abundant formation of trabecular mineralised bone matrix nodules began from $\sim$10 d. Zol selectively inhibited the mineralisation of bone nodules at low concentrations (IC50 10 nM); pam and clod exerted similar effects but at higher concentrations (IC50 $\sim$100 nm–1 muM and 1–10 muM, respectively). Complete abolition of organic matrix deposition occurred in the presence of 1 muM zol. Consistent with its low-dose effects on mineralisation, zol caused dose-dependent inhibition of OB alkaline phosphatase (ALP) activity (IC50 100 nM). In contrast, pam and clod stimulated ALP activity at high concentrations (100 nM–10 muM and 10 muM, respectively). For comparison, we also tested the effects of these BPs on the formation and activity of human osteoclasts (OC). Pam and clod progressively inhibited OC numbers and resorption pit formation, with $\sim$50% reductions evident at 1 nM. At higher concentrations (> 1 muM), pam exerted toxic effects. In contrast, 1 nM zol failed to inhibit OC formation or resorption but reduced OC numbers by $> 90\%$ at 10 nM.Our results indicate important differences in the mode of action of BPs on bone cells. Chronic exposure to zol, the most potent BP studied, inhibited bone formation in two distinct ways: first, a relatively non-toxic, selective inhibition of mineralisation at concentrations in the low nanomolar range and second, a cytotoxic inhibition of OB growth and function at concentrations 100 nM. Although no data are available on the BP concentrations that OB could be exposed to in vivo, our results are consistent with earlier reports that BPs may inhibit bone formation, including the anabolic response to PTH.

Conflict of Interest: None declared

Mo-P142

EXPRESSION OF GUT HORMONE RECEPTORS IN DIFFERENT STAGES OF OSTEOBLASTIC DEVELOPMENT

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Gut hormones constitute a group of gastro-entero-pancreatic hormones released during a normal physiological response to nutrient ingestion. Adequate nutrient intake and normal gastrointestinal function are critical to bone health, since bone is a tissue subject to constant forces that can cause its repairing/remodelling. These hormones may potentially integrate a connection between food intake and bone turnover.

The aim, was to study the presence of the expressed receptors for the gut hormones, glucose-dependent insulinotropic polypeptide (GIP), glucagon-like peptide 1 and 2 (GLP-1,GLP-2), ghrelin (GHR) and the obestatin receptor, (GPR39) and effects in vitro of GIP and GLP-1 in three osteoblastic cell lines, Mg-63, Te-85, and SaOS-2, which represent different stages of osteoblastic development.

Conventional PCR was used to screen for the presence of the receptors. Quantitative real time PCR was performed using an iCycler (iQ Bio-Rad) to examine the relative levels of expression of the receptors. The results were analysed and normalised to beta-actin. Cell cultures were treated for 5 days with varying concentrations of GIP and GLP-1 (1 to 1000 pM). After treatment, supernatants were harvested and alkaline phosphatase (ALP) was measured as a marker of osteoblast cell function. An assay for cell viability was performed 24 hours after exposure to the peptides, which utilises the fluorogenic, cell permeant, peptide substrate, glycil-phenylalanyl-amino-fluorocoumarin, Receptors for all five of the gut hormones were expressed in the three osteoblastic cell lines. The expression of GLP-1, GLP-2, ghrelin and obestatin receptors was higher in less differentiated cell lines (Te-85 and Mg-63) whereas the GIP receptor was expressed at a higher level in the most mature cell line SaOS-2.

To date only the SaOS-2 cell line demonstrated functional responses to GIP with concentrations from 10 to 1000 pM stimulating ALP production. Viability was greater when Te85 and Mg63 were stimulated with GLP-1, as indicated by the fluorescence signal proportional to the number of living cells.

These findings support the idea that osteoblast function might be modulated in the postprandial and post-absorptive states when gut hormones are released in response to changes in dietary intake.

Conflict of Interest: None declared

Mo-P143

RAP-011, A SOLUBLE ACTIVIN RECEPTOR TYPE IIA, STIMULATES OSTEOBLAST DIFFERENTIATION AND INHIBITS OSTEOCLAST DEVELOPMENT

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We previously reported that treatment with an activin antagonist, a soluble form of the extra cellular domain of activin type IIa receptor (ActRIIa) fused to a murine IgG–Fc fragment (RAP-011), increased bone formation in normal mice and restored bone loss in OVX mice. The mechanism responsible for this increased bone formation is unclear. For this reason, we studied the effects of RAP-011 on osteoblast and osteoclast precursor cells in vitro. Activin was shown to inhibit osteoblast differentiation in normal human osteoblast (NHOst) precursor cells based on a decrease in mineral deposition. The addition of soluble ActRIIa blocked activin inhibition and restored matrix mineralization. In osteoclast precursor cells (RAW246.7) stimulated to differentiate with RANKL + MCSF the addition of activin stimulated osteoclastogenesis based on increased TRAP5b expression. Addition of the soluble ActRIIA prevented this activin mediated enhancement of osteoclastogenesis. This study provides evidence that the use of an activin antagonist (RAP-011) acts as an anabolic bone agent by promoting osteoblast differentiation and inhibiting osteoclast development.

Conflict of Interest: All authors are full time employees of Acceleron Pharma, Inc.

Mo-P144

THE SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION (STAT) 3 LINKS IL-6 AND C-SRC PATHWAYS IN OSTEOBLASTS

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STAT proteins belong to a family of transcription factors which are downstream targets of cytokine signals. Among these proteins, STAT3 has been described to be a substrate of the tyrosine-kinase c-Src in tumorigenic cells, to be a component of the IL-6 pathway and to drive the IL-6 expression by a positive feedback. IL-6 action has been associated with a reduced osteoblast function and c-Src activity also maintains osteoblasts in a less differentiated status. However, the molecular mechanisms underlying these events are not yet understood. We hypothesise that IL-6 and c-Src work together to impair osteoblast differentiation and that such IL-6/c-Src interplay is mediated by STAT3. We observed that c-Src inhibition by PP1, c-Src mRNA down-regulation by siRNA and retroviral infection of a dominant negative kinase-dead c-Src variant caused reduction of IL-6 expression in cultured mouse calvarial osteoblasts. c-Src inhibition by PP1 also reduced STAT3-Y705-activating phosphorylation, with a lesser effect on STAT2-Y690-phosphorylation and no effect on other STAT family members. STAT3 down-regulation by siRNA caused transcriptional increase of osteoblast differentiation markers very similar to the effect induced by c-Src down-regulation. STAT3 down-regulation also decreased c-Src-Y416-activating phosphorylation, while treatment of osteoblasts with recombinant human IL-6 induced a robust induction of c-Src-Y416-activating phosphorylation only in long-term treatments (8 days), suggesting the involvement of intermediate factors. Treatment with IL-6 did not change IGF-I expression, but IGF-I metabolism appears to be implicated in the IL-6/c-Src interplay because the IGF-binding proteins IGFBP-3 and IGFBP-5 were transcriptionally up-regulated by treatment of osteoblasts with PP1. Combined treatment of osteoblasts with PP1 and IL-6 caused a reduction of alkaline phosphatase (ALP) activity versus PP1-alone treated osteoblasts very similar to the reduction of ALP observed in IL-6-treated versus vehicle-treated osteoblasts, indicating that IL-6 effects on osteoblast function is not entirely c-Src-mediated. In conclusion, we have

obtained evidence of an interplay between IL-6 and c-Src signals to maintain osteoblasts in a less differentiated status, which is mediated, at least in part, by the STAT3 transcription factor.
Conflict of Interest: None declared

Mo-P145

FISH AS AN ALTERNATIVE MODEL TO EXPLORE BONE BIOLOGY: ROLE OF FOUR AND A HALF LIM DOMAINS 2 IN TISSUE MINERALIZATION

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Bone diseases represent a major health problem nowadays, affecting millions of people worldwide. The molecular mechanisms governing bone homeostasis are still not fully understood, especially the basis for unbalanced osteopenic diseases (such as osteoporosis) characterized by a significant decrease in bone mass. In 2005, Günther and co-workers, while studying the role of the Four and a Half LIM domains 2 (FHL2) protein in cytoskeleton architecture and gene expression regulation, discovered that FHL2 knockout mice were osteopenic due to loss of osteoblastic function. Interestingly, FHL2 was also reported to interact with the runt-related factor, Runx2, a crucial transcription factor for bone regulation. Fish have been recently recognized as a suitable model organism to study vertebrate biology, especially skeleton development and metabolism. We propose to better characterize FHL2 role in bone formation using in vitro and in vivo tools recently developed for the marine teleost *Sparus aurata* (gilthead seabream). Seabream FHL2 cDNA was cloned and levels and sites of gene expression were determined by real-time PCR and *in situ* hybridization, during seabream development and in adult tissues. FHL2 mRNA was first detected in 48-hours post fertilization (HPF) embryos, when heart begins to beat and tissues and organs become differentiated. By *in situ* hybridization we could co-localize FHL2 transcription with the first cartilage structures formed in 96-HPF larvae. In adult tissues, FHL2 gene expression was observed in heart (as expected from previous studies in mammals) and also for the first time in mineralized tissues such as bone and cartilage, suggesting a role for FHL2 in fish tissue mineralization. This role is now being further investigated *in vitro* using S. aurata bone-derived cells engineered to over-express or knock-down FHL2 gene, and effects on extracellular matrix mineralization and bone-related gene expression are being evaluated. A similar *in vivo* approach using microinjected fertilized seabream eggs is also carried out in parallel. Since the molecular mechanisms behind tissue mineralization and FHL2 protein structure appear to be highly conserved in vertebrates, results obtained in fish, should provide new and interesting insights into FHL2 function for bone formation.
Conflict of Interest: None declared

Mo-P146

SETS OF GENES ARE REGULATED IN OSTEOBLASTS BY MODELLED MICROGRAVITY

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Mechanical unloading is detrimental for the skeleton, but the underlying molecular mechanisms are not fully elucidated. Global transcriptome analysis performed in mouse calvarial osteoblasts grown for 5 days under modelled microgravity ($0.008 \times g$) in the NASA-developed Rotating Wall Vessel bioreactor, revealed 30 up-regulated and 120 down-regulated genes relative to osteoblasts grown at unit gravity ($1 \times g$). Interestingly, the most up-regulated gene, LCN2 (Lipocalin 2), and the most down-regulated gene, PENK1 (preproenkephalin 1), were not so far associated with the bone metabolism. Real time RT-PCR and Western blot analysis of selected genes confirmed the microarray data both at mRNA and protein levels. Among the down-regulated genes we found some important bone metabolism-related transcripts, including osteomodulin, secreted frizzled related sequence protein 2, fibronectin 1 and connective tissue growth factor. A modest but significant decrease of alkaline phosphatase, Runx2 and osteocalcin was also observed. Remarkably, microarray data evidenced a significant up-regulation of RankL and interleukin 6 (IL-6) mRNAs and proteins, consistent with the observation that conditioned media from osteoblasts grown in microgravity stimulated osteoclast formation in mouse bone marrow cell cultures. In addition, IL-6 over-expression played a dual role also inhibiting osteoblast differentiation in an autocrine manner. In fact, treatment of osteoblast primary cultures with IL-6 significantly reduced their proliferation, differentiation and mineralization. Clustering the significantly modulated genes by the GOTM (gene ontology tree machine) software, which allows to identify the related biological processes, showed up-regulation of pathways involved in apoptosis, response to oxidative stress and inflammation, while pathways associated with Wnt signals, extracellular matrix production and cell growth were down-regulated. In conclusion, we believe that our global transcriptome analysis could contribute to shed light on the mechanisms affecting bone mass in unloading conditions and to identify new targetable molecules to prevent and/or cure bone pathologies.
Conflict of Interest: None declared

Mo-P147

CYCLICAL COMPRESSIVE LOADING INDUCES CHANGES IN CELLULAR BEHAVIOUR AND ATP RELEASE FROM OSTEOBLASTS GROWN ON 3-DIMENSIONAL SCAFFOLDS

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Extracellular nucleotides signalling via P2 receptors are important regulators of cellular processes in numerous tissues including bone. We have shown that multiple P2 receptors are expressed by bone cells and that extracellular ATP is released into the bone microenvironment via a non-lytic event modulating many functions of osteoblasts and osteoclasts. However, the exact mechanism by which ATP is released is still unknown. The aim of this study was to determine which type of mechanical stimulation induces ATP release from osteoblasts. SaOS-2 and Te85 osteoblastic cells were grown in standard monolayer culture and on 3-dimensional (3-D) polyurethane (PU) scaffolds (Caligen foams, UK). Monolayer cultures were subject to mechanical loading in the form of medium displacement, whilst cell-seeded PU scaffolds were subjected to cyclical compressive loading (1Hz, 5% strain, 30 minutes) in a biodynamic chamber mounted on a Bose Electroforce

3200 materials testing machine. The amount of ATP released into the medium was measured using the luciferein/luciferase bioluminescence assay. Following stimulation, the PU scaffolds were cultured for 1 week and processed for histology and Sirius red staining. We found that Te85 released more ATP than SaOS-2 cells and that mechanical stimulation increased ATP release in both cell lines when grown in monolayer. ATP release from Te85 seeded scaffolds peaked within 2 minutes and again after 16–20 minutes of constant cyclic loading, whereas ATP release from SaOS2 cells gradually decreased upon loading. When scaffolds were subjected to repeated bouts of shorter durations of cyclic loading (1Hz, 5% strain for 2 minutes interrupted by 6 mins rest periods) only the initial loading cycle induced ATP release. Detailed histological examination of the PU scaffolds demonstrates that both cell lines attach and proliferate within the PU scaffold. Cell proliferation within the scaffold was decreased for both cell lines in response to 30 minutes constant cyclic loading, whereas collagen production increased over time but was unaffected by loading. This novel method of cell culture will enable us to determine the effect of ATP release within a 3-D environment and to determine the mechanisms of release in response to specific loading patterns.

Conflict of Interest: None declared

Tu-P148

CONTROL OF OSTEOGENESIS BY POSTTRANSLATIONAL MODIFICATION OF RUNX2

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Background: BMP-2 induces heterotopic bone formation in muscle tissue. Similarly, BMP-2 treatment blocks myogenic differentiation and induces in vitro osteoblastic transdifferentiation of premyoblastic C2C12 cells. Previous reports suggested that BMP-2-stimulated the expression of osteogenic transcription factors such as Runx2 and Osterix. These upregulations of osteogenic transcription factors are mediated by canonical Smad signaling pathway and subsequent activation of Dlx5 expression. FGF2, another osteogenic agent also strongly stimulated Runx2 expression, probably independent of Smad pathway. In this case Runx2 expression is mediated by PKC pathway activation. Besides the upregulation of Runx2 expression, we also found that posttranslational modification (PTM) of this protein is very important.

Result: Phosphorylation of the protein clearly related with Runx2 activity. Moreover, phosphorylation also modified other type of PTM such as acetylation and ubiquitination of the protein. We found that both BMP-2 and FGF2 not only increased Runx2 expression but enhanced Runx2 protein acetylation which in turn decreased Runx2 degradation by ubiquitination. Runx2 acetylation could be stimulated by histone acetyl transferase (HAT). p300 plays the HAT role for the Runx2 protein acetylation and this process is mediated by BMP activated R-Smads. The acetylated Runx2 protein can be returned to original state by histone deacetylase (HDAC). Usually acetylation and ubiquitination occurs in free lysine residues of a protein. Thus deacetylated Runx2 protein is susceptible to degradation by ubiquitination.

Conclusion: Our findings suggest that post-translational modification of Runx2 protein will be a good target to develop osteogenic agent.

Conflict of Interest: Grant/Research support: Korea Health 21 R&D project of the Ministry of Health and Welfare of ROK

Tu-P149

GLUCOSAMINE AND ITS N-ACETYL PHENYLALANINE DERIVATIVE MODULATE MRNA EXPRESSION LEVELS IN CHONDROCYTES BY AFFECTING MAP KINASE PHOSPHORYLATION

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Glucosamine (GlcN) is used in the treatment of osteoarthritis as symptomatic slow-acting drug, but its mode of action is not completely known. We analyzed the influence of GlcN and its N-acetyl-phenylalanine derivative (NAPA) on mRNA transcription level, on mitogen-activated protein (MAP) kinase phosphorylation and on Activator Protein-1 (AP-1) transcription factor activation in human chondrocytes stimulated with inflammatory cytokines, TNF-alpha and IL-1beta.

Human immortalized cell line, lbpva55, and human healthy chondrocytes were challenged with 10 ng/ml TNF-alpha or IL-1beta cytokines after pre-treatment with 2.5 or 10 mM GlcN or NAPA. mRNA expression levels were evaluated by complementary DNA microarray (cDNA microarray) and by Quantitative-Real Time PCR (Q-RT-PCR). MAP kinase phosphorylation was evaluated by Western Blotting. AP-1 transcription factor activation was evaluated by measuring AP-1 DNA binding activity.

Several genes, whose mRNA level was increased by TNF-alpha treatment and significantly reduced by GlcN and NAPA in lbpva55 cells, were identified. These include cytokine receptors TNF-R1 and TNF-R2, their associated factor TRAF-6, signaling intermediates IGFB-6 and Rnd1, as well as cell cycle regulating proteins CUL-2 and G1S protein 1.

After IL-1beta stimulation, MMP-1, -3 and -13 productions were strongly increased. Treatment with 2.5 and 10 mM GlcN and NAPA reduced the expression of these metalloproteases. mRNA expression level is regulated by transcription factors such as AP-1 complex and Nuclear Factor-kB (NF-kB). We focused our attention on AP-1 transcription factor, which is activated by phosphorylated MAP kinases. IL-1beta stimulated phosphorylation of c-jun N terminal kinase (JNK), p38 MAP kinase and extracellular-signal regulated kinase (ERK) 1/2. GlcN and NAPA inhibited JNK and p38 phosphorylation and consequently c-jun binding activity.

These results demonstrated for the first time, in human chondrocytes, that GlcN and its derivative NAPA inhibit mRNA expression level by affecting MAP kinase phosphorylation and consequently transcription factor activation.

Conflict of Interest: None declared

Tu-P150

WISP3/CCN6 INDUCES GENES RELATED TO ANTIANGIOGENESIS, CELL SURVIVAL AND INTERFERON RESPONSE IN HUMAN MESENCHYMAL STEM CELLS

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WISP3/CCN6 (wnt1 inducible signalling pathway protein 3) is an extracellular matrix associated, secreted protein. WISP3 is linked to an inherited disease, progressive pseudorheumatoid dysplasia (PPD) due to loss of function mutations in the gene. Molecular data on WISP3 functions are limited, but suggest an antiangiogenic role and a tumor-suppressive function. Previously, we described WISP3 expression in human bone marrow-derived mesenchymal stem cells (MSCs). Aim of this study was to elucidate WISP3 functions in MSCs.

MSCs from human bone marrow were isolated by plastic adherence from the femoral head of patients undergoing total hip arthroplasty. Recombinant WISP3-Fc protein was expressed in insect cells and purified using protein G sepharose. MSCs were treated with WISP3, total RNA was isolated and RT-PCR analyses were performed according to standard procedures. The global gene expression pattern was analysed using the Affymetrix Chip HG-U133 2.0 Plus. Results were additionally evaluated using the PathwayAssist software and RT-PCR.

Silver gel analysis and western blotting revealed the purity of $> 95\%$ of WISP3. The function of the protein was proven in cellular migration studies. Affymetrix array analysis identified 111 regulated transcripts (107 up, 4 down) with at least 2.0-fold change, 23 selected genes were reevaluated by RT-PCR and regulations according to the array results was found in the vast majority of cases. A group of chemokines, namely the members CXCL10, CXCL11 and CCL5 as well as members of the TNFSF gene family and a series of interferon induced genes were upregulated by WISP-3. Additional RT-PCR showed that the majority of genes responded to WISP3 treatment already after 12 hours of treatment. Additional controls using the Fc-tag revealed no influence on RT-PCR intensities.

The upregulation of the chemokines CXCL10 and CXCL11 strengthens the view of WISP3 as an inhibitor of angiogenic processes. A series of interferon induced genes were upregulated. The family members IFIT1, IFIT2 and IFIT3 and two genes of the 2́-5́-oligoadenylate synthetase act in immune response processes. Although speculative, results might provide clues for WISP3 action in vivo in cartilage. Thus the clinical manifestation of PPD due to the loss of WISP3 might be explained by a missing protective role of WISP3 via anti-angiogenic, apoptotic and interferone-responsive genes.

Supported by a grant of the Interdisciplinary Center for Clinical Research, Würzburg, Germany.

Conflict of Interest: None declared

Tu-P151

AMP-ACTIVATED PROTEIN KINASE (AMPK) PLAYS A ROLE IN OSTEOBLAST FUNCTION

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In addition to the epidemiologic studies demonstrating that osteoporosis, obesity, and type 2 diabetes are linked, there is also increasing evidence for a direct hormonal link between bone mass, food intake and energy metabolism. Adenosine 5′-monophosphate-activated protein kinase (AMPK), a regulator of energy homeostasis, has a central role in mediating the appetite-modulating and metabolic effects of many hormones and neuromodulators involved in the regulation of bone mass, including leptin, ghrelin, cannabinoids and noradrenalin, as well as antidiabetic drugs metformin and glitazones. We tested if AMPK activity is regulated in osteoblasts and whether stimulation of AMPK activity in osteoblasts plays a role their function. Two osteoblastic cell lines UMR106 and ROS 17/2.8 rat osteosarcoma cell lines, as well as osteoblasts isolated from new-born rat calvaria, were cultured in the presence of various hormones and neuromodulators. Osteoblast cell proliferation was determined by cell counting using "In Cyto" system, while osteoblast differentiation was evaluated by alkaline phosphatase activity. AMPK activity in cell lysates was measured by a functional kinase assay using SAMS, a synthetic peptide substrate of AMPK, as well as by western blotting using an antibody recognizing the AMPKα phosphorylated at Thr-172. We first confirmed that AMPK was expressed in osteoblasts. Propranolol (1 μM), a non-specific beta-adrenergic antagonist inhibits AMPK activity in osteoblasts while ghrelin has a biphasic effect, stimulatory at low concentrations (10^{-10} M, 10^{-9} M) and inhibitory at higher concentrations (10^{-7} M, 10^{-8} M). Low concentrations of ghrelin also stimulate Thr-172 phosphorylation of AMPK in osteoblasts as determined by western blotting, while propranolol inhibited Thr-172 phosphorylation. We did not show any effect of estrogens on AMPK activity in osteoblasts. We showed that AICAR, a cell-permeable activator of AMPK, dose-dependently inhibits osteoblast cell proliferation and alkaline phosphatase activity. Preliminary results using a bone nodule assay suggest that AICAR also inhibits bone formation in vitro.

These results are consistent with AMPK playing a role in osteoblast function. Further studies will determine whether activation of AMPK is involved in bone formation in vivo and mediates the effects of hormones and antidiabetic drugs on the regulation of bone mass.

Conflict of Interest: None declared

Tu-P152

DIFFERENTIAL EFFECTS OF SECRETED FRIZZLED-RELATED PROTEINS (SFRPS) ON OSTEOBLASTIC DIFFERENTIATION OF MOUSE MESENCHYMAL CELLS AND APOPTOSIS OF OSTEOBLASTS

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Secreted Frizzled-related proteins (sFRPs) are modulators of Wnt signaling. This study was undertaken for definitive assessment of contribution of different sFRPs in osteoblastic differentiation of mesenchymal progenitor cells and apoptosis of osteoblasts. Treatment of C3H10T1/2 cells with sFRP-2 at concentrations of 10, 50, and 100 nM and sFRP-4 at low concentrations (5 nM) significantly increased Wnt-3A-induced alkaline phosphtase (ALP) activities, whereas sFRP-1 or 3 did not. Retroviral transduction of the sFRP-2 but not other sFRPs also significantly enhanced ALP activity induced by β-glycerophosphate and ascorbic acid. Furthermore, transfection of all the sFRP expression vectors significantly increased β-catenin/TCF reporter activity and the effects were most prominent with sFRP-2 and -4. In osteoblast apoptosis assay, only sFRP-3 increased etoposide-induced apoptosis in MC3T3-E1 mouse osteoblasts. In conclusion, we found that different repertoires of sFRPs exert differential effects on osteoblastic differentiation of mouse mesenchymal cells and cellular apoptosis of mouse osteoblasts in vitro.

Conflict of Interest: None declared

Tu-P153

THE EXPRESSION OF THERMO-REGULATED TRP CHANNELS IN PRIMARY CULUTURED MOUSE ODONTOBLASTS

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Odontoblasts are well-polarized columnar cells at the periphery of dental pulp and responsible for the dentin formation by synthesis and secretion of collagenous and non-collagenous matrix protein as well as participating in the Ca^{2+} transporting pathway to the dentin. In addition, odontoblasts have been believed to play a critical role in the perception of fluid displacement by the changes of temperature within dentinal tubules. Several types of the transient receptor potential (TRP) family are directly related to the cellular mechanism for temperature sensing and nociception. However, the expression of thermo/mechanical-sensing TRP channels in primary cultured mouse odontoblasts is unknown. Here, we investigated the expression types of thermo/mechanical-sensing TRP channels and its function in primary cultured mouse odontoblastic cells by RT-PCR and fluorometric calcium imaging. mRNA of TRPV1, TRPV2, TRPV3, TRPV4, and TRPM3 were expressed except that of TRPM8. To confirm these channels activities, 10 μM capsaicin (a TRPV1 agonist), 500 μM 2-APB (a TRPV2 and 3 agonist), 220 mOsm hypotonic stimulus (a TRPM3 and TRPV4 agonist), and 10 μM 4-phorbol-12,13-didecanoate (a TRPV4 agonist), and 1 mM menthol (a TRPM8 agonist) were treated, respectively. Stimulation of TRPV1–4, heat receptors, and TRPM3, a mechanical receptor, revealed intracellular calcium concentration increased, but activation of TRPM8, a cold receptor, did not. These results indicate that thermo/mechanical-sensing TRP channels express in primary cultured mouse odontoblasts and these channels may have to sense thermal and mechanical stimuli.

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Tu-P154

CANNABINOID RECEPTOR 2 SELECTIVE AGONISTS STIMULATE OSTEOCLAST FORMATION IN VITRO BUT ACT AS ANABOLIC AGENTS IN VIVO BY STIMULATING BONE FORMATION

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Recent studies have shown that the endocannabinoid system plays a role in regulating bone cell activity *in vitro* and bone mass *in vivo*. We have previously reported that cannabinoid receptor agonists stimulate osteoclast formation *in vitro*, but paradoxically, the cannabinoid receptor 2 (CNR2) selective agonist HU308 has also been found to partially protect against ovariectomy induced bone loss *in vivo*. In an attempt to resolve these discrepancies we studied the effects of HU308 and the CNR2 selective agonist JWH133 on bone cell activity *in vitro* and on ovariectomy-induced bone loss *in vivo*. Both HU308 and JWH133 significantly increased M-CSF/RANKL-induced osteoclast formation in cells prepared from wild type mice with a 50% increase at 10 nM ($p < 0.001$) and a 75% increase at 30 nM ($p < 0.001$). These agents had no effect in osteoclasts generated from CNR2 knockout mice. Further studies showed that HU308 and JWH133 stimulated bone nodule formation by about 10% in wild type cultures at concentrations of 300 nM and above. Partial stimulatory effects of HU308 and JWH133 on bone nodule formation were also observed ($\sim$5% increase) in cultures from CNR2 knockout mice indicating that at these concentrations, enhancement of bone nodule formation was mediated by CNR2-dependent and -independent effects. We went on to study the effects of HU308 and JWH133 in ovariectomised wild type and CNR2 knockout mice. Both HU308 and JWH133 at a dose of 1 mg/kg/day significantly protected from trabecular bone loss ($p < 0.05$) following ovariectomy and preserved trabecular number ($p < 0.01$) and thickness ($p < 0.01$). Bone histomorphometric analysis showed that both agents increased osteoblast number by about 20%, but osteoclast numbers were unchanged. No significant effects of HU308 were observed in CNR2 knockout mice. Interestingly, both agonists increased osteoblast number in sham operated wild type mice by about 20%, consistent with the hypothesis that CNR2 agonists exert anabolic effects by promoting osteoblast differentiation. These data shown that CNR2 agonists stimulate osteoclast formation *in vitro*, but do not stimulate osteoclast formation *in vivo*. Furthermore CNR2 selective agonists enhance osteoblast differentiation *in vitro* and protect against ovariectomy induced bone loss *in vivo* by promoting bone formation. We conclude that CNR2 agonists have anabolic activity in mice raising the possibility that these compounds might be of value as new treatments for osteoporosis.

Conflict of Interest: None declared

Tu-P155

ADIPOSE TISSUE DERIVED MESENCHYMAL STEM CELLS: OSTEOGENIC DIFFERENTIATION AND INTERATION WITH NANOSTRUCTURED TI6AL4V AND TI13NB13ZR

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Background: The aim of the present study was to evaluate the effect of different nanostructured Titanium alloys on osteogenic differentiation of adipose tissue mesenchimal stem cells (AMSCs). Previous work in this laboratory has demonstrated that AMSCs have the same ability to produce bone matrix as bone marrow derived stem cells (BMMSCs) and that Ti6Al4V surfaces exhibit an osteoinductive action on AMSCs, promoting their differentiation into functional osteoblasts and increasing bone formation. In this study in vitro tests were used to assay the ability of nanostructured Ti6Al4V and Ti13Nb13Zr to promote and to maintain the osteogenic differentiation on three primary cultures of preadipocytes (PA), using polystyrene (PS) and human osteosarcoma cell line (SaOS-2) for comparison.

Methods: PA cells were seeded onto Titanium alloys or PS and cultured for up to 40 days. Cell morphology, adhesion, proliferation and differentiation were evaluated by Laser Scanning Confocal Microscopy analysis, cell counting and alkaline phosphatase (ALP) activity evaluation.

Results and conclusion: The cells display a good adhesion and proliferation on all substrates, but the presence of nanostructure reduces cell proliferation and induces differentiation of AMSCs towards a phenotypic osteoblastic lineage, in agreement with the increase of the expression of ALP activity evaluated by a cytochemical staining and a fluorometric assay. Expression of osteopontin, osteocalcin and collagen type I was evaluated. Type I collagen expression was higher in all PA cells cultured on Ti6Al4V and on Ti13Nb13Zr compared to these cultured on PS, suggesting a more efficient extracellular matrix deposition. In addition, no differences exist between the two nanostructured alloys in terms of PA cells adhesion, proliferation and differentiation. Also together these findings that the nanonization of the Titanium alloy surfaces has an osteoinductive action on AMSCs.

Conflict of Interest: None declared

Tu-P156

SPHINGOSINE-1 PHOSPHATE IS AN IMPORTANT MEDIATOR OF OSTEOBLAST DYNAMICS

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Sphingosine-1-phosphate (SPP) has recently been shown to play an important role in osteoclast-osteoblast crosstalk. SPP, released by osteoclasts, augments osteoclastogenesis via the RANK-RANKL system by acting as chemo-attractant and survival factor for osteoblastic cells.

We have used primary osteoblastic cells derived from bone marrow as well as established osteoblastic cell lines to further investigate the effect of SPP on osteoblast motility. SPP released into the serum signals via five G-protein coupled receptors, S1P1 to 5. First, we used RT-PCR to establish expression of SPP receptors in the different osteoblastic cells and then correlated their SPP receptor profile with their migratory behaviour. To this end, bone marrow derived cells from 5 day old rabbits or 2 day old rat pups were cultured on dentine slices or glass-bottom Petri dishes in MEM medium supplemented with either 0.1–10 microM SPP or vehicle. Osteoblastic cells were identified by FastRed staining for alkaline phosphatase. To directly measure stromal cell motility, cells settled on glass-bottom dishes were allowed to adhere for 2 h in medium containing 10% FCS before exposure to SPP for 30 min to overnight.

Rabbit bone marrow cells express S1P1 and S1P2 whereas rat bone marrow cells express S1P1–3. Time course experiments showed that the SPP treated cells were less well spread and dissociated from the bone surface after 6 h of culture. Although the effect on the rat bone marrow cells was less dramatic, cells derived from both species showed a biphasic motility behaviour upon chronic exposure to SPP. The initial response of the cells SPP is an increase in motility, which is rapid and not sustained and followed by a marked decrease in cell motility after overnight treatment (36% for rat cells and 29% for rabbit cells compared to control). This decrease in motility was only observed after sustained (overnight) exposure to SPP, whereas when cells were treated when settling this interferes with cell adhesion to the substrate.

Our results clearly indicate that SPP plays an important role in modulating osteoblast behaviour. The length of exposure to SPP can dramatically alter cell motility. In this light, short bursts of SPP may act at the initial phases of resorption to induce osteoblast retraction, whereas at later stages of resorption, a sustained release of SPP may reduce osteoblast motility and induce cell proliferation.

Conflict of Interest: None declared

Tu-P157

AKT MEDIATED BETA-CATENIN ACTIVATION IN RESPONSE TO MECHANICAL STRAIN IN OSTEOBLASTS IS DEPENDENT ON IGF AND ESTROGEN RECEPTOR ALPHA BUT NOT PROSTAGLANDINS

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The ability of normal bone to regulate both mass and architecture in response to the prevailing load serves to prevent fracture. One pathway which has been implicated in the adaptive response of bone to load is the canonical Wnt pathway wherein engagement of the Wnt receptor results in inhibition of GSK3-β phosphorylation of the nuclear transcriptional regulator β-catenin, thereby allowing its nuclear translocation. Since AKT is also capable of regulating β-catenin via GSK3-β inhibition, we hypothesised that AKT may also be involved in mediating the strain response in osteoblasts. UMR106 rat osteoblast like cells were subjected to 4-point bending in vitro and protein expression examined. Western blotting demonstrated a transient increase in the expression of phosphorylated AKT (active) and GSK3-β (inactive) as well as hypo-phosphorylated β-catenin (active). To identify the mechanisms responsible for AKT activation, UMR106 cells were treated with autocrine growth factors known to mediate the strain response, namely insulin like growth factor-1 (IGF1), prostaglandin E2 (PGE2) and I2 (PGI2) as well as the NO donor SNAP. Whilst PGE2 resulted in a mild activation of β-catenin, there was no effect on AKT or GSK3-β, with only IGF-1 being capable of activating AKT and β-catenin in UMR106 cells. Blocking of IGF signalling with the IGF1R antagonist H1356 also prevented AKT mediated β-catenin activation by mechanical strain. Similarly, mechanical strain failed to stimulate AKT mediated β-Catenin activation in osteoblasts isolated from the long bones of ERα knockout mice, or UMR106 cells treated with the ERα inhibitor ICI 192780. Mechanical strain mediated activation of β-catenin signalling by AKT mediated inactivation of GSK3-β was blocked by the AKT inhibitor API-2. Similarly the mechanical strain induced nuclear translocation of β-catenin and β-catenin dependent transcriptional activation of an osteopontin promoter construct containing a Lef/TCF binding site were both inhibited by API-2 in UMR106 cells. Taken together these data suggest that AKT is a key mediator of the response of osteoblasts to mechanical strain, whose activation is dependent on both IGF and ERα signalling, and which serves to propagates the strain response by stimulating β-catenin and activating Lef/TCF transcription. However strain-related activation of AKT, and its subsequent effect on β-catenin, appears to be independent of prostaglandins or nitric oxide.

Conflict of Interest: None declared

Tu-P158

HEY1 REGULATES BONE MASS AND CARTILAGE HYPERTROPHY BY LINKING BMP SIGNALING WITH THE PTH RECEPTOR

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Hey1 transcription factor is strongly up-regulated upon BMP-2-induced osteoblastic differentiation in vitro. We examined the in vivo role of Hey1 in bone metabolism by analyzing mice deficient in, or overexpressing Hey1. Mice deficient in Hey1 showed almost no bone phenotype in vitro and in vivo. By contrast, Hey1 overexpression resulted in progressive low turnover osteopenia, predominantly from reduced osteoblast generation and performance in vitro and in vivo. PTH receptor, but not Notch or Wnt signaling, was found to mediate induction of Hey1 by BMP-2. Hey1 transgenics displayed increased collagen X expression and enlarged hypertrophic zone in the growth plates. Our data suggest that Hey1 has a dual role in chondro-osteolineage in bone: it is inhibitory for osteoblast function and stimulatory for chondrocyte hypertrophy. Thus, regulation of Hey1 by BMP via PTH receptor provides a link between these two pathways, both of which are active on cartilage and bone.

Conflict of Interest: The authors are employees of Novartis Pharma AG.

Tu-P159

EXTRACORPOREAL SHOCK WAVES ACTION ON MURINE OSTEOBLASTS

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Extracorporeal shock waves (ESW) have recently been used in orthopaedic treatments to induce bone repair, but their mechanisms of action are not sufficiently investigated. We studied the effect of shockwaves on murine osteoblastic cells in order to clarify the pathways of their responses and to determine suitable treatment settings. Osteoblast cultures were subjected to a single shockwave with combinations of low energy intensities (0.05 mJ/mm2) and 500 number of shocks (impulses), whereas control cells received no treatment. We found an immediate negative effect on cell viability, that occurs with an increase of Bax protein expression after 3 hours of treatment. After a longer time lapse a stimulatory effect on cell proliferation, as reflected by the increase of a G(1)-S phase marker, was observed. In fact, in the following 24, 48 and 72 hours after ESW treatment, we found a stronger association of Cyclin E2 and Cdk2, forming active cyclin E-Cdk2 kinase, compared to untreated cells at the same times.

We further explored the molecular mechanism for the ESW induction of osteogenesis: by Real Time PCR an enhancement of the osteogenic transcription factor (Runx2) mRNA, evident 48 hours after the treatment, was found. A link between physical ESW and core binding factor A1 activation has been already demonstrated (1). ESW-induced O2- production, followed by tyrosine kinase-mediated ERK activation and Runx2 activation, resulted in osteogenic cell growth and maturation. We also analyzed osteoblast expression of the cytokines RANK-L and OPG, regulating osteoclastogenesis. In Real Time PCR, both decreased after 24–48 hours following treatment, as expected considering the increase of osteoblast proliferation rate during this phase, but a later strong increase of OPG, higher than the simultaneous moderate rise shown by RANK-L. Thus, the RANK-L /OPG ratio decreases and can lead to a reduced osteoclastogenesis. This effect could probably explain one of the mechanism of ESW repair action on bone.

1. F.S. Wang et al. JBC, 277: 10931–10937, 2002.

Conflict of Interest: None declared

Tu-P160

VITAMIN A DEFICIENCY DELAYS HEALING PROCESS AFTER CORTICAL BONE AND BONE MARROW INJURY

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The necessity of vitamin A in chondrogenesis and generation of limbs has been reported. The role of vitamin A in bone regeneration has not been elucidated. Using three groups of 10-week-old male C57BL/6J mice, we investigated the effects of vitamin A deficiency on healing process after cortical bone and bone marrow injury. One was vitamin A deficiency (VAD) mice group, which was fed the diet without vitamin A from 10 day of gestation to the end of the experiments. Another was deficiency-sufficiency (VADS) mice group, which was fed the diet without vitamin A from 10 day of gestation until weaned and thereafter fed the standard diet. Last one was sufficiency (VAS) mice group, which was fed only the standard diet. We made drill-hole injury, 1 mm in diameter, at the anterior portion of the diaphysis of bilateral femurs. In vivo micro CT was performed for sequential analysis to measure the amount of regenerating bone in 5 mice of each group. The femurs were also harvested at 7, 14, 21 days after operation to do histomorphometrical and quantitative mRNA analysis of the injured site.

Regenerating bone was observed from 7 day after operation in VAS and VADS mice, and the cortical bone defects appeared to be almost healed until 28 day, while those defects in VAD mice were still apparent at 28 day. In histomorphometrical analysis, regenerated cortical bone volume in the drill-hole area in the VAD mice was lower than that in the VAS at each time point. Osteocalcin, Osterix, and Collagen-• mRNA expression in VAD mice apparently decreased compared with VAS mice at day 7.

These results clearly demonstrated that vitamin A deficiency suppressed cortical bone healing in association with the impairment of osteoblast maturation after cortical bone and bone mallow injury.

Conflict of Interest: None declared

Tu-P161

APICAL MICROVILLI OF OSTEOBLAST-LIKE SAOS-2 CELLS AS PRECURSORS OF CALCIFYING MATRIX VESICLES: A COMPARATIVE PROTEOMIC STUDY

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Physiological mineralization occurs during the fetal and early postnatal formation and development of bones and teeth. It continues throughout the life during the remodeling and repair of bone [1]. Hypertrophic chondrocytes, osteoblasts and odontoblasts from embryonic or growth plate cartilages, bones and teeth, respectively, initiate the mineralization process by releasing matrix vesicles (MVs) from their specialized areas of plasma membrane. These extracellular organelles are involved in the initial step of mineralization by promoting the formation of crystals of hydroxyapatite (HA) in their lumen [2]. Despite growing knowledge about the morphology and the functions of MVs, their biogenesis are not well understood. The purpose of this work was to determine the site of origin of MVs in osteoblast-like Saos-2 cells. Microvilli were isolated from the apical plasma membrane of Saos-2 cells and their proteome was compared with that of MVs. Respectively, 268 and 250 gene products were identified in microvilli and in MVs by mass spectrometry, and 162 of these gene products were common. More than 80% of MV membrane associated proteins were similar to those of microvillous membrane. Several MV protein markers were identified in microvilli such as Na + /K + ATPase, Ca2+ ATPase, tissue non specific alkaline phosphatase, Annexin A2, AnxA5, AnxA6, lactate dehydrogenase A and B. Furthermore, cytoskeletal markers of microvilli were present in MVs such as actin, ezrin, radixin, moesin, talin 1, transgelin 2 and other proteins including Ras-related Rab1B and Rab7, ADP-rybosylation factor 1 and 4, transferrin receptor protein 1 and 4F2 cell surface antigen. This comparative proteomic analysis suggests that apical microvilli of Saos-2 cells are the precursors of MVs.

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Conflict of Interest: None declared

Tu-P162

COLLAGEN CROSS-LINKING INFLUENCES OSTEOBLASTIC DIFFERENTIATION

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Osteoblasts synthesize a collagen matrix, which itself regulates the differentiation of precursor cells into mature osteoblasts. These cells also express lysyl oxidase (LOX), an enzyme involved in the collagen cross-linking process. Lathyrogens, like ß-aminopropionitrile (ßAPN), inhibit the formation of a stable matrix.

The aim of the present study was to investigate the influence of cross-linking on osteoblastic differentiation. MC3T3-E1 cells were seeded at a density of 20,000 cells/ cm^2 and cultured in α-MEM supplemented with 5% FCS, ascorbic acid, and treated without or with 400 μM ßAPN for one week. Thereafter, cells were removed by desoxycholate (0.5%) and onto this extracellular matrix (ECM), new MC3T3-E1 cells were seeded and cultured for one week as described above, without ßAPN. RNA was isolated and expression of specific marker genes was determined by RT-PCR. Changes in specific cross-links after ßAPN treatment were measured with Fourier-transform infrared spectroscopy (FTIR).

Compared to untreated cells, ßAPN treatment showed no visible changes in the appearance of the cells. The collagen matrix formed showed a reduction of two of the major cross-links of bone collagen, namely deH-DHLNL and pyr, compared to the matrix of non-treated cultures. Gene expression studies resulted in an increase of Collagen (I) alpha I (COL1A1). LOX and osteocalcin (OCN) showed a concomitant reduction of mRNA expression. When fresh MC3T3-E1 cells were seeded on this altered matrix in the absence of ßAPN, COL1A1 mRNA expression was upregulated, OCN downregulated, while LOX mRNA expression remained unaffected compared to control cultures.

These results indicate that ßAPN treatment not only disrupts collagen cross-link formation and maturation, but also affects osteoblastic activity and expression.

In conclusion, the disrupted matrix produced in the presence of the well-known lathyrogen has, even in its absence, a lasting effect on the expression of osteoblastic genes in new generations of cells.

Conflict of Interest: None declared

Tu-P163

MICE LACKING THE EPITHELIAL CALCIUM CHANNEL TRPV4 HAVE INCREASED BONE MASS AS A CONSEQUENCE OF ALTERED FUNCTION OF MULTIPLE BONE CELL TYPES

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We recently showed that the epithelial calcium channel TRPV5, a member of the TRP superfamily is crucial for proper osteoclastic bone resorption. TRPV6, a close homolog within this family, seems to be important during the mineralisation process mediated by osteoblasts. A third member of this family, TRPV4, was shown to respond to an array of stimuli, including osmolarity, heat, pH, temperature and pressure. Only very recently, it was postulated that TRPV4 deficiency leads to reduced sensing of mechanical stimuli. In this study we explored the role of TRPV4 in bone.

Real-time PCR studies demonstrated that TRPV4 mRNA is abundantly expressed in both osteoblasts and osteoclasts. μCT analyses showed an increased cortical and trabecular bone mass (thickness and volume) in TRPV4 null mice compared to wildtype mice, which was partly explained by increased femoral length. Bone marrow cultures from TRPV4 knockout bone marrow had reduced osteoclast number (TRAP staining) and less resorption pits (coomassie brilliant blue staining) after stimulation with MCSF and RANKL, indicating reduced osteoclastic activity. In contrast, osteoblast differentiation (alkaline phosphatase activity) and matrix mineralisation (alizarin red) were increased in TRPV4 knockout bone marrow cultures compared to wildtype cultures.

In conclusion, the current study demonstrates the involvement of an additional TRPV family member in bone metabolism. TRPV4 function is needed for proper development and function of both osteoblasts and osteoclasts. The apparent uncoupling of osteoclast and osteoblast function corroborates well with the in vivo high bone mass phenotype of mice lacking TRPV4.

Conflict of Interest: None declared

Tu-P164

IDENTIFICATION OF AN MGP/OC HYBRID GENE IN THE ADRIATIC STURGEON (ACIPENCER NACCARII), AN ANCIENT BONY FISH WITH A CARTILAGINOUS ENDOSKELETON

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Osteocalcin (OC) and Matrix Gla Protein (MGP) are members of the vitamin K-dependent protein family and play essential roles in controlling tissue mineralization. OC is a 5.6 kDa protein synthesized by osteoblasts and odontoblasts and is generally accepted to be specific for vertebrate calcified tissues. MGP is a 10 kDa protein produced and secreted mainly by vascular smooth muscle cells and chondrocytes and known to accumulate in bone, cartilage, and dentin from mammals, amphibians, cartilaginous and bony fishes. Conserved features between OC and MGP (transmembrane signal peptide, γ-glutamyl carboxylation recognition site and C-terminal Gla domain) and a similar gene structure, indicate a common ancestral origin but each of them also presents specific features: a propeptide in OC, removed after cleavage at the furin site, a phosphorylation domain and ANXF cleavage site in MGP.

Sturgeons are representative of ancient fishes possessing mainly cartilaginous skeleton, with absence of a calcified endoskeleton, suggesting a different mode of calcium homeostasis in these organisms. We present here the identification of a MGP-OC hybrid protein in sturgeon, combining features of both proteins, including a signal peptide, followed by a putative phosphorylation domain, a γ-glutamyl carboxylation recognition site, and ANXF-like and furin cleavage sites. Extraction and purification of OC from adult sturgeon branchial arches, through anionic exchange chromatography, allowed N-terminal identification of the mature protein, after the furin cleavage site. The C- terminal moiety contains the Gla domain, with three confirmed Gla residues, and the two conserved cysteines typical of a regular OC. Alignments of this ORF with known OC and MGP proteins confirmed homology of the N-terminal part of the protein, with MGPs, particularly with the shark protein, while remaining C-terminal features show higher homology with OCs. Moreover, the

broad range of tissue distribution obtained by real time PCR and the identification by in situ hybridization of osteoblasts and chondrocytes as target cells expressing this gene, suggests that this newly discovered protein represents an ancestral form of OC, which may have arisen after genome duplication from an ancestral MGP gene. These exciting results may provide new insights towards understanding the evolutionary relationship of these two proteins.

CSB Viegas is the recipient of a PhD fellowship SFRH/BD/9077/2002 from Portuguese Foundation for Science and Technology.

Conflict of Interest: None declared

Tu-P165

DIRECT EFFECT OF CELIAC PATIENTS SERA ON HUMAN OSTEBLAST LIKE-CELLS

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Background: Untreated celiac disease is associated with a low bone mass phenotype that is not necessarily linked to negative calcium balance and secondary hyperparathyroidism both in adults and in children. It is therefore likely that circulating factors are directly involved in the pathogenesis of the bone mass loss and or low acquisition. Methods and results:We have therefore exposed primary cultures of osteoblast-like cells (hOB) derived from normal human bone to the sera of untreated prepubertal patients with celiac disease, aged 8.5 $\pm$ 3.0 yrs, and to the sera of age-matched healthy controls. The sera of the patients induced a significant increase in hOB proliferation compared to controls. In the conditioned media obtained from hOB treated for 24 h with patients sera we observed a significant enhancement of the osteoprotegerin production, whereas the level of procollagen type I levels (PINP) remained unmodified.

The observation that osteoblasts express tissue transglutaminase (tTG) which is involved in mineralization, cell adesion and differentiation of these cells led us to raise the hypothesis that bone cell effects observed could be due to tTG autoantibodies-osteoblasts interaction. We have therefore shown that hOB display a positive staining for tTG, both in permeabilized and non permeabilized cells, indicating the presence of cytoplasmic and membrane exposed tTG. Similar staining pattern was obtained by incubating the cells with sera of patients. The staining was reduced to background signal in cells incubated with sera of healthy subjects, suggesting that an IgA present in the patients' sera is able to interact with hOB.

Conclusion: This study has shown that circulating factors in the sera of celiac patients have a direct effect on osteoblast proliferation and activity, potentially linked to the presence of IgA antibodies, that might contribute to the altered bone mass phenotype of celiac disease.

Conflict of Interest: None declared

Tu-P166

BONE MORPHOGENETIC PROTEIN-6 (BMP-6) IS AN ENDOGENOUS MEDIATOR OF BONE FRACTURE REPAIR

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Patients with osteoporosis often suffer from a delayed fracture healing. Osteoporotic bones lack BMP-6 and systemically administered BMP-6 restores bone volume in osteoporotic rats (Simic et al, J Biol Chem, 2006). In this study we tested the role of BMP-6 in the signaling cascade that governs fracture repair. Wild type (WT) and Bmp6 −/− mice were ovariectomized (OVX) and left for 6 weeks to allow the development of osteopenia when the left femurs were fractured (Fx) and immobilized by an intramedullary rod. Animals were divided into the following groups of 10 each: (1) WT sham, (2) WT OVX, (3) Bmp6−/− sham and (4) Bmp6 −/− OVX. Although OVX reduced the trabecular bone volume (BV) of WT mice by 52%, trabecular bone was not lost in Bmp-6 −/− mice, suggesting a specific requirement of BMP-6 in Bmp-6−/− mice. Three weeks following Fx, microCT analyses showed a 41% higher callus volume in Bmp6 −/− mice as compared to WT animals, implicating a delayed fracture healing. Although OVX of Bmp-6 −/− mice did not decrease the trabecular BV prior to Fx, it affected the healing of Fx by increasing the callus volume by 16% as compared to Bmp-6 −/− sham mice. At 3 weeks, OVX had no effect in WT mice on the Fx healing. The delay of the reparative response in Bmp-6 −/− long bones observed by μCT was paralleled at the cellular level by the reduced number of osteoblasts (38%) and osteoclasts (69%), and by the decreased bone formation rate (28%). Analyses of serum bone turnover markers subsequently showed reduced levels of C-telopeptide (23%), osteocalcin (24%) and osteoprotegerin (28%). Although mesenchymal progenitors capable of carrying out the repair process were present in Bmp-6 −/− bones, they remained undifferentiated in the absence of BMP6, as demonstrated by reduced Runx2 and osterix expression. Femurs of Bmp-6 −/− mice had a decreased expression of collagen type I, II, X, osteocalcin and alkaline phosphatase indicating a reduced bone repair. Mesenchymal cells present in femurs of Bmp-6 −/− mice had increased levels of BMP receptors, suggesting they are primed to differentiate but lack the appropriate BMP signal. Increased expression by RT-PCR of BMP-2, -4 and -7 (by 2.5–6 fold) in femurs of Bmp-6 −/− mice strongly implicate BMP-6 in the pathogenesis of delayed fracture healing in BMP-6 −/− mice. These results identify BMP-6 as an endogenous mediator in healing of acute bone fractures.

Conflict of Interest: None declared

Tu-P167

Abstract withdrawn

Tu-P168

NOVEL BIOMARKERS IN THE PLASMA OF PATIENTS WITH A BONE FRACTURE

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Following bone fracture various growth factors, cytokines and their potential receptors are active at the fracture site. To characterize their potential appearance in patients' blood we analyzed the plasma of 25 patients with an acute bone fracture following affinity plasma purification, SDS gel electrophoresis and liquid chromatography—tandem mass spectrometry. 213 non-redundant proteins were identified in the in-gel analysis of plasma proteins. Twelve proteins were potentially related to bone and cartilage metabolism and several have

not been previously identified, including: TGF-β induced protein IG-H(3), cartilage acidic protein 1 (CRTAC-1), procollagen C proteinase enhancer protein and TGF-β receptor III (TGF-β rIII). Next, we tested the relevance of TGF-β rIII and CRTAC1 as candidate biomarkers for monitoring the outcome of bone and cartilage healing following injury. Plasma samples of 30 patients (24–67 years of age) with a single tibial fracture were collected within 1, 3 and 7 days, and then at 2, 6, 10, 14, 18 and 24 weeks following fracture. A physical examination and radiographs were completed to assess the evidence of a bone union, and at 24 weeks 27 fractures were pronounced as healed by two independent radiologists, while 3 patients had a non-union. Six patients had an additional injury of the calcanear joint cartilage based on the nuclear magnetic resonance images. Specific human peptide antibodies against TGF-ßrIII and CRTAC-1 were raised in rabbits. We then developed an ELISA assay to measure their concentration in the patients' plasma throughout the follow-up period. In patients with a normal bone healing TGF-ßrIII increased immediately after the fracture and reached the maximum value at week 10 declining then gradually towards week 24. In 3 patients with a non-union the concentration of TGF-ßrIII was decreased by 30% throughout the period of 24 weeks. However, the concentration of CRTAC-1 was increased 3 fold in 6 patients with the articular cartilage injury and remained high until week 8 when it gradually decreased.

We suggest that following bone and cartilage injury novel molecules circulate in human plasma, among which TGF-ßrIII has a prognostic value in bone fracture rapair, while CRTAC-1 might become a novel biomarker for following joint cartilage injury and repair.

Conflict of Interest: None declared

Tu-P169

CIRCULATING BMP-1 ISOFORMS: NOVEL DIAGNOSTIC AND THERAPEUTIC CHALLENGES FOR BONE FRACTURE REPAIR

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Following organ injury different bone morphogenetic proteins (BMPs) are expressed in bone (BMP-2-7), kidney (BMP-7) and liver (BMP-9) recapitulating developmental regenerative processes. We hypothesized that during organ regeneration specific BMPs may be released into the circulation. Plasma from normal individuals and from patients with a long bone fracture, chronic renal failure (CRF) and liver cirrhosis were collected, purified and all protein bands from SDS gel electrophoresis have been analyzed by liquid chromatography—tandem mass spectrometry. Surprisingly, only small amounts of mature BMP-6 have been detected in healthy individuals, and GDF-15 was found in patients with CRF. However, BMP-1-3 isoform of the BMP-1 gene, a procollagen C-protease, circulates in healthy individuals and in patients with CRF. BMP-1-3 has not been previously detected at the protein level, while BMP-1-1, originally isolated with other BMPs from the bone matrix, does not circulate. As there is no information available on the function of BMP-1-3 isoform we cloned, expressed and purified the protein and raised specific polyclonal neutralizing antibodies for in vitro and in vivo experiments. BMP-1-3 isolated from plasma is active in processing the procollagen I, lacks the prodomain, and disappears from the plasma following a fracture in men and rats, in which I125 labeled BMP-1-3 accumulates at fracture bone ends and in the bone callus. Rats with a fractured femur injected intravenously with BMP-1-3 (25 μg/kg 5 × weekly for two weeks) had 156% bigger callus than control animal, and 96% bigger callus than rats injected with a similar amount of rhBMP-6. Interestingly, in rats with fractured femur inhibition of the circulating BMP-1-3 with an iv injected BMP-1-3 neutralizing antibody (12 μg/kg 2x weekly for two weeks) significantly delayed the healing suggesting that the presence of BMP-1-3 in blood is essential for the bone repair. In the rat calvariae assay addition of BMP-1-3 mediated the release of BMP-2 and -4 into the culture medium, which further stimulated the synthesis of procollagens I and III. In rabbits with an ulnar critical size defect BMP-1-3 in a collagen carrier stimulated the rebridgement of the defect, while the bone did not heal in control animals. In conclusion, we discovered that BMP-1 isoforms, and not BMPs, circulate in humans at a physiologically relevant level and affect bone regeneration, providing both novel diagnostic and therapeutic challenges.

Conflict of Interest: None declared

Tu-P170

CP-533,536 ENHANCES BONE HEALING IN A RABBIT HIP FRACTURE

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Hip fracture occurs most commonly in osteoporotic patients with more than 300,000 hip fractures occurring each year in America and Europe. Hip fractures are associated with high morbidity and mortality rates. These fractures are treated surgically or by hip replacement. Recently, an agonist of the EP2 receptor of prostaglandin E2, CP-533-536-02, was efficacious in preclinical models of regeneration of critical size bone defects and acceleration of long bone fractures. The effects of CP-533,536 have been reported in diaphyseal fractures but not metaphyseal fractures such as hip fractures. We developed a hip fracture model in the rabbit to assess the ability of CP-533,536 to enhance healing in a metaphyseal fracture. In New Zealand rabbits ($n = 24$) a 3 mm wide trochanteric surgical fracture was created using an electrical saw while maintaining intact muscle insertions. The surgical fracture was stabilized with an intramedullary pin and two nylon bands or a robust osteosynthesis screw. During surgery under aseptic conditions fractures ($n = 8$/group) were stabilized and untreated (standard care of treatment); stabilized and treated with the PLGH carrier alone; and PLGH carrier with either 4 or 40 mg of CP-533,536. The healing was then monitored by X-ray biweekly up to 22 weeks post surgery. Upon termination of the study, both femurs were removed and submitted to microCT analysis and biomechanical testing. Results indicated that fractures treated with the low dose of CP-533-536-02 at all time points showed enhanced healing compared to both the PLGH or untreated groups. In the two groups treated with CP-533, 536 animals treated with 4 mg of CP-533,536 showed healed faster compared to the 40 mg group. MicroCT analyses in the 4 mg group, showed an increase bone volume by 34% at 4 weeks, and 48% at 16 weeks following surgery. The biomechanical testing at 22 weeks showed that in the 4 mg group, the maximal breaking strength in a three point bending test was similar to the intact femur while the indentation test indicated a similar amount of trabecular bone in the control and treated femurs. These results indicate that CP-533-536-02 accelerated bone healing in a hip fracture model in rabbits and suggests that CP-533,536 is able to enhance bone healing in both metaphyseal and diaphyseal bone sites.

Conflict of Interest: V. Paralkar, T. Brown and D. Thompson are employees of Pfizer

S. Vukicevic, Pfizer, Grant Research Support

Tu-P171

EFFECT OF ENDOGENOUS TSH ON SERUM BONE REMODELING PARAMETERS IN THYROIDECTOMIZED WOMEN

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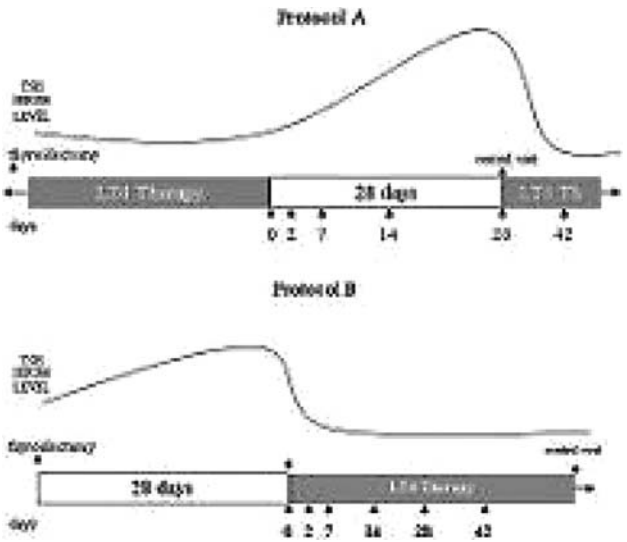
We recently reported that TSH directly affects bone volume in osteoporotic rats (Sampath TK et al., J Bone Miner Res, 2007). In addition, a single super-physiological dose of TSH (Thyrogen®) increased the bone formation and reduced the bone resorption in euthyroid postmenopausal osteoporotic patients with low endogenous TSH levels (Mazziotti G et al., J Bone Miner Res, 2005). In this study we evaluated the effect of low and high endogenous TSH levels in 11 women (29 to 64 years of age) with a total thyroidectomy due to the thyroid cancer by measuring serum osteocalcin, C-telopeptide (CTx), bone alkaline phosphatase, 1,25(OH)2 vitamin D3, PTH, calcium and phosphate values. For this purpose we monitored for a period of 28 days 6 women with a high endogenous level of TSH, due to stopped L-thyroxin suppressive treatment (protocol A), and 5 women with low endogenous TSH who underwent L-T4 suppressive therapy (protocol B) (Figure 1). In women from protocol A, TSH gradually increased from 0.6 mU/L on day 0 to 147.8 mU/L on day 28 ($P < 0.01$) with a progressive suppression of serum osteocalcin from 22.1 μg/L on day 0 to 13 μg/L on day 28 ($P < 0.056$), and serum CTx from 0.305 μg/L on day 0 to 0.21 μg/L on day 28. In women from protocol B, TSH gradually decreased from 94.8 mU/L on day 0 to 10.3 mU/L on day 28 with an increase of osteocalcin from 9.28 μg/L on day 0 to 16.29 μg/L on day 28, as well as CTx from 0.11 μg/L on day 0 to 0.19 μg/L on day 28. There were no changes in the serum levels of bone specific alkaline phosphatase, 1,25 (OH)2 vitamin D3, PTH, calcium and phosphate in neither women from the protocol A nor B. We conclude that, unlike exogenously administered TSH, high endogenous levels of TSH did not enhance the bone formation serum parameters, while TSH' effect on suppressing the bone resorption could not be separated from the effect of the thyroid hormone deficiency.



Conflict of Interest: RA Sendak, JM McPherson, KT Sampath, Genzyme Corporation, Grant/Research Support.

Tu-P172

PURIFICATION OF HOMODIMERIC RECOMBINANT BONE MORPHOGENETIC PROTEIN 6

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Bone morphogenetic protein 6 (BMP-6) is a growth and differentiation factor within the TGF-beta superfamily and induces new bone formation both when implanted locally and administered systemically (Simic et al, JBC, 2006). We developed a practical method of BMP-6 purification after establishing a stable producer CHO cell line by lipofectamine transfection. Harvest media were processed over Cobalt-IMAC resin from which the soluble form of BMP-6 was eluted, comprising a dimer of pro-domains and TGF-beta domains. This soluble form of pro-BMP-6 showed biological activity in the alkaline phosphatase assay using C2C12 cells. In order to isolate the mature TGF-beta domain we subjected the soluble BMP6 to ion exchange chromatography on either DEAE or Heparin-sepharose (Hi-Trap) in urea containing buffer (4 M or 6 M). Surprisingly, mature dimeric BMP6 was found eluting from Heparin resin (HiTrap) in low salt whereas additional forms of BMP-6, including immature BMP-6 were bound more tightly, eluting with 1M NaCl. Similar results were found using DEAE Sepharose. The immature BMP-6 in the more tightly bound fraction appears to contain at least one chain of uncleaved pro- and mature domain. On the other hand, the weakly bound mature form of BMP-6 may be N-terminally truncated, lacking its heparin binding site. We also used another method for isolating the mature domain without the pro-domain, namely the limited digestion with trypsin which results in destruction of the pro-domain leaving behind a biologically active BMP-6 dimer. The mature BMP-6 preparation was used to induce new bone formation in the rat ectopic bone formation bio assay.

Conflict of Interest: None declared

Tu-P173

COMPARISON OF THE OSTEOINDUCTIVE POTENTIAL OF DIFFERENT BONE GRAFTS

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Introduction: Different grafting materials for the filling of large bony defects are used in clinic. In a previous study we quantified different growth factors in different bone grafts [Wildemann et al. 2007a;b]. Aim of the present study was the comparative analysis of their osteoinductivity in vitro.

Material & Method: Spongiosa & cortical allografts and demineralized bone matrix (DBM) were provided by the tissue bank. The material was sterilized with the peracetic acid-ethanol procedure (PES) [Pruss et al. 2001]. Unprocessed spongiosa was also analyzed. DBX Putty (Putty, Synthes) is a combination of DBM with sodium hyaluronate carrier ($n = 5$). AlloMatrix Putty (AMP, Wright) is DBM mixed with a calcium sulphate carrier and carboxymethylcellulose. All materials were received from 5 different donors. Cell culture: C2C12 cells were cultivated in DMEM. Grafting materials were

placed into the culture wells with cell culture inserts (Nunc) and the cells were cultured for 6 days. After this period, the grafts were removed and cultured for further 6 days with C2C12 cells. Positive control: rhBMP-2, Negative control: cells only. Analysis: Cell vitality: AlamarBlue Assay (Biozol). Alkaline phosphatase (AP): p-NPP (Sigma). Statistical Analysis: Kruskal-Wallis, Mann-Whitney, Bonferroni-Holm.

Results: The highest osteoinductivity was seen for BMP-2 by significantly enhanced AP-activity. The five different samples from each grafting material showed individual differences which are donor related. For PES processed spongiosa from the tissue bank, 2 of the 5 materials had a negative effect on the AP-activity in the first incubation period. The incubation for further 6 days, however, revealed no negative effect of these samples. A significant enhancement of osteogenic differentiation (AP) was seen for the two commercial DBM-products (DBX putty and AlloMatrix). The PES sterilized spongiosa showed a significant reduction in the AP-activity. No effect on AP-activity of the different materials was seen in the second incubation.

Discussion: The results of the present study showed an osteoinductive potential of the analyzed commercial available DBM preparations, with a donor dependent variability. The PES sterilized allografts or the native spongiosa, however, revealed no osteoinductive effect. Important for the use in vivo is beside the osteoinductivity also the osteoconductivity and further comparative studies are necessary.

Conflict of Interest: B. Wildemann, BMBF, Grant Research Support

Tu-P174

HYPO-OSMOLALITY INDUCED MECHNICAL STRESS INCREASES RANKL EXPRESSION BY ACTIVATING TRPM3 AND TRPV4 IN PRIMARY CULTURED OSTEOBLASTIC CELLS

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RANKL, a member of tumor necrosis factor family, plays an essential role in osteoclastic differentiation to precursors of osteoclast and in osteoclast activation and cell survival. On the other hand, bone remodeling is also regulated by mechanical stresses such as fluid shear stress, or hypo-osmotic pressure, which induce increases in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in osteoblasts and change the function of osteoblasts. However, the mechanism of osmolality-induced Ca^{2+} response and effect of osmolality on RANKL expression in osteoblastic cells are not known. In the present work, RANKL expression and Ca^{2+} signaling with the changes of osmotic pressure and the type of Ca^{2+} channel regulated by osmolality in osteoblastic cells were investigated using RT-PCR, $[Ca^{2+}]_i$ measurement, and RNA interference. Hyper-osmolality reduced $1\alpha,25(OH)_2D_3$-induced RANKL expression, and hypo-osmolality itself increased RANKL expression. $1\alpha,25(OH)_2D_3$-induced $[Ca^{2+}]_i$ increases were blocked by hyper-osmolality. In contrast, $[Ca^{2+}]_i$ was increased by hypo-osmolality, and then it was a reversible response. Removal of extracellular Ca^{2+}, or treatment with Gd^{3+}, a non-specific blocker of plasma Ca^{2+} channel, inhibited hypo-osmolality-induced $[Ca^{2+}]_i$ increases, suggesting that hypo-osmolality-induced $[Ca^{2+}]_i$ increases are evoked from Ca^{2+} influx across the plasma membrane. Ruthenium red, an inhibitor of TRPV4, and 2-APB, an inhibitor of TRPM3 partially inhibited hypo-osmolality-induced $[Ca^{2+}]_i$ increases, respectively. In addition, knockdown of TRPM3 and TRPV4 using siRNA inhibited hypo-osmolality-induced $[Ca^{2+}]_i$ increases, respectively. Moreover, expressions of TRPM3 and TRPV4 were increased by hypo-osmolality, not by hyper-osmolality. These results suggest that hypo-osmolality increases RANKL expression by activating Ca^{2+} entry via TRPM3 and TRPV4 in osteoblastic cells.

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Su-P175

IMPAIRED BONE RESORPTION ASSOCIATED WITH PYCNODYSOSTOSIS

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Background/aims: Cathepsin K is supposed to play a major role in the osteoclast-driven bone resorption. However, we describe a pycnodysostosis patient with a total cathepsin K deficiency who in spite of that developed extensive bony erosions and acro-osteolysis. Methods: Pycnodysostosis patient and control monocytes were isolated from peripheral blood and stimulated to fuse and form multinuclear osteoclast like-cells. The mRNA expressions of four osteoclastic markers (cathepsin K, TRAP, calcitonin receptor, integrin beta 3) were measured using quantitative RT-PCR. Their ability to resorb bone was assessed measuring pit formation and collagen degradation products. Results: Patient cells fused normally to form osteoclasts, but could not produce cathepsin K or cat K-mediated bone degradation products. Surprisingly, also expression of integrin beta 3 was degreased to an almost non-detectable level. These multinucleated cells were able to resorb bone, but in contrast to control osteoclasts, these were immobile and formed deep pits on bone slice. Conclusion: Integrins are heterodimeric cell surface receptors that mediate cell-cell and cell-matrix interaction. Osteoclast exhibits high expression of integrin beta 3, which binds to a variety of extracellular matrix proteins. This integrin may play an important role in regulating osteoclast function, especially in inhibition of osteoclastic movement.

Conflict of Interest: None declared

Su-P176

EXPRESSION OF CYSTIC FIBROSIS TRANSMEMBRANE REGULATOR (CFTR) IN HUMAN BONE CELLS

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Cystic fibrosis (CF) is the most common lethal autosomal recessive disease in Caucasians and affects 1 in 2500 neonates. Life expectancy in individuals with CF has increased from a median of around 5 years to 35 years of life over the past 6 decades. This increase in survival has unmasked osteoporosis as a common and clinically important complication of the disease, and alteration of CFTR function in bone cells may be a contributory factor. Inactivation of the CFTR gene in mice is associated with severe osteopenia, and in adults with CF the ΔF508 mutation is an independent risk factor for low bone mineral density. Furthermore, chloride channels are important for normal osteoclast function; the CLC-7 chloride channel mediates the excretion of chloride ions across the osteoclast membrane, resulting in the formation of hydrochloric acid and subsequent mineral dissolution.

Osteoblastic cell lines (MG63 and SAOS), primary human osteoblasts isolated from bone samples obtained as surgical waste during routine surgical procedures and human osteoclasts cultured from peripheral blood mononuclear cells purified by CD14+ magnetic bead

separation were examined for the expression of CFTR protein by Western blotting using lysates and TCA precipitates and immunofluorescence using a mouse anti-human CFTR (C-terminus) monoclonal antibody (clone 24).

Primary human osteoblasts and osteoblastic cell lines were found to express mature (fully glycosylated ~180 kD), ER-glycosylated (~150 kD) and newly-synthesized non-glycosylated (~130 kD) CFTR. Non-glycosylated and ER-glycosylated CFTR was detected in Western blots of osteoclast lysates, but minimal (< 0.5% of total CFTR) fully glycosylated protein was found in these cells. Immunofluorescence suggested a cytoplasmic rather than membrane-associated distribution in all cell types examined. In situ expression of CFTR in neonatal and adult human bone was seen in osteoclasts, osteoblasts and osteocytes but not chondrocytes. Expression in osteocytes was strongest in newly embedded osteocytes.

CFTR protein is present in human osteoblasts and osteoclasts, although in the latter cell type the expression of mature, fully glycosylated CFTR is limited. The functional significance of these findings and their implication for CF-associated bone disease remain to be determined.

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Conflict of Interest: None declared

Su-P177

DEXAMETHASONE REDUCES OSTEOCLAST FORMATION BY AFFECTING ADHESION BETWEEN OSTEOCLAST PRECURSORS AND OSTEOBLASTS

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Glucocorticoids and $1,25(OH)_2VitD_3$ are known to play important roles in bone remodeling. $1,25(OH)_2VitD_3$ stimulates the differentiation of osteoblasts whereas glucocorticoids promote the differentiation of osteoclasts and their binding to the bone surface, this way supporting bone resorption. However, studies addressing the effects of glucocorticoids and $1,25(OH)_2VitD_3$ on the formation of osteoclasts show conflicting findings.

During osteoclast formation, adhesion between osteoclast precursors and osteoblasts is essential. In the present study we investigated the effect of dexamethasone (10^{-8} M), a synthetic glucocorticoid analog, and $1,25(OH)_2VitD_3$ (10^{-7} M) on the adhesion of peripheral blood mononuclear cells (PBMCs) to osteoblasts. We further analyzed the formation of TRACP-positive, multinucleated cells. After 3 days of co-culture we quantified the number of PBMCs attached to a confluent layer of osteoblasts. In the presence of $1,25(OH)_2VitD_3$ the number of adherent PBMCs was not altered whereas dexamethasone significantly decreased the number of attached PBMCs. Cultures with both dexamethasone and $1,25(OH)_2VitD_3$ showed a decrease comparable to the culture where only dexamethasone was added. The presence of dexamethasone in co-cultures, alone or with $1,25(OH)_2VitD_3$, significantly decreased the number of TRACP-positive multinucleated cells that were formed after three weeks. The number of osteoclast-like cells formed proved to directly relate to the number of attached cells under all conditions tested. Our data suggest that dexamethasone reduces the adhesion of osteoclast precursors to osteoblasts which leads to a lower number of osteoclast-like cells. The presence of $1,25(OH)_2VitD_3$ did not affect adhesion nor did it influence the effects of dexamethasone on adhesion. These data together could indicate that dexamethasone and not $1,25(OH)_2VitD_3$ reduces osteoclast formation by affecting the adhesive capabilities of osteoclast precursors and osteoblasts.

Conflict of Interest: None declared

Su-P178

OSTEOCLAST SIZE AND SURVIVAL IS CONTROLLED BY FRA-2 THROUGH LIF/LIF-RECEPTOR SIGNALING AND HYPOXIA

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Osteoclasts are multinucleated hematopoietic cells essential for bone resorption. The inhibitory role of the c-Fos transcription factor in osteoclast formation is well documented, however, little is known about the Fos related protein Fra-2.

Here we show that Fra-2 controls osteoclast survival and size through a novel pathway involving LIF/LIFR signaling and hypoxia. Fra-2-deficient bones exhibit numerous giant osteoclasts with impaired LIF/LIFR signaling. Moreover, the LIF cytokine is a transcriptional target of Fra-2. Importantly, newborns lacking LIF also display giant osteoclasts. In vivo, Fra-2 or LIF-deficient long bones are hypoxic and express increased Bcl-2 levels. Bcl-2 over-expression in hematopoietic cells is sufficient to induce giant osteoclasts in mice. In vitro, Bcl-2 expression and the survival of Fra-2 and LIF-deficient osteoclast precursors are sensitive to oxygen levels.

These findings linking oxygen tension to LIF/LIFR signaling offer potential therapeutic targets for the treatment of bone syndromes associated with increased osteoclastogenesis.

Conflict of Interest: None declared

Su-P179

EXTRA RENAL HYDROXYLATION IN HUMAN BONE CELLS

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1, $25(OH)_2D_3$ stimulates differentiation and function of osteoblasts. Hydroxylation of the main circulating vitamin D metabolite, $25(OH)_3$, into 1, $25(OH)_2D_3$ occurs in the kidney and is under tight regulation to avoid hypercalcaemia. Extra renal hydroxylation is demonstrated in osteoblasts. The primary aim of this study was to test wether bone cells can actively convert $25(OH)_3$ into 1, $25(OH)_2D_3$. Therefore, the effect of Vitamin D metabolites (1, $25(OH)_2D_3$ & $25(OH)D_3$) on proliferation and differentiation of bone cells was investigated.

For proliferation experiments, human osteosarcoma cells and primary human bone cells were plated out into 69-well plates containing 2.5 μL/well either 10 nM (10^{-8}), 100 nM (10^{-7}) of 1, $25(OH)_2D_3$ or 100 nM (10^{-7}),200 nM (2×10^{-7}),400 nM (4×10^{-7}) of $25(OH)D_3$ for 8 days. Incubation with 2.5 μL/well pure ethanol served as a negative control. Cell proliferation was assayed on day 1,4,5,6,7 and 8 using a Cell Proliferation Kit II (XTT) (© Roche Diagnostics GmbH, Roche Applied Science, Mannheim, Germany).

For differentiation experiments human primary bone cells were plated out into 6-well plates at a density 5×10^4 cells/well. Alkaline Phosphatase (ALP) staining was performed at day 1, 7 and 14. Total RNA was extracted after 96 hrs and 72 hrs respectively using RNeasy Mini Kit (QIAGEN GmbH, Hilden, Germany). Osteocalcine gene expression was tested by RT-PCR.

25(OH)D3 inhibited proliferation of primary osteoblasts as well as MG63, with a maximum of 40 % (400 nM $25(OH)D_3$) in a dose

dependant manner. 1, 25$(OH)_2D_3$ inhibited proliferation up to 50 % (100 nM 1, 25$(OH)_2D_3$). Osteocalcine and alkaline phosphatase showed increased response to either 25$(OH)D_3$ and 1, 25$(OH)_2D_3$.

This data support the previous results which suggested that autocrine and possibly paracrine pathways of vitamin D3 metabolism may regulate osteoblasts functions independently of circulating 25$(OH)D_3$.

In conclusion, the present investigation demonstrates that both Vitamin D metabolites 25$(OH)D_3$ and 1, 25$(OH)_2D_3$ display antiproliferative effects and enhance differentiation in human primary osteoblasts and human osteosarcoma cells (MG63) in vitro. This suggests that both MG63 and primary human bone cells contain enzymes for the hydroxylation of 25$(OH)D_3$ into 1, 25$(OH)_2D_3$.

Conflict of Interest: None declared

Su-P180

GREEN TEA CATECHIN (-)-EPIGALLOCATECHIN-3-GALLATE INHIBITS OSTEOCLASTIC DIFFERENTIATION VIA SUPPRESSION OF NF-KB

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Background: Osteoporosis is a significant disease in developed countries. A nutritional approach to prevent bone loss would be a future goal to achieve an inexpensive way for managing osteoporosis. Previous epidemiological studies also found that people with a habit of tea drinking have higher bone mineral density and less chance to get hip fracture. Green tea catechin, (-)-epigallocatechin-3-gallate (EGCG), increased the osteogenic function in mesenchymal stem cells was reported. The effect of EGCG on osteoclastogenesis remains to be elucidated.

Methods: RAW 264.7 mouse macrophage/monocytes were seeded in 96-well plates at a density of 103 cells/well supplemented with 100 ng/ml recombinant murine RANKL. Osteoclastogenesis of RAW 264.7 cells were examined by TRAP staining. Viability of RAW 264.7 cells were measured by the colorimetric assay MTT. Leakage of LDH from cells was measured to quantify the cytotoxicity. The resorption ability of RAW 264.7 cell-derived osteoclasts was assessed on plates coated with calcium-phosphate apatite. AW 264.7 cells were co-transfected with 10 μg/well of pNFkB-luc reporter construct and 1 μg/well of a pGL 4.74 (TK) construct for luciferase activity.

Results: EGCG was capable of inhibiting osteoclastogenesis by decreasing osteoclast formation, activity and pit formation. Under 10–100 μM EGCG, receptor activator of NFκB ligand (RANKL)-induced osteoclastogenesis was suppressed significantly by EGCG in a dose-dependent manner, accompanied by a decrease in the number of mature osteoclasts and TRAP activity. EGCG also inhibited RANKL-induced pit formation, but was non-toxic to the RAW 264.7 pre-osteoclasts. It was observed that EGCG inhibited TRAP activity in RANKL-induced RAW 264.7 cells during the early stage of osteoclastogenesis. Furthermore, EGCG inhibited osteoclast differentiation via suppression of NF-kB transcriptional activity, which was mediated by a decrease in the intranuclear translocation of NF-kB.

Conclusion: The present results demonstrate for the first time that EGCG is a potent inhibitor of osteoclastogenesis, via a mechanism involving NF-kB.

Conflict of Interest: None declared

Su-P181

INHIBITION OF RAB GERANYLGERANYL TRANSFERASE AND RAB PRENYLATION BY PHOSPHONOCARBOXYLATE DRUGS

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Phosphonocarboxylates (PCs) specifically inhibit Rab geranylgeranyl transferase (RGGT), the enzyme responsible for the prenylation of Rab proteins, a large family of small GTPases that regulate vesicular trafficking. Since the first PC inhibitor that we identified (3-PEHPC) also inhibits bone resorption, it is likely that PCs disrupt osteoclast function by inhibiting Rab-dependent vesicular trafficking pathways in these cells. To confirm that the cellular effects of PCs are the result of inhibition of Rab prenylation, we compared the potency of several PCs for inhibiting RGGT and Rab prenylation, with their cellular effects. We found that inhibition of RGGT by PCs correlated with reduction in the viability of J774 macrophages, and the accumulation of large, actin-bound vacuoles in osteoclasts, indicating that these effects are the result of disrupting the prenylation and function of Rab GTPases. In support of this, macrophages from gunmetal mice, which have an 80% decrease in RGGT activity, were much more sensitive to PCs (in terms of loss of cell viability) than macrophages from wild-type animals. However, we found that the most potent RGGT inhibitor (3-IPEHPC; the PC analogue of the bisphosphonate minodronate) exhibited unexpectedly poor anti-resorptive potency, both in vitro and in vivo. This raises the possibility that additional mechanisms may be involved in the anti-resorptive activity of these drugs. We have also found that the potency of PCs for inhibition of RGGT activity varies with different Rab substrates, which are prenylated on one or two cysteine residues in characteristic -CC, -CCXX, -CXC and -CaaX motifs at their C-terminus. Rabs prenylated on adjacent cysteine residues are extremely susceptible to inhibition of RGGT-catalysed prenylation by 3-IPEHPC in vitro, and to disruption of prenylation and membrane localization in HEK293 cells. Some Rabs with -CXC motifs are equally sensitive, although there is much more variation between Rabs with this motif. By contrast, Rab proteins with monocysteine -CAAX motifs are unaffected by PCs. Interestingly, using HPLC analysis of a series of Rab38 mutants with different C-termini, we found that only the second step of prenylation of Rabs is inhibited by PCs, offering an explanation for the resistance of mono-prenylated Rabs to inhibition of prenylation. This data indicates that rather than affecting all Rabs, the profile of inhibition of Rab function by PCs is likely to be dependent on the concentration used.

Conflict of Interest: F. Coxon, P&G, Research Grant
M. Rogers, P&G, Research Grant
C. McKenna, P&G, Research Grant

Su-P182

FLUORESCENTLY LABELED RISEDRONATE AND RELATED ANALOGS: DESIGN AND EVALUATION AS IMAGING PROBES

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It has become clear that the pharmacological activity of nitrogen-containing bisphosphonate (N-BP) drugs is influenced by both skeletal distribution and enzyme inhibitory activity in osteoclasts. Variations in mineral affinity among N-BPs is likely to influence their skeletal distribution, and may help to explain potential emerging differences in the clinical performance of drugs such as alendronate, risedronate and zoledronate in osteoporosis. In addition, some BPs and their phosphonocarboxylate (PC) analogs have anti-neoplastic effects. However, it is unclear whether these effects are due to direct effects on tumour cells or a consequence of inhibition of osteoclastic resorption. To address these questions, we have synthesized fluorescent conjugates of N-BPs and some PC analogs, enabling visualization of the distribution and cellular uptake of these drugs within the bone microenvironment in vivo. Activated esters of fluorophores are ideal tools for labeling molecules that contain a primary amine group (through formation of a stable amide bond). However, since many N-BPs, including risedronate (RIS) lack this group, we have chemically modified RIS to introduce an appropriate site for this conjugation. Using this approach, a fluorescein-labelled analog of risedronate (FAM-RIS), as well as other pyridine-containing N-BP and PC analogs have been designed. FAM-RIS retained relatively high affinity for mineral in a hydroxyapatite affinity column assay, albeit lower than RIS itself. FAM-RIS also displayed evidence of strong binding and recycling on mineral surfaces in vitro, and was internalized by cells by fluid phase endocytosis. Surprisingly, FAM-RIS was as active as RIS at inhibiting Rap1A prenylation in J774 cells, and it also inhibited prenylation in rabbit osteoclasts in vivo. Moreover FAM-RIS inhibited bone resorption in the Schenk model (LED = 0.1 mg P/kg). These effects are unlikely to be solely due to inhibition of farnesyl diphosphate synthase (FPPS), since in isolated enzyme assays FAM-RIS was a much less potent inhibitor of this enzyme than RIS (IC50 = 2700 vs 5.7 nM). Indeed, FAM-RIS was also able to inhibit geranylgeranyl diphosphate synthase (IC50 = 2500 nM), which may help to explain the potency of FAM-RIS for inhibition of Rap1A prenylation. Fluorescent N-BPs and PCs will be valuable tools for examining potential differences in skeletal distribution and cellular uptake in vivo between drugs with different mineral affinity.

Conflict of Interest: F. Coxon, P&G, Research Grant
G. Russell, P&G, Research Grant
M. Rogers, P&G, Research Grant
C. McKenna, P&G, Research Grant

Su-P183

MUTATIONS ASSOCIATED WITH PAGETIC DISEASES CAUSE LACK OF SIGNAL PEPTIDE CLEAVAGE OF RANK, AND ACCUMULATION WITHIN ORGANISED SMOOTH ENDOPLASMIC RETICULUM WHEN OVEREXPRESSED

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Heterozygous insertion mutations in the signal peptide region of the protein RANK, are responsible for early onset forms of Pagetic diseases. Early onset Paget's disease of bone (ePDB), Familial Expansile Osteolysis (FEO) and expansile skeletal hyperphosphatasia (ESH) are characterised by focal areas of increased bone resorption associated with hyperactive osteoclasts. We have previously shown that when RANK containing the mutations is overexpressed in HEK293 cells it does not localise to the plasma membrane, but accumulates within organised smooth endoplasmic reticulum (OSER) and prevents RANKL-mediated signalling. The aim of this study was to determine the localisation of the proteins when overexpressed in osteoclasts and to investigate whether this mislocalisation could be due to lack of signal peptide cleavage.

The mutant RANK proteins were overexpressed in human osteoclasts by adenoviral transduction. Immunostaining and confocal microscopic analysis demonstrated that FEORANK does not localise to the plasma membrane, but accumulates within spherical structures in the cytosol. Transmission electron microscopy of transduced osteoclasts revealed the presence of OSER within osteoclasts overexpressing FEORANK, but not WTRANK.

To investigate whether the FEO, ePDB or ESH mutations result in lack of signal peptide cleavage of the RANK protein, two in vitro translation assays were used. The mass of translated proteins produced in the Xenopus egg extract translation system (XEE; in which post-translational modification can take place) were compared to the mass of proteins translated in a rabbit reticulocyte lysate (RRL; in which no post-translational modification can take place). The mass of WTRANK was greater when translated in the RRL system, suggesting signal peptide cleavage occurred as expected in the XEE system. By contrast, there was no difference in the sizes of FEO-, ePDB- and ESHRANK between the RRL and XEE systems, demonstrating lack of signal peptide cleavage.

Taken together, in human osteoclasts, overexpression of RANK proteins containing these disease-associated mutations induces OSER formation. Since overexpression of ER-resident membrane proteins can result in the formation of OSER, we propose that the lack of signal peptide cleavage effectively converts RANK into an ER-resident protein and prevents its localisation to the plasma membrane. How these observations relate to the hyperactivated osteoclast phenotype is under further investigation.

Conflict of Interest: None declared

Su-P184

REDUCED BONE DENSITY IN XLA PATIENTS, AN EFFECT OF B-CELL DEFICIENCY ON OSTEOCLAST DIFFERENTIATION AND OSTEOLYTIC ACTIVITY

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X-linked agammaglobulinemia (XLA) is caused by mutations in the gene for Bruton's tyrosine kinase (Btk), resulting in impaired B-cell receptor signaling and maturational arrest of B cells. XLA patients have profoundly decreased peripheral B cells, virtually undetectable serum immunoglobulins (Ig), and hypoplastic or absent lymphoid tissue. T-cell numbers and function in XLA are normal. Crosstalk between the immune and bone system is well acknowledged, but most studies have focused on the effects of T cells on bone. Recent evidence in mice suggests that B cells play a role in normal bone physiology and maintenance of peak bone mass. Therefore we investigated the effect of the systemic absence of B cells on the bone density of XLA patients.

We utilized quantitative ultrasound (QUS) of the heel to investigate the BMD of 6 ambulatory XLA patients (ages 36–53 years). In 13 XLA patients and age-matched controls, serum markers of bone metabolism and inflammatory cytokine levels were quantified using

ELISA. Osteoclast assays were performed using either B-cell depleted or XLA patient PBMC, in order to assess the effects of B cell deficiency on TRAP positive multinucleated cell (MNC) formation and the expression of osteoclast proteolytic enzymes.

QUS showed that XLA patients, compared to age matched data, had significantly decreased bone density ($p = 0.02$) and increased susceptibility for osteoporosis with advancing age. While no difference was found in the serum markers of bone metabolism, we found profoundly increased expression of the pro-inflammatory cytokines IL-1 and IL-6 ($p = 0.006$ and $p = 0.0001$, respectively) in the serum of XLA patients compared to controls. PBMC-osteoclast cultures of XLA patients showed a significant increase in the number of TRAP positive MNC ($p = 0.04$) and an increase in cathepsin K expression (> 3 fold) compared to controls. Furthermore, depletion of B-cells from PBMC-osteoclast cultures resulted in significantly increased PBMC-osteoclast differentiation ($p = 0.02$) and expression of cathepsin K (> 3 fold).

We provide unique evidence that B-cell depletion of PBMC-osteoclast cultures is stimulatory of markers of osteoclast differentiation and activation in vitro. Our novel finding of reduced bone density in XLA patients, suggest that absence of B-cells in the peripheral blood, in combination with increased serum cytokine levels give rise to the overriding increase in osteoclast activity and decreased bone density in vivo. Funded by ARC.

Conflict of Interest: None declared

Su-P185

CHARACTERIZATION OF THE ROLE OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR (PPAR)-DELTA IN OSTEOCLAST BIOLOGY

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Peroxisome Proliferator-Activated Receptors (PPARs) are part of the nuclear steroid hormone receptor superfamily and three isoforms have been isolated: PPARα, PPARγ and PPARδ, each having characteristic tissue distributions and specific roles. PPARs are better known for their regulatory roles in lipid metabolism, but there is increasing evidence that they have roles in other pathways and tissues, including bone. The PPARδ agonist, carbaprostacyclin, has been reported to stimulate osteoclast (OCL) activity. In this study, we investigated the role of PPARδ in osteoclast biology.

We propose that a conditional null of PPARδ in OCLs will dysregulate their differentiation and activity thereby altering the skeletal structure of affected mice. Using this approach together with gene expression profiling we can identify novel targets of PPARδ that are functional in bone resorption. We have investigated PPARδ function in OCLs in vitro using, GW501516, a highly specific PPARδ agonist, in RAW/C4 cells, a sub-clone derived from the RAW264.7 macrophage cell line with increased potential to differentiate into OCL in vitro with the cytokine, RANKL. PPARδ is the prevailing isoform in RAW/C4-derived OCL and its expression is markedly upregulated during OCL differentiation whereas expression of the other isoforms decreases. GW501516 increases the expression of the typical OCL markers tartrate acid phosphatase (TRAP), cathepsin K and calcitonin receptor (CTR) but decreases the levels of expression of all PPAR isoforms during OCL differentiation. Stable over-expression of PPARδ in RAW/C4 cells also upregulates expression of these genes and this effect is further increased by the presence of GW501516.

Using bone-marrow progenitor cells from floxed-PPARδ mice we knocked-out PPARδ expression ex vivo using recombinant transducible HNC-Cre and showed that there is a marked decrease on the ability to form giant multinucleated TRAP-positive cells. Also these cells display a decreased expression of TRAP, CTR and NFAT2.

We have generated transgenic mouse lines with the objective of conditionally knocking-out PPARδ in OCL by using the Cre-LoxP system and use these to characterize PPARδ-null phenotype in bone. The characterization of PPARδ function and potential target genes will aid our understanding of osteoclast and bone biology and will aid the development of new therapies for bone-resorbing related disorders.

Conflict of Interest: None declared

Mo-P186

RELATIONSHIP BETWEEN BONE RANKL MRNA LEVELS AND BONE STRUCTURAL AND TURNOVER MARKERS IN MALE OSTEOARTHRITICS

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We have previously shown a strong relationship between the expression in bone of mRNA encoding receptor activator of NFkB ligand (RANKL) and the histomorphometric resorption parameter, ES/BS (1). We also reported elevated RANKL mRNA levels in the bone of female hip fracture patients, compared with age-matched controls (2). In the present study, a male cohort was studied at the time of total hip replacement for primary osteoarthritis to investigate the relationship between bone RANKL mRNA and other parameters of bone turnover. Samples were obtained from 15 male patients, age range 50–79 years. Intertrochanteric trabecular bone biopsies were collected intra-operatively and divided for analysis of RANKL mRNA, using real time RT-PCR, and histomorphometric analysis following plastic embedding of undemineralised bone. Fasting blood samples were obtained on the day of surgery and serum total RANKL levels were measured using ELISA. In this male cohort, bone RANKL mRNA levels were positively related to age ($r = 0.73$, $p = 0.003$). Interestingly, RANKL mRNA levels were inversely related to serum total RANKL levels ($r = -0.70$, $p = 0.007$). RANKL mRNA levels were negatively associated with BV/TV and Tb.Th and positively associated with ES/BS, OS/BS, and with the biochemical markers of bone turnover, serum alkaline phosphatase and osteocalcin, and urinary deoxypyridinoline. These relationships, which were found between parameters, measured using very different methods of analysis, strongly support the concept of a pro-resorptive role for RANKL in the human bone microenvironment. In addition, the mechanism that gives rise to the inverse relationship between RANKL mRNA and serum RANKL requires further investigation. Since we measured total serum RANKL, this cannot be explained by differential complexing of serum RANKL with OPG or other serum proteins. A possible explanation involves shedding of cell surface RANKL to release soluble RANKL into the serum, and that this shedding activity is somehow decreased with increasing expression of RANKL mRNA.

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Mo-P187

EFFECTS OF MELATONIN ON PRO-INFLAMMATORY CYTOKINE PRODUCTION IN PGE2-ACTIVATED HUMAN GINGIVAL FIBROBLASTS IN VITRO

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The present study examined the effects of melatonin on PGE2-induced pro-inflammatory cytokine production in human gingival fibroblasts in vitro.

Human gingival fibroblasts were cultured in DMEM and exposed for 1 h to melatonin in the presence/absence of 10(super-5) M PGE(sub2) for up to 24 h. The levels of mRNA for IL-1 beta, Il-8 and osteoprotegirin/osteoclastogenesis inhibitory factor (OPG/OCIF) were quantitatively evaluated by reverse transcriptase-PCR using a LightCycler method.

While melatonin did not significantly alter the levels of OPG/OCIF mRNA, either in the presence or absence of PGE2, it markedly decreased the levels of IL-1 beta and Il-8 mRNA in PGE2-treated fibroblasts.

These results suggest that melatonin has a potency to inhibit the production of pro-inflammatory cytokines from PGE2-activated human gingival fibroblasts in the periodontal disease tissue. It is conceivable that melatonin therapy could be developed into an effective and safe treatment for periodontal disease.

Conflict of Interest: None declared

Mo-P188

ACIDOSIS AUGMENTS THE RESORPTIVE CAPACITY OF HUMAN OSTEOCLASTS BY INCREASING LYSOSOMAL ACIDIFICATION

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Metabolic acidosis shows detrimental effects on the bones, and local acidosis as seen in metastatic bone disease leads to localized bone destruction. The mechanism underlying the increased resorption involves a combination of upregulation of RANKL expression by osteoblasts, and upregulation of the resorptive activity by the osteoclasts. To further clarify the mechanism underlying the increased resorption, we investigated the effect of acidosis on cultures of human osteoclasts.

We used CD14+ sorted monocytes cultured in the presence of RANKL and M-CSF to generate mature human osteoclasts. We seeded the mature osteoclasts on bone slices and exposed them to medium with pH values ranging from 6.5 to 8.0 and investigated bone resorption by measurement of calcium release and osteoclast number by TRACP activity after 5 days of culture. We then measured lysosomal acidification using both the acid influx assay in membranes isolated from osteoclasts exposed to pH 6.8 and 7.5, and finally we quantified lysosomal acidification in osteoclasts using acridine orange. I addition, we measured V-ATPase activity in the membrane preparations.

We found that osteoclasts exposed to low pH values resorbed 200% of the level observed in control osteoclasts at normal pH values. Furthermore, we found that the TRACP activity increased, but that the increase did not fully explain the increase in resorption. Finally, we found the low pH treatment increased acid influx by 40% and V-ATPase activity in the membrane fractions. Finally, we found that the lysosomal pH was reduced mildly by the low pH when incubating the cells at low pH for 24 hours, but not 45 minutes.

In conclusion, we found that lowering pH leads to an increase in osteoclastic resorption via increased V-ATPase activity leading to increased acidification of the lysosomes. These data for the first time show that human osteoclasts are activitated by low pH, which correlates well with the findings in disorders involving both systemic and local acidosis.

Conflict of Interest: None declared

Mo-P189

IL-6 INHIBITS RANKL-INDUCED OSTEOCLASTOGENESIS BY DIVERTING CELLS

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Osteoclasts are bone-resorptive cells which differentiate from hematopoïetic precursors upon RANKL activation. Previous studies demonstrated that IL-6 indirectly stimulated osteoclastogenesis through the production of RANKL by osteoblasts. However, few data described the direct effect of IL-6 on osteoclast. To investigate this effect, we used several models: murine RAW264.7 cells and bone marrow, human blood monocytes. In all model used, the addition of IL-6 inhibited RANKL-induced osteoclastogenesis. Furthermore, IL-6 decreased the expression of osteoclast markers but up-modulated macrophage markers. To elucidate this inhibition, STAT3, the main signaling molecule activated by IL-6, was analyzed. Addition of two STAT3 inhibitors completely abolished RANKL-induced osteoclastogenesis, revealing a key role of STAT3 during osteoclastogenesis. We demonstrated that a basal level of phosphorylated-STAT3 on Serine727 associated to an absence of phosphorylation on Tyrosine705 is essential for osteoclastogenesis. Furthermore a decrease of Serine727-phosphorylation leaded to an inhibition of osteoclast differentiation whereas an increase of phosphorylation of Tyrosine705 upon IL-6 stimulation induced the formation of macrophages instead of osteoclasts. In conclusion, we showed for the first time that IL-6 inhibits RANKL-induced osteoclastogenesis by diverting cells into the macrophage lineage and demonstrated the functional role of the activated-STAT3 level and its form of phosphorylation in the control of osteoclastogenesis.

Conflict of Interest: None declared

Mo-P190

OSTEOBLAST-MEDIATED INHIBITION OF OSTEOCLASTOGENESIS BY TUMOR NECROSIS FACTOR-ALPHA

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Tumor necrosis factor-alpha (TNFalpha) is a major inflammatory cytokine and has been implicated in local and systemic bone loss in diseases such as rheumatoid arthritis and osteoporosis. We have shown that TNFalpha induces primary murine osteoblasts in vitro to release (a) soluble factor(s) that efficiently inhibit(s) the development of osteoclasts from bone marrow cells (BMC). In the present study, the effects of TNFalpha on osteoblasts were further characterized.

To assess the effects of TNFalpha on osteoblastic gene expression, primary calvarial osteoblasts from ddy mice were cultured $\pm$1,25VitD3 (10^{-8} M) and $\pm$TNFalpha (1 ng/ml) for 24 h. Total RNA was prepared and analyzed either on whole genome arrays (Agilent) or low density arrays (ABI). Subsequently, the dose and time dependent expression of genes of interest was further

investigated by real-time PCR in C57Bl/6 wt and p55TNFR/p75TNFR double ko osteoblasts. Since TNFalpha is a stimulator of osteoclast development, emphasis was placed on the regulation of osteoclastogenic growth factors and cytokines.

In primary murine osteoblasts, RANKL and OPG mRNA levels were highly responsive to 1,25VitD3, with only little (RANKL) or no (OPG) response to TNFalpha. Levels of transcripts encoding other factors known to modulate the development of osteoclasts such as IL1, IL6, IL11 were upregulated by TNFalpha, and further stimulated by 1,25VitD3, while 1,25VitD3 alone was not effective. CSF-1, which is an essential growth factor for monocyte and osteoclast lineage cells was not affected. Extending these studies to dose and time dependence, RANKL mRNA levels were found, (a) to be downregulated by TNFalpha at concentrations of > 3 ng/ml in the presence of 1,25VitD3 and, (b) to be induced by TNFalpha in the absence of 1,25VitD3 during osteoblast differentiation. Levels of OPG mRNA were decreased by TNFalpha. Transcripts encoding IL6 were increased with culture time and increasing TNFalpha concentrations. Osteoblasts from p55TNFR/p75TNFR double ko mice maintained their responsiveness to 1,25VitD3, while the TNFalpha response was blunted.

The main response of osteoblasts to TNFalpha consists in the release of growth factors and cytokines stimulating the development of osteoclasts. The effects of TNFalpha often are further increased by 1,25VitD3. In the light of the upregulation of numerous stimulators of osteoclast development, it is surprising that TNFalpha can exert an inhibitory action on osteoclastogenesis in vitro.

Conflict of Interest: None declared

Mo-P191

STIMULATION BY TOLL-LIKE RECEPTOR 5 MODULATES OSTEOCLAST DIFFERENTIATION THROUGH STAT1/IFN-BETA

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Osteoclasts, bone resorbing cells, are differentiated from hemopoietic precursor cells of monocyte/macrophage lineage. Stimulation of Toll-like receptors (TLRs) has been shown to positively or negatively modulate osteoclast differentiation, depending on experimental condition. However, the molecular mechanism by which stimulation by TLRs modulates osteoclast differentiation still remains to be elucidated. In the present study, we examined the effect of flagellin, a specific microbial ligand of TLR5, on receptor activator of NF-kB ligand (RANKL)-stimulated osteoclastogenesis. Flagellin suppressed RANKL induction of c-Fos protein expression in bone marrow-derived macrophages (BMMs), without affecting c-Fos mRNA expression. Ectopic overexpression of c-Fos and an active form of NFATc1 reversed flagellin-induced anti-osteoclastogenic effect. The inhibitory effect of flagellin was mediated by IFN-beta; production. Flagellin stimulated IFN-beta; expression and release in BMMs and IFN-beta-neutralizing antibody prevented flagellin-induced c-Fos down-regulation and anti-osteoclastogenic effect. IFN-beta gene induction by flagellin, LPS, or RANKL was dependent on STAT1 activation. Treatment with flagellin or RANKL stimulated STAT1 activation, and STAT1-deficiency or a JAK2 inhibitor AG490 dramatically prevented IFN-beta; induction in response to flagellin or RANKL. In addition, STAT1-deficiency abolished anti-osteoclastogenic effect induced by flagellin or LPS. On the other hand, flagellin rather stimulated osteoclast differentiation in cocultures of osteoblast and bone marrow cells, without induction of IFN-beta. Taken together, these results demonstrate that IFN-beta acts as a critical modulator for osteoclastogenesis in response to TLR5 activation.

Conflict of Interest: None declared

Mo-P192

IS BACTERIAL LIPOPOLYSACCHARIDE CAN MEDIATE OSTEOCLASTOGENESIS INDEPENDENTLY OF RANKL?

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The differentiation of osteoclast precursors into bone-resorbing osteoclasts is mainly mediated by the RANK/RANKL pathway. However, some other factors can substitute to RANKL (TNF-alpha, IL-1beta, LIGHT…) and initiate osteoclastogenesis independently of RANKL. Bacterial lipopolysaccharide (LPS) is known to induce greater bone resorption when it is injected in vivo, and the proposed mechanism is that LPS induces the expression of RANKL at the surface of osteoblasts. The aim of this study was to assess if LPS can induce directly osteoclastogenesis from human osteoclast precursors.

PBMC were isolated from 3 healthy volunteers and cultured on glass coverslips and dentine slices in the presence of M-CSF. At day 7 human sRANKL (50 ng/ml) and/or LPS (10 microg/ml) were added to the cultures still in the presence of M-CSF. Osteoclast differentiation and activity was assessed respectively, by determining the number of tartrate resistant acid phosphatase (TRAcP) positive multinucleated cells after 14 days and the extent of bone resorption after 21 days. Non-parametric Mann-Whitney test was used to compare the differences between the groups. Differences at $p < 0.05$ were considered significant.

RANKL induced an 8.25-fold increase in the number of newly-generated osteoclast ($p = 0.05$ for 111.5 ± 5 vs. 13.5 ± 6) and a 28.2-fold increase in the percentage of total area of resorption ($p = 0.009$; $23.7\% \pm 6.3\%$ vs. 0.8% vs. 0.08%), compared to M-CSF alone. When LPS is added to M-CSF, a 5.2-fold increase in the number of osteoclast ($p = 0.05$; 70.5 ± 23 vs. 13.5 ± 6) and a 2-fold increase in the percentage of total area of resorption ($p = 0.025$; $1.7\% \pm 0.06\%$ vs. $0.8\% \pm 0.08\%$) was evidenced, compared to M-CSF alone. Moreover, when RANKL and LPS were added together, an 18.1-fold increase in the number of osteoclast was found ($p = 0.05$ for 244.5 ± 6 vs. 13.5 ± 6). Interestingly, the combination of RANKL and LPS increased the total area of resorption by 5.3-fold compared to M-CSF alone ($p = 0.025$; $4.5\% \pm 0.7\%$ vs. $0.8\% \pm 0.08\%$) but decreased it by 5.3-times compared to RANKL alone ($p = 0.025$; $4.5\% \pm 0.7\%$ vs. $23.7\% \pm 6.3\%$).

These results indicate, for the first time, that LPS can directly stimulate the differentiation of human osteoclast precursors into bone-resorbing osteoclasts in the absence of RANKL. It is still unclear why the combination of RANKL and LPS increases the number of newly-formed osteoclasts but decrease the capability of these cells to resorb bone.

Conflict of Interest: None declared

Mo-P193

ACID EXTRUSION AND INTRACELLULAR PH REGULATION IN PRIMARY OSTEOCLASTS CULTURED ON GLASS

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The acidification of the resorption lacuna, mediated by vectorial proton extrusion, has an important role in bone resorption and is critical for the pH regulation of the osteoclasts. The main contributor to acid extrusion is the vacuolar H^+-ATPase (V-ATPase); however, other mechanisms are involved in the acidification of the bone matrix and in the ionic homeostasis, such as sodium/hydrogen (Na^+/H^+) exchanger and hydrogen-potassium (H^+-K^+) ATPase. The present study was performed to evaluate the combined activity of mechanisms of hydrogen transport across the plasma membrane, such as sodium/hydrogen (Na^+/H^+) exchanger, hydrogen-potassium (H^+-K^+) ATPase and vacuolar H^+-ATPase, during intracellular pH (pHi) recovery, in the absence of HCO_3^-, after acid load by superfusion with NH_4Cl (20 mM, 2 min.). Mature osteoclasts, their precursors, other bone cells and bone marrow cells were removed from long bones of 2–6 days-old, newborn rats (Wistar). Cells were maintained in DMEM, 10% FBS (pH 7.4), at 37°C, 5% CO_2, for two hours, or until they exhibit adherence to glass, before the experiments. In the time of the analysis, the osteoclasts were precisely selected based on their morphology, using a differential interference contrast filter system. Just multinucleated cells were considered. The pH measurements were performed by fluorescence microscopy, using BCECF-AM (12 muM), a fluorescent probe sensitive to the intracellular [H^+]. The emitted light at 440 nm and 490 nm was collected and the pixel intensity of the areas of interest (from two to six areas for each osteoclast) at 490 nm was divided by the pixel intensity at 440 nm. The excitation fluorescence ratios were converted into pHi values using the high-K^+ nigericin technique. The pHi recovery to basal values was registered during ten minutes. The rate of hydrogen extrusion was defined by the angular coefficient of the linear regression (dpHi/dt) of the pH values registered at the two first minutes after the acid load. The preliminary results indicated that the mean pHi of osteoclasts is 7.29 ± 0.326 ($n = 16$). However, these cells individually exhibit diverse pH values, which varies between 6.61 and 7.93, and diverse rates of H^+ extrusion, which varies from 0.01 to 0.58 pH units/minute. Such variations are probably related to the functional stage of the cell in the resorption cycle and will be futurely investigated. (Supported by: CNPq)

Conflict of Interest: None declared

Mo-P194

THE SEVERE OSTEOPETROTIC PHENOTYPE IN CLC-7 DEFICIENT MICE IS CAUSED BY DECREASED LYSOSOMAL ACIDIFICATION IN THE OSTEOCLASTS

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ClC-7 knock out (KO) mice display a very severe type of osteopetrosis, which closely resembles autosomal recessive osteopetrosis in humans. ClC-7 is a chloride channel, which is highly expressed in mature osteoclasts, localizes to the ruffled membrane and is suspected to mediate acidification of the resorption lacuna. To examine the underlying mechanism causing the osteopetrotic phenotype, osteoclast and osteoblast phenotypes were evaluated.

ClC-7 KO mice and their corresponding wildtype (WT) littermates were sacrificed at 4–5 weeks of age. Biochemical markers of resorption (CTX-I), osteoclast number (TRACP 5b), and osteoblast activity (ALP) were measured in serum. Spleens were dissected and osteoclasts were generated by use of M-CSF and RANKL. Mature osteoclasts were seeded on calcified or decalcified bone slices and supernatants collected for measurements of CTX-I and Ca2+. The acidification of membrane vesicles from bone cells deriving from KO and WT mice was examined using acridine orange. Histological studies were performed to examine bone formation and osteoclast number. Osteoblastogenesis in vitro was investigated using calvarial osteoblasts. Nodule formation was examined by alizarin red staining after 14 days of culture. The serum ALP level was increased by 30%, the TRACP level was increased by 250% in KO mice, whereas the resorption per osteoclast was reduced to 50% of the WT level. ClC-7 −/− osteoclasts were unable to resorb calcified bone in vitro, measured both by CTX-I and Ca2+. However, the cells were able to resorb decalcified bone to similar levels as WT osteoclasts. Equal numbers of osteoclasts were verified. Furthermore, the acid influx in bone membrane vesicles was reduced by 70% in KO mice, $p < 0{,}01$. Histological evaluations of KO bones showed high osteoclast numbers and ongoing bone formation. Finally, ClC-7 −/− osteoblasts showed no increased osteogenic capacity in vitro.

In summary, we present evidence supporting the hypothesis of ClC-7 having a pivotal role in acidification. First, the inability of the ClC-7 −/− osteoclasts to resorb calcified bone, but sustained ability to resorb decalcified bone, and second, the decreased acid influx in bone membranes from KO mice. Both increased ALP levels and histological stainings verified increased bone formation in the KO mice, and it has now been shown that this is not due to a de novo osteogenic effect of the ClC-7 −/− osteoblasts. This indicates, that the osteoclasts, despite their non-resorptive phenotype, are capable of sending signals for sustained bone formation.

Conflict of Interest: None declared

Mo-P195

THE ACUTE CHARCOT FOOT IS CHARACTERISED BY INCREASED PROINFLAMMATORY CYTOKINES, TNF-ALPHA AND IL-6, WHICH CORRELATE WITH ITS PATHOLOGICAL BONE TURNOVER

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Charcot osteoarthropathy or Charcot foot is a disabling complication of diabetes and is characterised by pathological fractures, joint dislocations and severe foot deformity. It classically presents acute inflammation but its role in the pathogenesis is poorly understood. This study shows that the proinflammatory cytokines, tumour necrosis factor-alpha (TNF-alpha) and interleukin-6 (IL-6) are both raised in acute Charcot patients and are significantly associated with markers of bone resorption (serum C-Terminal Telopeptide (CTx)) and bone formation (bone specific alkaline phosphatase-BAP).

We studied 3 groups of patients: 27 presenting with acute Charcot osteoarthropathy; 14 with chronic Charcot osteoarthropathy and 24 diabetic control patients.

In acute Charcot patients, there was a significant increase in the serum levels of TNF-alpha (1.74 ± 0.94 pg/ml) compared with chronic Charcot patients (1.25 ± 0.38) and diabetic control patients (1.12 ± 0.38 pg/ml), $p = 0.008$. Similarly, there was a significant rise in serum IL-6 in acute Charcot patients (3.84 ± 3.55 pg/ml) compared with chronic Charcot patients (1.73 ± 1.23 pg/ml) and diabetic controls (1.86 ± 1.52 pg/ml), $p = 0.025$. Serum Osteoprotegerin (OPG), a cytokine that modulates bone resorption and osteoclastic activity, was significantly raised in acute Charcot patients

(5.52 ± 1.74 pmol/l) compared with chronic Charcot patients (4.86 ± 1.42 pmol/l) and diabetic patients (4.37 ± 1.38 pmol/l), p = 0.048. Serum CTx, a breakdown product of type 1 collagen, was significantly raised in patients with acute Charcot foot (0.409 ± 0.395 ng/ml), compared with patients with chronic Charcot foot (0.095 ± 0.042 ng/ml) and diabetic patients (0.107 ± 0.68 ng/ml), p = 0.001. Serum BAP was also significantly raised in acute Charcot patients (18.5 ± 9.2 μg/L) compared with chronic Charcot patients (12.7 ± 4.42 μg/L) and diabetic controls (14.7 ± 5.13 μg/L), p = 0.041. Serum TNF-alpha levels were significantly associated with serum CTx (r = 0.526, p < 0.001) and serum BAP (r = 0.512, p < 0.001), and similarly serum IL-6 levels were significantly associated with serum CTx (r = 0.664, p < 0.000) and serum BAP (r = 0.680, p < 0.001). Serum TNF-alpha and IL-6 were also significantly associated with serum OPG (r = 0.431, p = 0.003 and r = 0.348, p = 0.005 respectively).

This study indicates that inflammation, as reflected by proinflammatory markers TNF-alpha and IL-6, plays an important role in the pathological bone turnover of the acute Charcot foot.

Conflict of Interest: None declared

Mo-P196

RANKL-DEPENDENT DIFFERENTIATION OF HL-60 CELLS INTO FUNCTIONAL OSTEOCLASTS

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Knowledge of bone remodeling mechanisms has greatly advanced with the findings of RANK/RANKL/OPG proteins that allowed, for instance, in vitro culture of osteoclasts from human blood monocytic precursors. However, experiments with such primary cells depend on several non-foreseeable parameters like low number of these blood precursors, and variability of functional responses. From that point of view, a well-characterized osteoclast-like cell line remains a useful tool to decipher molecular mechanisms of bone remodeling. Studies have shown that the human promyelocytic cell line HL-60 (ATCC: CCL240) can acquire phenotypic and functional features of various myeloid cell types. In the present study, we provide evidence that, under specific experimental conditions, the HL-60 cell line can differentiate into functional osteoclast-like cells. The optimal conditions to obtain HL-60 osteoclast-like precursor cells are the concomitant presence of recombinant soluble RANKL and MCS-F for 7 days. RANKL is indispensable for the process of differentiation. The transformation of these precursors into functional osteoclast-like cells is favored by adherence on type I collagen. Such differentiated HL-60 cells are multinucleated TRAP positive cells with the capacity to adhere to, and resorb artificial calcium hydroxyapatite discs and dentine. They form actin rings, as demonstrated by confocal laser microscopy. In contrast to undifferentiated cells, HL-60 cells differentiated into bone resorbing cells expressed RANK at the mRNA and protein levels (real time PCR, flow cytometric and western blot analyses). They also express other osteoclast specific molecules like the adaptor protein TRAF6 and receptors for calcitonin. Agonists of FcγRI and FcγRII enhance resorptive capacity of HL-60 osteoclast-like cells, as do PMA, a PKC activator, and ionophore A23187, an intracellular calcium mobilizing agent. Stimulation of HL-60 osteoclast-like cells by soluble or cell surface RANKL is associated with activation of intracellular protein tyrosine phosphorylation, NF-κB and NFATc1 pathways. In conclusion, the HL-60 osteoclast-like cells could serve as an easily accessible and reproducible model for the study of bone immunobiology and pharmacology. (Funded by the CIHR)

Conflict of Interest: None declared

Tu-P197

HYPERACTIVITY OF OSTEOCLAST PRECURSORS IN ACUTE CHARCOT OSTEOARTHROPATHY

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Although Charcot osteoarthropathy is characterised by increased bone resorption mediated by multinucleated osteoclasts, the exact cellular mechanism by which this process is initiated remains unknown. The aim of this study was to determine the activation state of the osteoclast precursors isolated from patients suffering with acute Charcot osteoarthropathy as compared to age/sex-matched diabetic and healthy controls.

Peripheral blood mononuclear cells (PBMC) were isolated from 9 patients with acute Charcot osteoarthropathy and 16 control patients (8 with diabetes but exhibiting no osteoarthropathy and 8 age/sex-matched healthy volunteers) and cultured on coverslips and dentine slices in the presence or absence of M-CSF and sRANKL. Osteoclast differentiation and activity was assessed by determining the number of TRAcP positive multinucleated cells and the extent of bone resorption. Experiments were repeated at least three times and the differences between groups were compared using Mann-Whitney and Kruskall-Wallis tests.

In the presence of M-CSF alone, the mean number of newly-formed TRAcP+ osteoclasts in Charcot patients (48.6 ± 18.2) was increased significantly as compared to diabetic (6.75 ± 2.7, p = 0.010) and healthy controls (5.0 ± 0.7, p = 0.003). Similar increase was also observed in the extent of bone resorption in these cultures. In the presence of M-CSF and sRANKL, the mean number of osteoclasts was markedly increased in Charcot patients compared to diabetic and healthy controls (by 1.7 and 3.3 fold, respectively). These findings were mirrored in the mean percentage area resorption where there was a 3–4fold increase in Charcot patients (41.6 ± 8.1) compared to diabetic (14.2 ± 6.6, p = 0.008) or healthy controls (10.4 ± 1.9, p = 0.002). Addition of excess concentrations of OPG to these cultures did not affect the extent of bone resorption in Charcot patients as compared to the controls.

These results indicate, for the first time, that osteoclast precursors isolated from acute Charcot patients are capable of differentiating into mature osteoclasts in the presence of M-CSF alone and in the absence of any osteoclastogenic factors such as RANKL or TNF?. Moreover, the extent of osteoclast differentiation and bone resorption was increased in the presence of sRANKL and this effect was not suppressed by the addition of OPG, suggesting that an alternative pathway to RANK/RANKL is involved in the pathogenesis of acute Charcot ostoarthropathy.

Conflict of Interest: None declared

Tu-P198

CARBON DIOXIDE RICH WATER BATHING INCREASE LOCAL MATRIX METALLOPROTEINASE-3, BONE GLA PROTEIN SECRETIONS IN ISCHEMIC LOWER LIMBS OF DM HEMODIALYSIS PATIENTS

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Background: Carbon dioxide rich water bathing (COWB) known to dilate local veins, increase local vascular blood flow, decrease local tissue pressure by increasing NO synthesis. COWB improves numbness, edema, skin ulcer of ischemic tissues in Diabetes Mellitus(DM),

and necrosis of Arteriosclerosis Obliterans(ASO) patients. We reported increments of Vascular Endothelial Growth Factor(VEGF) in Davos Workshop 2006, increments of Angiopoietin-I in 33th ECTS 2006, and increments of Angiopoietin-I/opposite decrements of Angiopoietin-II in the 6th CECR, Montreal 2007. We recognize plasma MMP-3 levels are high in some cases.

Methods: About 36 degree centigrade, 1100–1200 ppm carbon dioxide enriched water has made by CO2 diffusion method using follow fiber, which CO2 gas inside, and circulating warm water outside. In 6 DM hemodialysis patients, we executes COWB of lower limbs for 15 minutes. We collect blood samples from the local vein where COWB executed, and evaluate plasma matrix metalloproteinase-3(MMP-3) and Bone Gla Protein(BGP,OC).

Results: In hemodialysis patients similarly, MMP-3 levels are about twice high at pre-COWB. After once COBW, BGP are significantly increased (pre-COWB 5.6 ± 3.1 ng/ml to Post-COWB 12.1 ± 6.2 ng/ml (mean±SD); $p < 0.05$), also MMP-3 are increased (pre-COWB 124.3 ± 51.7 ng/ml to Post-COWB 144.6 ± 45.8 ng/ml; $p < 0.05$). Otherwise MMP-3 and BGP of 12 healthy controls have no significant changes.

Conclusion: COWB, which increase local VEGF, increments of Angiopoietin-I/ opposite decrements of Angiopoietin-II , is suggested to improve clinical symptoms of DM, ASO. Increments of MMP-3 and BGP are suggested to absorb surrounding tissues and bone matrix.

Conflict of Interest: None declared

Tu-P199

THE EFFECT OF RECOVERY DURATION BETWEEN REPEATED BOUTS OF EXERCISE ON HUMAN BONE METABOLISM

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Bone resorption increases in response to running exercise at 65–70% VO_{2max}. Short recovery periods between repeated bouts of exercise may accentuate this response. Therefore, the aim was to investigate the metabolic response of bone to repeated bouts of running exercise, separated by 3 and 23 h.

Ten active men, aged 21 to 35 y, completed two, 9 d, experimental regimens. On days 1 to 3 and 6 to 9 subjects consumed a prescribed diet and performed no exercise. In regimen 1 (LONG), subjects completed two 60 min bouts of treadmill running at 65% VO_{2max}, one on day 4 at 1430 (Bout A) and another 24 h later (Bout B). In regimen 2 (SHORT), subjects completed the two exercise bouts at 1030 (Bout A) and at 1430 (Bout B) on day 5. Blood (fasted) was withdrawn at 0800 on days 4 to 9 and second void urine samples collected. On days 4 and 5, blood samples (non-fasted) were taken before and immediately after exercise, and after 1, 2, and 3 h of recovery. Plasma was analysed for markers of bone resorption (beta-CTX) and bone formation (P1NP, bone-ALP), PTH and ACa. Urine was analysed for the bone resorption markers UfPYD and UfDPD, corrected by volume.

Beta-CTx, UfPYD, UfDPD and bone-ALP concentrations did not change in response to exercise in either SHORT or LONG. In SHORT, mean (1SD) P1NP concentrations were higher ($p < 0.05$) on day 6 (60 (18) mug $\times$ L^{-1}), but lower ($p < 0.05$) on day 9 (54 (17) mug $\times$ L^{-1}), compared with day 4 (56 (17) mug $\times$ L^{-1}). There was no significant change in P1NP in LONG. PTH concentration increased immediately (on average between 1.5 and 1.9 fold) following all bouts of exercise ($p < 0.05$), but the magnitude of change was not significantly different between regimens. ACa concentration did not increase following Bout A in SHORT, but increased following all other bouts of exercise ($p < 0.05$). There were no significant differences between regimens for any variable.

In conclusion, despite the transient increase in P1NP concentration in response to repeated exercise in SHORT, reducing the recovery between repeated exercise bouts from 23 to 3 h had no significant effect on bone metabolism, PTH or ACa concentrations. The exercise intensity and duration might have been too low to elicit a change in bone metabolism, or feeding subjects prior to exercise might have suppressed the metabolic response of bone to exercise.

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Conflict of Interest: None declared

Tu-P200

HUMAN OSTEOPETROSIS ASSOCIATED TRANSMEMBRANE PROTEIN 1 (OSTM1) SHOWS A LYSOSOMAL EXPRESSION PATTERN WHEN CO-TRANSFECTED WITH CHLORIDE CHANNEL 7 IN HUMAN OSTEOCLASTS

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Mutations in Osteopetrosis Associated Transmembrane Protein 1 (OSTM1) have been found in autosomal recessive osteopetrosis patients showing a phenotype strongly resembling that of osteopetrosis patients bearing Chloride Channel 7 (ClC-7) mutations. Although initially considered a transcription factor protein, co-immunoprecipitation and co-localization evidences presented in a further study in mice suggest that OSTM1 could instead be an interaction partner for ClC-7 and that the former needs the chloride channel protein to reach the lysosomes. The aim of the current study was to investigate whether the human homologues of OSTM1 and ClC-7 show a similar behaviour. Two expression vectors, pGFP2N1/OSTM1 and pCMV-HA/CLCN7, containing the human cDNA sequences of both proteins were engineered in order to transfect cells of human origin. When transiently transfected in human cells the former was expressed as a OSMT1 fused to green fluorescent protein while the latter was expressed as a ClC-7 protein fused to an hemagglutinin epitope. Both proteins were detected by western blotting showing the expected molecular weights. When monitored by fluorescence microscopy, HEK293 cells transfected with pGFP2N1/OSTM1 alone showed accumulation of GFP signal into big intracellular vacuoles. However, when co-transfected with pCMV-HA/CLCN7 the GFP signal was distributed in small intracellular dots consistent with lysosomal expression. Remarkably, such phenomenon could also be reproduced in human osteoclasts. In this case, most of the cells showed the vacuolar pattern although a small cell subpopulation presented a lysosomal distribution. We attribute this result to the presence of endogenous ClC-7 in osteoclast inducing correct localization of OSTM1 in cells expressing low level of transfected DNA. When osteoclasts were co-transfected with ClC-7, the vacuolar pattern disappeared and lysosomal localization of OSTM1 was ubiquitous. A DNA concentration effect in transfected cells was dismissed as the underlying cause of the phenomenon by using the proper negative controls. Our results suggest that the human form of OSTM1 interacts with ClC-7 and its lysosomal localization depends on the expression of the channel protein. Implicitly, our results seem to discard the hypothesis that OSTM1 is a transcription factor and favour the notion of a lysosomal protein.

Conflict of Interest: None declared

Tu-P201

THE EFFECT OF CALCITONIN ON OSTEOCLASTIC BONE RESORPTION IS NOT RESTRICTED TO CYCLIC AMP

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Calcitonin binds to its G-protein coupled receptor on the surface of the osteoclasts resulting in acute inhibition of bone resorption. The common signal transduction pathway of G-protein coupled receptors is through induction of higher levels of cyclic AMP. We asked the question whether cAMP modulators would result in similar decreases in bone resorption in purified human osteoclasts, i.e. if calcitonin signal transduction in human osteoclasts was restricted to modulation of cAMP.

We used a non-specific phosphodiesterase inhibitor (IBMX), which prevents cAMP degradation, and Forskolin a PKA inducer, which increases the levels of cAMP. Human osteoclasts were generated from CD14+ monocytes cultured with 25 ng/ml M-CSF and RANKL for 12 days to mature. The mature human osteoclasts were seeded on bovine bone slices for 5 days. The effect of calcitonin (1 nM–100 nM), IBMX and Forskolin (1 μM–100 μM) on bone resorption by osteoclasts was measured as release of CTX-I into the supernatants. The cell viability was measured by the AlamarBlue assay. Bafilomycin A1 (10 nM) was used as a positive control of the bone resorption.

We found that neither IBMX nor Forskolin inhibited bone resorption by the human osteoclasts, whereas calcitonin as expected reduced resorption by 50% ($P < 0.01$). Neither IBMX nor Forskolin showed any toxic effects on the cell viability at the tested concentrations.

In conclusion, the inhibitory effect of calcitonin in human osteoclasts is not restricted to cAMP modulation. This may be in contrast to that observed in chondrocytes suggesting divergent and cell type specific pathways. These findings suggest that further studies are needed to clarify the exact signal transduction of calcitonin in human osteoclasts.

Conflict of Interest: None declared

Tu-P202

VERY LOW CLC-7 CHLORIDE CHANNEL EXPRESSION IS ABLE TO RESCUE FUNCTION OF CLCN7−/− OSTEOCLASTS

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While recessive loss-of-function mutations in the osteoclast chloride channel ClC-7 lead to infantile malignant osteopetrosis (ARO) dominant negative mutations, which statistically reduce the number of functional chloride channel dimers to 25%, evoke the milder Albers-Schoenberg disease (ADOII). As carriers of heterozygous recessive mutations do not have any overt phenotype it is obvious that ClC-7 becomes rate limiting for osteoclast resorption if channel function is reduced to in between 25 and 50%. We recently demonstrated that the osteopetrosis phenotype can be rescued by osteoclast-specific expression of ClC-7 in Clcn7−/− mice under the control of the TRAP-promoter. While transgenic line F1 leads to an intermediate, ADOII-like phenotype with 2-fold increased BV/TV, transgenic ClC-7 expression in F3 was capable to reduce BV/TV almost to wildtype levels. The high BV/TV in F1 rescue animals was due to an increased trabecular number as can be expected for a resorption defect. Although we did not find evidence for an altered cortical mineral apposition rate osteoid thickness and osteoblast numbers were reduced in the trabecular bone of F1 rescue animals.

In order to delineate the threshold at which ClC-7 expression becomes rate-limiting for osteoclast resorption we determined mRNA and protein expression levels. ClC-7 expression was barely detectable in F1 and reduced in F3 rescue animals in immunohistology and in Western blots of osteoclast lysates. This surprisingly low expression was paralleled by a strong reduction of mRNA levels in both rescue lines to app. 1%. No significant differences were found in expression of TRAP or the a3 subunit of the osteoclast proton pump.

Our data demonstrates that already very low expression of ClC-7 in osteoclasts of Clcn7−/− animals is able to rescue the osteopetrotic phenotype. The resulting partially active osteoclasts show an impaired coupling behaviour leading to reduced bone formation, which could be due to unphysiological expression driven by the TRAP-promoter. These results may have implications for gene therapy approaches for osteopetrosis.

Conflict of Interest: None declared

Tu-P203

ASSOCIATIONS BETWEEN BIOMARKERS OF CARTILAGE AND RADIOGRAPHIC FEATURES IN KNEE OSTEOARTHRITIS (KOA)

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The main radiographic features of knee OA are joint space narrowing (JSN), adjacent subchondral sclerosis and pre-sence of marginal ostephytes (OPHs). The diagnosis is expressed as a summary measure -OA grade. In our study we focused on two main features of KOA—OPH and JSW. OPHs are the hallmark of OA and can be seen prior to JSN (Watt & Doherty, 2003). Enhanced production of some collagenous and non-collagenous components of the cartilage matrix may offer early insight into the turnover of joint tissues. AIM of the study was to explore the extent to which the cartilage biomarkers present in serum and/or urine could be associated with radiographic changes in early knee OA. METHODS. We studied 99 women from the Elva population based cohort, aged 34–54 (mean 45) years. BMI (> 26) was observed in 66% of them. Weight-bearing radiographs of the tibio-femoral compartment(TF) according to Spector et al.(1992), and axial radiographs of the patello-femoral(PF) compartment according to Nagaosa et al.(2000) were used to diagnose KOA. The degradation of cartilage was assessed by urinary excretion of the C-telopeptide fragments of type II collagen, U-CTX-II (Nordic Bioscience Diagnostics) and by the serum cartilage oligomeric matrix protein, S-COMP (AnaMar Medical), both measured by ELISA. The level of S-COMP is associated with several structures of the knee joint (Kumm, 2006). Spearman's rank correlation was used for data analysis. RESULTS and COMMENTS. Grade 1 OA was found in more than half (53 %) of the subjects, and grade 2 or 3 OA was found in 9 %. Both biomarkers had weak correlat-ions with TF-OA as a summary radiographic diagnosis, but not with PF-OA (Table 1). In case we segregated the main fea-tures of OA, concentrations of the biomarkers were associ-ated with OPHs in TF compartment, but not with TF JSN. The contribution of PF compartment into pool of S-COMP was not significant, and it was minimal for U-CTX-II. TABLE. As TF OPHs predict also cartilage defects detectable by MRI of the knees (Boegard et al. 1998), the observed correlations between increased production of the cartilage markers and OPHs, may refer to the presence of cartilage defects in some of our patients.

Table 1

RADIOLOGICAL PARAMETERS	S-COMP	U-CTx-II
TF-OA	0.219 ($p = 0.03$)	0.274 ($p = 0.008$)
TF OPH	0.220 ($p = 0.03$)	0.334 ($p = 0.001$)
TF JSN	n.s.	n.s.
PF-OA	n.s.	n.s.
PF OPH	n.s.	0.228 ($p = 0.03$)
PF JSN	n.s.	n.s.

Conflict of Interest: None declared

Tu-P204

MYELOMA CELLS UNDERGO FUNCTIONAL OSTEOCLAST-LIKE TRANSFORMATION IN VITRO THROUGH THE ALPHAVBETA3 INTEGRIN ACTIVATION

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Accelerated osteoclastogenesis is a major event for the skeletal destruction in multiple myeloma (MM) and marrow osteoclasts (OC) are directly activated by myeloma cells (MC). MC may undergo to functional OC-like transformation and produce bone erosion in vitro. Since OC promote osteoclastogenesis through activation of many adhesion molecules as AvB3, we investigated the role of this integrin expressed by MC in their OC-like activity in vitro.

Marrow MC were purified from 8 patients with skeletal disease (group A) and from 2 without bone lesions (group B). U266 and peripheral monocyte-derived OC were the control. Semi-nested PCR assessed the CDR3 rearrangement whereas OC markers as TRAcP, cathepsin-k, vATPase and RANK were evaluated by PCR. The rearrangement of F-actin was analyzed by IF. AvB3 expression was evaluated on MC by flow whereas bone erosion by measuring number of pits on dentine discs. The effect of AvB3 on OC function was investigated by evaluating the phosphorylation and activation of both ERK1/2 and either cFos or NFATc1. The role of AvB3 in OC-like transformation was explored in MC by B3 siRNA.

Derivation of MC from the B-cell lineage was confirmed by the monoclonal CDR3 rearrangement and Pax-5 down-regulation. Cells from A expressed OC markers similarly to OC in contrast with those of B. Rearrangement of F-actin confirmed the differentiation of MC to the OC-like phenotype. Cells from A expressed AvB3 ($85 + 7\%$) similarly to U266 and OC whereas a minimal expression occurred in B ($6 + 2\%$). AvB3+ MC produced a high number of pits at variance from AvB3- MC ($35 + 8$ vs $4 + 1$ pits/cm^2). Highest phosphorylation of ERK1/2 and cFos transcription were revealed in A as compared to B ($p < 0.0001$) in parallel with NFATc1 over-expression. Finally, the $\beta 3$ silencing inhibited the erosion by AvB3+ MC with reduced number of pits ($7 + 2$ pits/cm^2) with a pattern similar to AvB3- MC. Inhibition of ERK1/2 and cFos was demonstrated in silenced MC from A with values similar to B. RANK and fms were negative in both groups.

Since AvB3 drives adhesion of OC to extracellular matrix resulting in activation of osteoclastogenesis, it is conceivable that AvB3+ MC acquire a OC-like behaviour after their transformation into functional OC induced by the contact with stromal cells within microenvironment. Our data suggest that MC may drive OC-like functions by AvB3 and independently of RANK and fms. This is supported by the B3 silencing that reduces the OC-like activity in vitro.

Conflict of Interest: None declared

Tu-P205

THE NOVEL BIPHENYL KETONE ABD345 INHIBITS NF-KAPPA-B ACTIVATION, INFLAMMATION AND JOINT DESTRUCTION IN COLLAGEN INDUCED ARTHRITIS

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We have previously shown that the butanediol ester of biphenyl-carboxylic acid (ABD56) inhibits bone resorption by inhibiting RANKL- and TNF-induced NFκB signaling. As TNFα and RANKL are important mediators of inflammation and bone destruction in diseases such as rheumatoid arthritis (RA), ABD56 and related compounds might inhibit both the inflammation and bone loss in RA. We therefore investigated the effects of ABD345, a more potent and metabolically stable derivative of ABD56, in the collagen induced arthritis (CIA) mouse model of RA.

DBA1 mice were injected intradermally at the base of the tail with chicken collagen type II in complete Freund's adjuvant. Once the first signs of paw swelling were observed (typically after 2 weeks), treatment was started, and the progression of the inflammation was measured by scoring the level of paw swelling. After 3 weeks of treatment, the animals were killed, and the paws analysed by μCT analysis using a Skyscan 1172 μCT scanner. Data are presented as mean ± SEM, $N = 10$ per group.

Like ABD56, ABD345 potently inhibited TNF- and RANKL-induced NFκB activation in bone marrow macrophage cultures and prevented RANKL induced osteoclast formation. However, ABD345 (IC50: 1 μM) was considerably more potent then ABD56 (IC50: 18 μM). In the CIA model, treatment with ABD345 lead to a dose dependent inhibition of inflammation. Inflammation was almost completely absent at a dose of 10 mg/kg/day ($95 \pm 3\%$ inhibition of paw swelling, $p < 0.001$). Treatment with 5 mg/kg/day and 2 mg/kg/day lead to a $79 \pm 15\%$ ($p < 0.01$) and $49 \pm 23\%$ ($p = 0.14$) decrease of the score respectively. When the paws were scanned using the μCT scanner, the vehicle control group displayed severe bone erosions near the affected joints, whereas these erosions were virtually absent in the 10 mg/kg ABD345 treated group and strongly reduced in the 5 mg/kg group.

In conclusion, ABD345 is a potent inhibitor of inflammation and joint destruction in the CIA model of RA.

Conflict of Interest: Rob van't Hof, Stuart Ralston, Iain Greig and Aymen Idris are named inventors on patents for the use of ABD56 and ABD345 in the treatment of bone and joint disease. The patents are held by the University of Aberdeen.

Tu-P206

XYLITOL, SUGAR ALCOHOLS, DOWN-REGULATES 1ALPHA,25-DIHYDROXYVITAMIN D3-INDUCED OSTEOCLASTOGENESIS VIA IN PART THE INHIBITION OF RANKL PROTEIN EXPRESSION IN A MOUSE CO-CULTURE SYSTEM

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Xylitol, sugar alcohol has a variety of function to cells, such as bacteriocidal, and anticariogenic effects. However, understanding of cellular mechanism for the role of xylitol on bone metabolism remains to be solved. In this study, we exploited the physiological role of xylitol in osteoclastogenesis in a co-culture system, osteoblastic cells plus RAW 264.7 cell. Xylitol treatment (1,10,30,50, and 100 mM) to the co-culture medium, reduced dose dependently the number of tartrate-resistant acid phosphatase (TRAP) positive multinucleated cells induced by 10 nM 1alpha,25$(OH)_2D_3$. With viability test, we confirmed that there were no cellular damages up to 100 mM of xylitol. In order to investigate the mechanism by which xylitol inhibits 10 nM 1alpha,25$(OH)_2D_3$-osteoclastogenesis, the mRNA expressions of receptor activator of NF-kappaB ligand (RANKL) and osteoprotegerin (OPG) were analyzed by RT-PCR. Exposure of osteoblastic cells to a medium containing of xylitol decreased RANKL mRNA expression induced by 10 nM 1alpha,25$(OH)_2D_3$ in a dose-dependent manner, whereas did not change OPG mRNA. Further, bone resorption activity, which was performed on the bone slice in co-culture system, was dramatically decreased as xylitol concentration was increased. In addition, RANKL and OPG protein were also analyzed with ELISA using anti-RANKL and anti-OPG antibody. The quantity of soluble RANKL (sRANKL) protein was decreased with the increase of xylitol concentration, whereas the OPG protein was not changed at the whole range of xylitol concentration. Thus, these results imply that xylitol inhibits osteoclast differentiation by reducing sRANKL/OPG ratio in osteoblastic cell.

Conclusively, these findings suggest that xylitol could inhibits bone resorption via the suppression of RANKL expression from the osteoblastic cell.

Conflict of Interest: None declared

Su-P207

TRANSCRIPTIONAL PROFILE OF IMMUN SYSTEM-RELATED GENES IN POSTMENOPAUSAL OSTEOPOROTIC VERSUS NON-OSTEOPOROTIC HUMAN BONE TISSUE

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Introduction: The functional interaction between the immune system and bone metabolism has been established both in molecular and cellular levels. We have used non-paramentric and multidimensional expression pattern analysis to determine significantly changed mRNA profile of immune system-associated genes in postmenopausal osteoporotic vs. non-osteoporotic bone tissue. These marked changes might result different local immune regulation processes.

Materials and methods: Seven bone tissue samples from postmenopausal osteoporotic patients (age range: 56–75 years, T-score < -2.5 SD) and ten bone tissue samples from non-osteoporotic healthy women (age range: 55–77 years, T-score > -1.5 SD) were examined in our study. The mRNA transcription differences of selected 44 genes were analyzed in Taqman probe-based quantitative real-time RT-PCR system. Statistical evaluation was performed utilizing Mann-Whitney U test and canonical variates analysis (CVA).

Results: Mann-Whitney U test indicated significantly ($p \leq 0.09$) down-regulated expression activity of 6 genes (FCGR2A, ITGAM, SCARA3, IL6, NFKB1 and TGFB3) in osteoporotic bone tissue which have prominent role in (antibody) clearance, phagocytosis, pathogen recognition and inflammatory response. Applying CVA the groups of postmenopausal osteoporotic and non-osteoporotic women are separable by 14 genes coding for cytokines, co-stimulator and cell surface receptor molecules affected in T-cell dependent activation which have the best discriminatory power.

Conclusion: Based on a complex transcription pattern of the selected 44 genes in human bone cells, we could distinguish osteoporotic and non-osteoporotic states from an immunological aspect. Our data might provide further insight into the changes of the inter-system crosstalk between immune and bone tissue, as well as local immune response in the altered microenvironment of osteoporotic bone.

Conflict of Interest: None declared

Su-P208

CALCIUM-SENSING RECEPTOR GENE "A986S" POLYMORPHISM: GENOTYPE FREQUENCIES AND RELATION TO BONE MINERAL DENSITY IN A SPANISH EARLY POSTMENOPAUSAL WOMEN POPULATION

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Background: Calcium-sensing receptor (CaSR) is a candidate gene for osteoporosis susceptibility. There are only a limited number of studies available regarding the relationship between the CaSR gene A986S polymorphism and bone mineral density.

Objectives: 1) To assess the distribution of genotype frequencies of CaSR gene A986S polymorphism in a Spanish population of early postmenopausal women. 2) To evaluate whether CaSR gene A986S polymorphism affects bone mineral density.

Subjects and methods: 158 women with natural menopause were randomly selected in the province of Albacete, Spain (mean age 52.7 ± 1.6 yr). They did not have any diseases to affect bone metabolism. Genomic DNA was extracted from peripheral blood leukocytes by the Higuchi method. A fragment of exon 7 of CaSR gene containing the A986S polymorphism was amplified by polymerase chain reaction (PCR). After amplification, all samples were digested whit Bsa H1 restriction enzymes, and the fragments were separated by agarose gel electrophoresis. Lumbar spine BMD (L2–L4) was measured by DXA.

Results: The distribution of genotype frequencies of CaSR gene A986S polymorphism was: AA, 121 (76.6%); AS, 34 (21.5%), and SS, 3 (1.9%). We found no association between CaSR gene A986S polymorphism and BMD. Women with the AS genotype had lower BMD than those with genotype AA, but there was no significant difference (AS, 0.880 ± 0.118; AA, 0.931 ± 0,141 g/cm^2; $p = 0{,}301$).

Conclusions: 1) The distribution of CaSR genotypes in this Spanish women population was similar to other European women population. 2) We found no association between CaSR gene A986S polymorphism and BMD.

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Conflict of Interest: None declared

Su-P209

EP2/EP4 MEDIATED PGE2 SIGNALLING INCREASES GENE EXPRESSION AND THE SYNTHESIS OF OSTEOPROTEGERIN (OPG) AND RECEPTOR ACTIVATOR OF NF-KAPPA B LIGAND (RANKL) IN HUMAN OSTEOARTHRITIC CHONDROCYTES

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Aims: Cartilage degeneration is the central feature in osteoarthritis (OA), but it is associated with concomitant changes in all the structures of the joint, in particular the subchondral bone. Articular chondrocytes express and synthesize OPG, RANK and RANKL. Our aim was to explore the role of PGE2, the eicosanoid found at the higher concentration in OA joints, in the expression and synthesis of OPG and RANKL in human osteoarthritic chondrocytes (HOC) in culture.

Methods: HOC were obtained from the joint specimens of OA patients who underwent total knee replacement surgery. The gene expression of OPG and RANKL were assessed in HOC stimulated with PGE2 by quantitative PCR, and the corresponding protein synthesis was studied by western-blot. We also examined which of the four different PGE2 receptors (EP receptors) was involved in the PGE2 action using specific EP agonists.

Results: PGE2 elicited a dose and time-dependent increase in the gene expression and protein synthesis of OPG, with a peak at 24 hours of incubation (gene expression: 2.8-fold increase; protein: 3.5-fold increase, both for 10–6M PGE2 vs. unstimulated HOC). Exposure to PGE2 also resulted in a dose and time-dependent increase in the presence of RANKL, peaking at 24 h of incubation (gene expression: 8-fold for 10–6M PGE2 vs. unstimulated HOC). Although the levels of RANKL were increased for all the doses of PGE2 tested, compared to unstimulated HOC, the amount of RANKL anchored to cell membrane was inversely proportional to the amount of PGE2 in the culture medium, with a peak at 10–8M PGE2 (20-fold vs. unstimulated HOC). 11-Deoxy-PGE1, an EP2/EP4 agonist, reproduced PGE2 actions on OPG and RANKL expression. Butaprost, a selective EP2 agonist was less potent than PGE2. Sulprostone, an EP1/EP3 agonist, had no effect on OPG neither RANKL expressions. An inhibitor of adenylate cyclase completely abolished the up-regulation of OPG and RANKL exerted by PGE2, pointing to an EP2/EP4 dependent signalling, that requires cAMP for this action.

Conclusions: The activation of EP2/EP4 receptors up-regulated the expression and synthesis of OPG and, to a significantly higher extent, those of RANKL in HOC. These data suggest that PGE2, a key mediator in the development of OA, could regulate the in vivo expression and the release of the key mediators of bone metabolism by articular chondrocytes. This mechanism, still unknown, would allow the chondrocytes to control the activity of subchondral bone cells.
Conflict of Interest: None declared

Su-P210

NSAID TREATMENT DECREASES COX-2 AND MPGES-1 PRODUCTION BY OSTEOARTHRITIC CHONDROCYTES OF PATIENTS WITH SEVERE KNEE OSTEOARTHRITIS

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Aims: To simultaneously study the effect of a selective COX-2 inhibitor and that of a traditional NSAID on the expression of pro-inflammatory genes in the articular cartilage of patients with severe knee osteoarthritis (OA) and in human osteoarthritic chondrocytes in culture.

Methods: A 3-month controlled, open clinical trial was carried out on 30 patients with severe knee OA scheduled for total knee replacement surgery. They were randomized in two groups: patients treated with celecoxib (CBX) (200 mg/24 h) and patients treated with aceclofenac (ACF) (100 mg/12 h). Patients with OA who did not wish to be treated with NSAIDs served as the control group (CTR). After knee surgery, the articular cartilage was processed for molecular studies performed by western blot and real time PCR. In vitro studies were also conducted in chondrocytes isolated from OA joints. At second passage, these cells were used to examine the effects of CBX and ACF on proinflammatory gene expression in cells stimulated with 10 u/ml IL-1b.

Results: The gene expression of COX-2, mPGES-1 and iNOS was lower in the joint cartilage from patients treated with CBX and ACF. In the same way, at the protein level there was a reduction in COX-2 (CBX 0.3 ± 0.1*; ACF 0.8 ± 0.3; CTR 2.1 ± 0.6; *$p < 0.05$ vs. CTR), mPGES-1 (CBX 0.3 ± 0.1*; ACF 0.3 ± 0.1*; CTR 1.0 ± 0.4; *$p < 0.05$ vs. CTR) and iNOS (CBX 0.4 ± 0.1*; ACF 0.3 ± 0.1*; CTR 2.2 ± 0.3; *$p < 0.05$ vs. CTR) in cartilage of OA patients. In cultured OA chondrocytes, we observed that both NSAIDs decreased the COX-2 and mPGES-1 synthesis as well as the PGE2 release induced by IL-1b. On the other hand, no effect was observed on NO or iNOS synthesis. With respect to the proinflammatory cytokines TNFa and IL-1b, which are involved in joint destruction, only CBX decreased the expression of both molecules (for TNFa: CBX 1.0 ± 0.1*&; ACF 3.6 ± 0.8; CTR 3.4 ± 0.8; *$p < 0.05$ vs. CTR; & $p < 0.05$ vs. ACF; for IL-1b: CBX 1.9 ± 0.6*&; ACF 3.4 ± 1.1; CTR 4.3 ± 0.7; *$p < 0.05$ vs. CTR; & $p < 0.05$ vs. ACF). However, both NSAIDs down-regulated IL-1b expression induced by cytokines in cultured OA chondrocytes.

Conclusion: Both NSAIDs diminished PGE2 release and unexpectedly, induced a decrease in COX-2 and mPGES-1 gene expression. Even more, prolonged therapy with PGE2 blocking agents decreases PGE2 production not only by the direct inhibition of COX-2 activity, but also down-regulating COX-2 and mPGES-1 expression and synthesis in the articular cartilage.
Conflict of Interest: None declared

Su-P211

CHARACTERISATION OF A NEW MOUSE LINE DISPLAYING OSTEOARTHRITIS, CHONDRODYSPLASIA AND ECTOPIC BONE FORMATION

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We utilise the Munich N-ethyl-N-nitrosurea (ENU) mutagenesis program to identify and characterise genes and alleles that regulate bone and cartilage development and homeostasis in mouse. The *ALI34* (abnormal limb) autosomal dominant mutant line was isolated, indicating features for early-onset osteoarthritis (OA), chondrodysplasia and ectopic bone formation in the adult stage resulting presumably from anomalies within chondrification. Here, we present preliminary results on *ALI34* focusing on the bone and cartilage phenotype and the genetic mapping of the *ALI34* locus. 8-week-old *ALI34/+* show a shorter tibia and fibula with a stronger pronounced bending, while the fibula is misarranged to subchondral epiphysial bone struts at the tibia. A decrease of metaphyseal trabecular bone in the femur was verified. Furthermore, the growth plate shows disorganization in the avascular zone. Articular cartilage of *ALI34* lacks the transitional zone, whereby OA-like degeneration is evident. Possible *ALI34* homozygous embryos occasionally show calvarial aplasia and malformed or splitted thoracic and lumbar vertebral bodies. In addition, embryos show a crooked arrangement of sternal bones. Aberrant bone formation in the hindlimbs is not evident at this age. Linkage analysis of the *ALI34* locus reveals a 3Mb region on chromosome 6. In summary the *ALI34* mutation suggests a novel mechanism involved in cartilage and bone development and preservation. Further studies are planned unravelling the *ALI34* mutation and focusing on the developmental process of the observed bone phenotypes.

Conflict of Interest: None declared

Su-P212

TRANSCRIPTIONAL REGULATION OF THE XENOPUS LAEVIS MATRIX GLA PROTEIN GENE

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Matrix Gla Protein (MGP) belongs to the family of vitamin K-dependent Gla proteins and is known to be involved in regulation of extracellular matrix calcification and maintenance of cartilage and soft tissue integrity during growth and development. In order to understand the regulation of Xenopus laevis MGP (XlMGP) gene expression we have cloned and characterized two functional XlMGP promoters. Sequence analysis of the promoters revealed the presence of putative binding sites for Runx family of transcription factors that are known to regulate chondrocyte maturation and differentiation. Using promoter-reporter assays we have shown that Runx2 significantly transactivates the XlMGP promoters by at least 2 fold indicating that XlMGP is a potential downstream target of this factor. This up-regulation was abrogated when the Runx2 responsive elements on the XlMGP were mutated. Finally, we show that Runx2 specifically binds to these DNA elements in the XlMGP promoters. Thus our results provide, for the first time, clear evidence for a direct XlMGP transcriptional regulation through the bone and cartilage related transcription factor Runx2.

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Conflict of Interest: None declared

Su-P213

PROGNOSTIC AND THERAPEUTIC VALUE OF AUTOSOMAL RECESSIVE OSTEOPETROSIS GENETIC DISSECTION

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Autosomal recessive osteopetrosis in humans represents a heterogeneous group of diseases, including osteoclast-rich and osteoclast-poor forms. The osteoclast-rich form, in which a normal or even elevated number of non-functional osteoclasts is present, is due to defects in genes involved in the osteoclast effector function (TCIRG1, ClCN7, OSTM1, PLEKHM1). The osteoclast-poor form, in which no mature osteoclasts are present, has remained poorly understood until recently, when our group identified RANKL mutations in some patients.

The individuation of the exact molecular defect not only allows a precise and early diagnosis but also identifies subsets of patients with different features, response to therapy and prognosis. We have been able to show that among the various ARO subsets, the TCIRG1-dependent form is quite homogeneous in presentation, is not associated to primary defect in the CNS and responds to BMT, if a suitable donor is available. In addition, the earlier the transplant, the better the result and studies in the oc/oc mouse which bears the same defect, suggest that this form is potentially curable if diagnosed and transplanted in utero. Biallelic abnormalities in CLCN7 and OSTM1-dependent forms, on the contrary, are associated with severe primary involvement of CNS, have a very poor prognosis and BMT is not deemed appropriate, since the CNS defects are not cured even when the hematological defect is ameliorated. In contrast, all the RANKL patients described so far are still alive, although with severe stigmata, but those who had been transplanted did not benefit from BMT. RANKL-dependent patients are not expected to be cured by transplantation of hematopoietic stem cells alone, since although some RANKL is probably produced by T cells in specific occasions, the defect is mainly in the osteoblasts which originate from mesenchymal (stromal) stem cells (MSC). This suggests that RANKL-dependent patients could benefit from transplantation of purified (and in vitro expanded) MSCs, but this possibility must be tested in experimental models and in humans, since so far MSCs are still quite elusive. RANKL-dependent ARO is unique among osteopetroses since the cell defect is non-autonomous and this could pave the way to a replacement therapy which could theoretically provide a complete rescue of the phenotype. In conclusion, the identification of specific subsets of patients is not only of biological significance but also of clinical relevance.

Conflict of Interest: None declared

Su-P214

NEW MOUSE MODELS AND MECHANISMS FOR BONE AND CARTILAGE DISORDERS

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Background: The aim of the Dysmorphology, Bone and Cartilage Screen of the German Mouse Clinic (GMC) is the identification and characterization of mouse models for bone related human diseases like osteoporosis, osteoarthritis, osteogenesis imperfecta, scoliosis or limb defects. Our screen takes over the analysis of mouse mutants for medically relevant bone and cartilage parameters and supports the discovery of underlying genes.

Methods: We have implemented an experimental set-up utilizing DXA, X-ray imaging, a 54-parameter protocol for the rapid morphological observation of animals, micro-CT and pQCT, markers of bone metabolism and hormonal regulation, fracture/stress parameters and an osteoblast cell culture system to describe potential cellular causes of bone diseases.

Results: Since the beginning of the German Mouse Clinic (GMC) the Core Facility provided 83 mutant mouse lines and 19 inbreeding or hybrid lines for the primary screen. Most of the mutant lines have already finished the phenotypic analysis in the Bone and Cartilage module. In 19 mutant lines, a bone specific phenotype was known before the GMC screen, and we could confirm all of them. In 16 lines out of the 19 lines, we were able to detect additional phenotypes. In 13 mutant lines where no bone phenotype was known, we detected new phenotypes. In 15 mutant lines, we found few subtle changes with unknown scientific relevance. 31 mutant lines did not show any alterations in bone parameters. We were able to characterize new mouse models for Osteogenesis imperfecta, inflammatory arthritis, osteoarthritis and osteoporosis. For example, the *Aga2* mutant mouse line represents a new murine model for type II osteogenesis imperfecta. We provide evidence that the *Col1a1*^{Aga2} mutation initiated an endoplasmic reticulum stress-specific cascade involving caspases 12 and 3 causing apoptosis of osteoblasts. We recently published the *Ali18* mouse mutant line, the first non-induced mouse model for psoriatic arthritis, dermatitis and osteoporosis. *Ali18* mice exhibit rubor and swelling of footpads in hindlimbs in adults. Histological analysis revealed infiltration of mixed populations of inflammatory cells into bone marrow, peripheral joints, and skin in the affected areas of *Ali18* mice.

Conclusion: The Dysmorphology, Bone and Cartilage Screen of the GMC is an efficient and powerful platform to identify and characterize new mouse models for bone related human diseases.

Conflict of Interest: None declared

Su-P215

GENOTYPES AND HAPLOTYPES OF THE PPARGAMMA GENE ARE ASSOCIATED WITH OSTEOPOROTIC FRACTURE THROUGH MOLECULAR HETEROSIS

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Osteoporosis is a common disorder with a multifactorial pathogenesis. Stimulation of PPARgamma forces mesenchymal stem cells to develop in an adipocyte direction at the expense of osteoblasts leading to decreased osteoblasts number and BMD. Previously, polymorphisms of the PPARgamma-gene have been associated with osteoporosis.

The aim of this study was to thoroughly investigate the effect of PPARgamma polymorphisms on BMD and the risk of vertebral fractures. On the basis of patterns of linkage disequilibrium between SNPs throughout the PPARgamma gene we choose 17 polymorphisms including the C161T and pro12ala polymorphisms for this investigation.

The polymorphisms were investigated in a case-control study comprising 462 osteoporotic patients and 336 controls. Polymorphisms were examined using Taqman assays and BMD was examined by DXA.

rs1373641 A/G, rs4135263 T/C and rs1151999 A/C were found to be significantly associated with osteoporotic fracture ($p < 0.05$ for all). The increased fracture risk was mainly found in individuals heterozygous for the polymorphisms. The frequencies of heterozygosity were higher in patients with osteoporostic fractures versus normal controls: rs4135263: 29.9 % vs 21.9 %, $P = 0.03$ rs1151999: 54.0 % vs 41.0 %, $P = 0.002$ and rs1373641: 44.7 % vs. 37.8 %, $P = 0.098$. BMD was lower in heterozygous individuals; however the difference did not reach statistical significance for any of the polymorphisms. The three polymorphisms were in strong linkage disequilibrium ($\acute{D} = 1.0$, $P < 0.002$).

None of the other polymorphisms were associated with fracture risk, BMD or biochemical markers of bone turnover.

Molecular heterosis, i.e. the phenomenon that only heterozygosity for a given genotype influences a certain trait has been described for a number of human conditions including the effect of the PLOD1 polymorphisms on lsBMD and fnBMD. We performed haplotype analyses and explored the influence of heterozygosity of the genotypes on fracture risk. We found that patients with osteoporotic fractures were significantly more likely to be heterozygous in 1 or 2 of the genotypes compared with normal controls ($P = 0.002$).

On basis of these results, we suggest that polymorphisms of the PPARgamma gene affect the risk of osteoporotic fracture through molecular heterosis.

Conflict of Interest: None declared

Su-P216

POLYMORPHISMS IN THE CHEMOKINE RECEPTOR 3 GENE ARE TESTED FOR ASSOCIATION WITH BONE MINERAL DENSITY VARIATION IN A FAMILY-BASED STUDY

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Previous studies have observed suggestive evidence of linkage between low bone mineral density (BMD) and the 3p22–21.1 locus. The chemokine receptor 3 (CCR3) gene, located at 3p21.3, has been linked to a novel pathophysiological mechanism for osteoporosis involving the increased recruitment of monocytes into the bone microenvironment (1). We investigated whether polymorphisms in the CCR3 gene are associated with variation in BMD at the lumbar spine (L2–L4) and femoral neck in a Caucasian family-based study.

Haploview was used to determine CCR3 linkage disequilibrium structure and select tagSNPs. The Kbioscience Genotyping service was employed to determine the SNP genotypes in 551 individuals from 249 families (192 pedigrees) ascertained through probands with low BMD ($T < -1.5$). Genotypes were tested for Mendelian errors and Hardy-Weinberg Equilibrium using PEDSTATS. The Kolmogorov-Smirnov test implemented in SPSS v12 was used to test for normal phenotype distribution. We used a combination of MERLIN, QTDT and P2BAT to test for linkage, total association and family-based association between SNPs and haplotypes with variation in BMD at the lumbar spine and femoral neck.

Four tagSNPs captured 83.3% of the HapMap validated variation across the CCR3 gene. None of the SNPs significantly deviated from HWE. Age, height, weight and sex were included as covariates in all subsequent statistical analysis. There was no evidence of linkage between the CCR3 SNPs and BMD variation using Merlin (LOD

scores < 1.0). Suggestive evidence of within family association was observed between the CCR3_03 SNP with variation in lumbar spine BMD (P = 0.05). The CCR3_02/03 (GA) haplotype (26.3%) approached significant association at the lumbar spine (P = 0.05). However, the estimated SNPSpD experiment-wide significance threshold to correct for multiple testing requires a p-value of < 0.02 to keep the type I error rate at 5.0%.

To our knowledge, this is the first study to investigate the possible association between CCR3 SNPs and BMD variation in a Caucasian cohort. There was suggestive evidence of association between the CCR3 SNPs and variation in BMD at the lumbar spine, though replication in a larger cohort is needed to support these results.

1. Liu et. al., 2005 J Biol. Chem. Aug 12;280(32):29011–6

Conflict of Interest: None declared

Su-P217

THE T29C POLYMORPHISM IN THE SIGNAL PEPTIDE OF TRANSFORMING GROWTH FACTOR-BETA1 INCREASES SECRETION IN VITRO

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Osteoporosis is a common age-related bone disease. The pathogenesis is multifactorial but genetic factors are known to exert a significant influence. Transforming growth factor (TGF)-β1 is one of the most extensively studied candidate genes. It is expressed by osteoblasts and is involved in the control of bone formation and resorption. Some studies have suggested that the T29C variant, which causes an amino acid change from leucine to proline at codon 10, is associated with increased bone mineral density. Furthermore, an association has been found between this variant and increased serum level of TGF-β1. The polymorphism is located in the signal peptide and it could be speculated that the clinical effects seen could be due to increased TGF-β1 secretion. The aim of this study was to investigate whether the T29C variant affects TGF-β1 secretion *in vitro*.

Wild-type (wt) and T29C variant TGF-β1 cDNA was cloned into the pcDNA3.1+ expression plasmid. Three independent clones of both the wt and the T29C variant and an empty pcDNA3.1+ control vector were used for transfection of mammalian cell lines. TGF-β1 secretion was measured by ELISA.

In HEK 293 cells we did not find any difference in TGF-β1 secretion between cells transfected with wt and variant constructs (n = 27). This was surprising because a previous study using similar constructs but HeLa cells has demonstrated a 2.8 fold increased secretion of the variant. To confirm these results we also transfected HeLa cells and found a 1.39 ± 0.03 fold increased secretion of the variant ($p < 0.001$, n = 27). Because there seemed to be a difference between cell lines we also transfected human osteosarcoma cell lines. In U2OS cells the secretion of the variant was increased by 14 ± 6 % (p = 0.02, n = 27) compared to the wt and in SaOS-2 cells it was increased by 80 ± 7 % ($p < 0.001$, n = 27). The differences found between cell lines may be explained by differences in the ability of the cell lines to process and transport TGF-β1.

In conclusion, we found that the substitution of leucine with proline in position 10 in the TGF-β1 signal peptide is associated with significant increased secretion of TGF-β1 in several cell lines including osteosarcoma cell lines. We speculate that this increased secretion may be causing the previously demonstrated clinical effects of this polymorphism.

Conflict of Interest: None declared

Mo-P218

POLYMORPHISMS IN THE PROMOTER REGION AND INTRON 1 OF THE ESTROGEN RECEPTOR ALPHA GENE INCLUDING A NOVEL GLUCOCORTICOID RESPONSIVE SITE ARE ASSOCIATED WITH BONE MINERAL DENSITY IN 75-YEAR OLD SWEDISH WOMEN

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It is well recognized that estrogen plays an important role in the maintenance of bone mass. Polymorphisms in the estrogen receptor alpha [ESR1] have been associated with BMD previously, but results have been conflicting, differing between populations studied. The purpose of this study was to evaluate the association between polymorphisms covering a 38 kbp proportion of the promoter region of the ESR1 gene, including a novel polymorphism recently showed to be glucocorticoid responsive, to variation in BMD. We investigated the genotypes for the novel ERNE-145, the PvuII, XbaI and promoter TAn repeat polymorphisms of the ESR1 gene in subjects from a large, well characterized population based cohort of 1044 Swedish women all aged 75, of the Malmö Osteoporosis Prospective Risk Assessment (OPRA) study.

Of the studied SNPs, the ERNE-145 was associated to adjusted BMD of the lumbar spine L2L4 (p = 0.01), and femoral neck ($p < 0.05$). The PvuII polymorphism was associated to adjusted BMD of the lumbar spine ($p < 0.05$), and femoral neck ($p < 0.05$). The XbaI polymorphism had no statistically significant association to adjusted BMD at any site. For the TAn repeat polymorphism when divided into short (e) or long repeats (E) according to the naturally occurring repeat lengths, long alleles had a 5% lower BMD (p = 0.001) and 3% lower BMD at the femoral neck (p = 0.03). A trend for effects was seen for ultrasound parameters. In conclusion, BMD in elderly women, particularly of the spine, is related to ESR1 genotype, including a novel glucocorticoid responsive element (ERNE-145).

Conflict of Interest: None declared

Mo-P219

LRP5 A1330V POLYMORPHISM IS ASSOCIATED WITH HIP BMD IN OSTEOPOROTIC MEN, BUT NOT WITH RESPONSE TO BISPHOSPHONATE TREATMENT

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Polymorphisms of the LRP5 gene have been found to contribute to the variation in bone mass and fracture risk in both men and women but the relationship between LRP5 polymorphisms and response to treatment of osteoporosis is unknown. In this study we examined the relationship between common polymorphisms of LRP5 and BMD and also evaluated the relationship between LRP5 polymorphisms and the therapeutic response to treatment with the bisphosphonate risedronate. The study subjects comprised a subgroup of 249 men who were participants in a randomised double blind placebo-controlled trial of risedronate treatment for osteoporosis and who consented to provide samples for genetic analysis. Patients received risedronate 35 mg

once-a-week or placebo for 24 months and were also given daily supplements of calcium and vitamin D. Bone mineral density was measured by DXA at the lumbar spine (LS), total hip (TH), femoral neck (FN) and trochanter (TR) at baseline and after 6, 12 and 24 months follow-up. We analyzed three polymorphisms of LRP5 gene: *V667M* (rs4988321), *A1330V* (rs3736228), *IVS4-4T/C* (rs314776), and the genotypes were in Hardy-Weinberg equilibrium. The *A1330V* polymorphism was associated with BMD at baseline for all hip measurement sites but there were no differences at LS. Patients with *1330Val/Val* genotype (n = 14) had 10.8% higher BMD at TH compared with the other genotype groups (n = 224) (P = 0.009). Corresponding values for FN and TR were 10.3% (P = 0.016) and 14.1% (P = 0.002) respectively. There were no significant association between the other LRP5 SNPs and BMD at baseline. We also studied the relationship between LRP5 alleles and response to treatment with risedronate (n = 156) but found no significant association between any SNP and change in BMD. We conclude that the LRP5 *A1330V* polymorphism is associated with hip but not lumbar spine BMD in osteoporotic males confirming the importance of this candidate gene in the regulation of bone mass. However we found no evidence to suggest that LRP5 alleles are associated with response to risedronate treatment in this group of osteoporotic men.

Conflict of Interest: Stuart H. Ralston, P&G Pharmaceuticals, consultant

This study was supported by P&G Pharmaceuticals research grant

Mo-P220

VDR FOKI POLYMORPHISM IS ASSOCIATED WITH BMD AND AN IMPAIRED RESPONSE TO RISEDRONATE TREATMENT IN OSTEOPOROTIC MEN

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Polymorphisms of the VDR gene have been widely studied as possible genetic predictors of BMD and fracture risk. In a previous study of 24 postmenopausal women the *BsmI* polymorphism was reported to be associated with BMD response to etidronate therapy. In this study we examined whether VDR polymorphisms might play a role in regulating BMD and the response to treatment with the bisphosphonate risedronate in osteoporotic males. The study subjects comprised a subgroup of 249 men who were participants in a randomised double blind placebo-controlled trial of risedronate treatment for osteoporosis and who consented to provide samples for genetic analysis. Patients received risedronate 35 mg once-a-week or placebo for 24 months and were also given daily supplements of calcium and vitamin D. Bone mineral density was measured by DXA at the lumbar spine (LS), total hip (TH), femoral neck (FN) and trochanter (TR) at baseline and after 6, 12 and 24 months follow-up. We conducted genotyping for the *Cdx2* (rs11568820), *FokI* (rs10735810), *BsmI* (rs1544410), *ApaI* (rs 7975232) and *TaqI* (rs731236) polymorphisms of the VDR gene. Genotypes for all SNPs were in Hardy-Weinberg equilibrium. There was a significant association between baseline LS-BMD and the VDR *Cdx2* and *FokI* polymorphisms. Homozygotes for the *Cdx2* AA genotype (n = 8) had 11.7% higher LS-BMD compared with the other genotype groups (n = 230; P = 0.02) whereas homozygotes for the *FokI* CC genotype (n = 71) had a 3.5% lower LS-BMD than the other genotype groups (n = 168; P = 0.017). We also found a significant association between the *FokI* SNP and response to risedronate treatment; LS-BMD increased by 4.6% ± 0.7 (mean BMD change from baseline ± SEM) in *FokI* CC homozygotes (n = 46) compared with 6.2% ± 0.4 in the other genotype groups (n = 110; P = 0.02). Moreover, the *FokI* CC genotype was found to be an independent predictor of the change in LS-BMD by linear regression analysis (P = 0.02), contributing to 3.3% of the variance in response when additional factors such as age, weight, height and baseline BMD were taken into account. There were no significant differences in response to the therapy in relation to other studied SNPs. We conclude that the response to risedronate treatment in men who are homozygous for the *FokI* C allele is reduced by about 26% as compared with other genotype groups. This suggests that *FokI* polymorphism genotyping might be of value in predicting the response to risedronate therapy in osteoporotic males.

Conflict of Interest: Stuart H. Ralston, P&G Pharmaceuticals, consultant

This study was supported by P&G Pharmaceuticals research grant

Mo-P221

SINGLE NUCLEOTIDE POLYMORPHISMS IN NEW CANDIDATE GENES ARE ASSOCIATED WITH BONE MINERAL DENSITY AND FRACTURE RISK

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Background: Osteoporosis (OP) is a multifactorial disease with high heritability but its exact genetic background is still poorly understood. We examined the effect of twenty four single nucleotide polymorphisms (SNPs) located in five genes (ALPL, FABP3, FGFR1, MMP2, TIMP2) previously not associated with OP.

Methods: 360 Hungarian postmenopausal women were involved in the study. Bone mineral density was determined at three skeletal sites (spine, hip and distal radius). Genomic DNA was extracted from venous blood samples and a high-throughput genotyping method based on single-based primer extension was applied for allelic discrimination. Robust statistical tools were utilized for multiplex data analysis.

Results: SNP rs6996321 in FGFR1 was significantly related to adjusted spine BMD (p = 0.002, power = 0.893) and rs10914367 in FABP3 was associated with adjusted hip BMD (p = 0.028, power = 0.86). Fracture risk was significantly increased in carriers of 'A' allele of rs9900972 in TIMP2 (OR = 2.06, p = 0.018, power = 0.877). We could also identify validated gene-gene interactions significantly affecting BMD and fracture risk.

Conclusions: We identified previosuly not reported SNPs in five genes associated with bone mineral density or risk for osteoporotic fractures. Multiplex genotyping and data processing methods including haplotype- and gene-gene interaction analyses might contribute to the understanding of the pathogenesis of this multifactorial disease.

Conflict of Interest: None declared

Mo-P222

RANDOMIZED DOSE COMPARISON OF PAMIDRONATE IN CHILDREN WITH TYPES III AND IV OSTEOGENESIS IMPERFECTA: 3 VS 6 MONTH CYCLES

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Background/aims: Controlled trials of bisphosphonates in children with osteogenesis imperfecta (OI) demonstrated significant increases in vertebral BMD, height and area, while effect on fracture rate is

controversial. High cumulative doses of pamidronate are associated with side effects, including abnormal bone modeling, decreased bone material properties, slow osteotomy healing and jaw osteonecrosis. The purpose of this study is to determine whether the vertebral benefits of q3m infusion cycles can be attained on q6m cycles, with a lower cumulative dose.

Methods: Twenty-seven children with types III and IV OI were randomly assigned to receive 1 mg/kg/3d IV pamidronate in q3 or q6 month cycles. All patients had spine radiographs, L1–L4 DXA, and musculoskeletal and function testing.

Results: L1–L4 DEXA increased significantly after 1 year of q3m cycles, with average change in z-score = +1.41 SD, but did not improve significantly with further treatment. In the q6m group, the average change in DEXA was not significant. Repeated measures analysis of DEXA z-scores yielded a z-score rate change of 0.064 SD/m for q3 vs 0.036 SD/m for q6 group ($p = 0.13$). T12–L4 vertebral area and percent central compression were determined from radiographs. On repeated measures analysis, there was significant improvement of q6m group average LI–L4 and T12–L2 vertebral height ($p = 0.05$, 0.01) and area ($p = 0.002$, 0.006). The rate of improvement of the q3 group did not differ from the q6m for L1–L4 area or height ($p = 0.52$, 0.86) or T12–L2 area or height ($p = 0.28$, 0.77). The OI children had no significant improvement in fracture incidence, manual muscle testing or BAMF motor scores in either group after two years. It is noteworthy that response to treatment was highly variable in each treatment group. Also, improvement in vertebral area did not correlate with change in DEXA z-score, including 5 children (4 from q6m group) with $\geq$100% increase in L1–L4 area despite modest DEXA change of < 1 SD, and 10 children (9 from q3m group) with less than 50% increase in L1–L4 area despite DEXA changes of 1-2 SD.

Conclusions: Equivalent gains in vertebral height and area are obtained with q6m and q3m pamidronate cycles. For individual OI children, gain in DEXA does not correlate with extent of vertebral response.

Conflict of Interest: A. Obafemi, Research year was made possible through the Clinical Research Training Program, a public-private partnership supported jointly by the NIH and Pfizer Inc (via a grant to the Foundation for NIH from Pfizer Inc).

Mo-P223

GENE EXPRESSION IN TRABECULAR BONE: BMP2 EXPRESSION IS HIGHER IN HIP FRACTURE PATIENTS COMPARED TO OSTEOARTHRITIS AND RHEUMATOID ARTHRITIS

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Background: Regulation of bone turnover in malfunctions of bone metabolism manifesting as osteoarthritis (OA), rheumatoid arthritis (RA) and osteoporosis are likely to be different and hypothetically, gene expression should differ between osteoporosis or OA and bone disease arising from chronic inflammation. Expression studies have commonly been performed in cell culture rather than directly from bone tissue and even then, not from trabecular rich bone from the proximal femur. Our aim was to investigate RNA expression in biopsies from patients exhibiting hip fracture, OA and RA. In this preliminary study, we selected potentially regulatory genes, reflecting: bone remodeling, BMP2; osteoblast activity, COLIA1 and osteocalcin (BGLAP); osteoclast activity, TCIRG1. To detect adipocyte involvement, we analysed adiponectin (ADIPOQ).

Methods: Patient biopsy samples were collected from individuals undergoing hip replacement surgery due to OA, RA and hip fracture. Selection criteria included: female, no underlying malignancy, pathologic fracture or use of bone active medications. This preliminary study comprises: 9 low-impact hip fracture patients with presumed osteoporosis; 7 individuals each with OA and RA. Following RNA isolation, gene expression profiles of the 5 selected RNAs were determined by qRT-PCR. Non-parametric tests were used to identify differences between groups. Data is reported as median [range] at significance level $p = 0.05$.

Results: There was no statistical difference in age between the 3 groups: RA: 66.3 [21.3–88.6]; OA: 72.0 [51.5–90.1]; fracture: 76.7 [73.1–88.7] ($p = 0.28$). Standardised BMP2 RNA levels in the fracture group were 4–6 fold higher than RA and OA ($p = 0.002$) while RNA levels did not differ statistically between groups for BGLAP, TCIRG, COLIA1 and ADIPOQ. We found that ratios of BGLAP to ADIPOQ, TCIRG, BMP2, COLIA1 and BMP2 were significantly lower (range 2–6 fold) in fracture compared to RA and OA ($p = 0.003$–0.04). There were no differences in RNA levels between RA and OA.

Conclusion: We have established methods allowing RNA levels to be determined reproducibly in human bone biopsies. We found that samples from the fracture group had higher BMP2 RNA levels in comparison with the other groups. Despite large within-group variation, the ratio between BGLAP and TCIRG (markers for osteoblast and osteoclast activity, respectively) was significantly lower in the fracture group, suggesting rapid activation of bone remodelling.

Conflict of Interest: None declared

Mo-P224

MICROARRAY TECHNOLOGY IN MEDICAL ANALYSIS AND RESEARCH OF BONE METABOLISM GENES

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Studies of hormonedependent patients suffering from bronchial asthma have shown significant variability in the mineral bone density (MBD) regardless of daily dozes and duration of system glucocorticoids (SGC) intakes. To estimate the undesirable effects of SGC and determine the optimal terms for antiosteoporotic therapies, it is possible to carry out of genetic study for revealing predisposition to the development of glucocorticoid-induced osteoporosis.

The purpose of research: To study the association of gene polymorphisms and particulary collagen 1α1 (Col1a1), osteocalcine gene (BGP), interleucine 6 (IL-6), tumor necrosis factor alpha (TNF α), vitamin D receptor (VDR), estrogene receptor (ER) and glucorticoid receptor (GR). Proteins which are coding by these genes are characterized by significant genetic polymorphism, which is thought to be responsible for genetic predisposition to osteoporosis and with parameters BMD and with the development of axial and peripheral skeleton fracture in hormonedependent patients suffering from bronchial asthma.

We have started to introduce in practice a new molecular approach developed using multiplex PCR followed by allele-specific hybridization on biochip for SNP detection to diagnose the osteoporosis earlier, allowing genotyping these genes in individuals. A fluorescently labeled amplified DNA is hybridized with oligonucleotide DNA probes immobilized in gel pads on a biochip. At this moment, we chose these loci Col1a1(-1997G/T, 1245G/T), VDR (-3731A/G,

61968T/C) to compare the results of the biochip-based approach and the established PCR protocol, which showed 100% concordance. These results suggest that this approach may well be established for the other loci.

The genotyping procedure is faster, reliable and can be used for rapid screening and the biochip is suggested to be used for genetic predisposition analysis to multifactorial diseases like osteoporosis, as well as for screening the polymorphic loci associated with individual drug sensitivity.

Conflict of Interest: None declared

Mo-P225

GENOTYPE FREQUENCIES OF CYP 17 AND CYP 19 POLYMORPHISMS IN A SPANISH EARLY POSTMENOPAUSAL WOMEN POPULATION. RELATION TO BONE MINERAL DENSITY

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Background: Osteoporosis is a polygenic disorder resulting from the interaction of common polymorphic alleles and environmental factors. Linkage studies and association studies have identified several genetic loci and candidate genes related to the pathogenesis of osteoporosis. The CYP 17 and CYP 19 genes encode 17α-hydroxylase/17,20-lyase and aromatase, respectively, both involved in androgens and estrogens synthesis, that they are important for the development and maintenance of bone mass.

Objectives: 1) To assess the distribution of genotype frequencies of 17-hydroxylase/17,20-lyase gene (CYP 17) and aromatase gene (CYP19) polymorphisms in a Spanish population of early postmenopausal women. 2) To evaluate whether CYP 17 and CYP19 polymorphisms affects bone mineral density.

Subjects and methods: 158 women with natural menopause were randomly selected in the province of Albacete, Spain (mean age 52.7 ± 1.6 yr). They did not have any diseases to affect bone metabolism. Genomic DNA was extracted from peripheral blood leukocytes by the Higuchi method. The areas of interest were amplified with specific primers by polymerase chain reaction (PCR) technique. After amplification, all samples were digested whit Msp A1 (CYP 17) and Rsa1 (CYP 19) restriction enzymes, and the fragments were separated by agarose gel electrophoresis. Lumbar spine BMD (L2–L4) was measured by DXA.

Results: The distribution of genotype frequencies of CYP 17 polymorphism was: TT 59 (37.3%), TC 82 (51.9%) and CC 17 (10.8%). The distribution of genotype frequencies of CYP 19 polymorphism was: AA 49 (31%), AG 83 (52.5%), GG 26 (16.5%). The CYP 17 genotype was significantly associated with BMD at lumbar spine ($p = 0.035$). Women with the TT genotype had significantly lower BMD, compare to CC genotype. (mean ± SD: TT, 0.904 ± 0.141; TC, 0.919 ± 0.135; CC, 1,002 ± 0.096 g/cm^2, $p = 0{,}031$). Regarding to CYP 19 polymorphism, women with the GG genotype had the lowest BMD, and those with genotype AG had the highest BMD, but there was no significant difference (GG, 0.887 ± 0.126; AG, 0.934 ± 0.148; AA, 0.921 ± 0,120 g/cm^2).

Conclusions: 1)The distribution of genotype frequencies of CYP 17 and CYP 19 polymorphisms in this Spanish women population is similar to the other European women populations. 2)The CYP 17 genotype is associated with BMD at lumbar spine in early postmenopausal women.

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Conflict of Interest: None declared

Mo-P226

EFFECTS OF BMP2 IN THE IN VITRO REGULATION OF THE HUMAN COL1A1 PROMOTER

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Two polymorphisms in the promoter of human COL1A1 (-1997 G/T and -1663 indelT) were found associated with osteoporotic phenotypes. Transcription factor CIZ/NMP4 was identified as one of the proteins that binds a COL1A1 promoter site encompassing the -1663 indelT polymorphism. CIZ/NMP4 knock-out mice were reported to present increased bone mass due to raised bone formation. BMP2-induced osteoblastic differentiation was found to be enhanced, suggesting that CIZ is a suppressor of the BMP2 effects. We decided to study the effect of BMP2 on 2.5 kb of the human COL1A1 promoter.

Saos-2 cells were transiently transfected 24 h after plating with different COL1A1 promoter constructions and with the phRG-TK Renilla Luciferase normalization vector. The main constructs analysed were SP (220 bp, short promoter), LP (2,500 pb, long promoter), IR (LP with a 1 kb internal deletion encompassing the two polymorphisms) and the IRD (LP with 1 kb deletion encompassing a repressor element). BMP2 treatment (2 nM) was added 6 hours after transfection, in a medium essentially depleted of Bovine Serum (0.1%). Luciferase activity was measured 24 h after BMP2 (or mock) treatment using Dual-Glo Luciferase System.

The baseline transcriptional pattern of these constructs was similar to the one described previously in MG-63 cells, in which SP and IRD displayed activities twice as strong as those of LP and IR, explained by the lack of repressor. Upon BMP2 treatment, transcription from the LP, IR and IRD constructs was stimulated. However, stimulation of the IR construct was approximately 20%, whereas those of either the LP or the IRD constructs were about 60%. These two last constructs contain the region where the polymorphisms are located as well as putative Smad1 and Dlx5 binding sites. To test whether any of them mediate BMP2 stimulation of COL1A1 promoter activity, new constructs were prepared containing mutant or deleted sites. Preliminary results showed that deletion of a region containing the -1663 indelT and Smad1 sites resulted in a reduced response (40% stimulation versus 60% stimulation of the wild type). A similar reduction was observed for a construct including mutagenised Dlx5 and a Dlx5/Smad1 double mutant construct. Neither Smad1 mutation alone, nor deletion of a region encompassing -1997 G/T and Dlx5 did produce any effect,. Taken together, these results point to a role for Dlx5 site and probably the -1663 indelT polymorphism in mediating BMP2 stimulation.

Conflict of Interest: This work was supported by a grant of the Spanish Ministerio de Educación y Ciencia and the Instituto Carlos III.

Mo-P227

ASSOCIATIONS OF THE VITAMIN D RECEPTOR, CALCITONIN RECEPTOR AND OSTEOPROTEGERIN GENES AND THEIR INTERACTIONS WITH BONE DENSITY, BONE TURNOVER MARKERS AND FRACTURE INCIDENCE IN SLOVAK POSTMENOPAUSAL WOMEN

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Osteoporosis is a multifactorial disease with a strong genetic component. Various genes have been reported to be involved in the pathology of this disease. The vitamin D receptor (VDR) and the calcitonin receptor (CALCR) mediate hormonal action in target tissues including bone. Osteoprotegerin (OPG) belongs to OPG/RANK/RANKL system regulating osteoclast activity and differentiation. Therefore, genetic variability in these genes could affect the variability of bone mineral density (BMD) and fracture risk.

In the present study we analysed associations of FokI polymorphism in the VDR gene, AluI polymorphism in the CALCR gene, and AseI polymorphism in the OPG gene with variability of femoral and spinal BMD, as well as circulating alkaline phosphatase (ALP) and osteocalcin (OC; formation markers), beta-CrossLaps (CTX; resorption marker), and fracture incidence in 121 Slovak postmenopausal women (63.4 $\pm$ 7.5 years). Women were selected according to strict inclusion criteria. Genetic polymorphisms were detected by PCR-RFLP method. The differences between the genotypes were analysed by GLM procedure and covariance analysis after correction of the measurements for age and BMI. Gene-gene interactions were also evaluated within the statistical analysis. Frequencies of fractures were tested using the chi-square test.

We found a significant effect of VDR gene interactions on BMD. The VDR-CALCR interaction was associated with femoral BMD ($P = 0.033$) and spine BMD ($P < 0.001$). Moreover, associations of VDR-CALCR-OPG with spine BMD ($P = 0.014$) were reported. No polymorphism alone affected any of the analysed traits significantly, however; effects of VDR gene on spine BMD ($P = 0.070$) and ALP ($P = 0.086$) were not far from the significance level. We did not find significant associations between the genes and OC, CTX. Comparison of fracture incidence between the genotype groups showed significant differences ($P < 0.01$) for VDR polymorphism. The f-allele carriers had significantly higher frequency of fracture than non-carriers.

The analysis of associations between candidate genes (their interactions) and BMD or bone turnover markers can extend our knowledge about molecular background of bone remodeling and loss. The results could be also applicable in osteoporosis susceptibility prediction.

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Conflict of Interest: None declared

Tu-P228

TRANSCRIPTION PROFILING IN MURINE OSTEOBLAST-LIKE CELLS REVEALS NOVEL MECHANICALLY INDUCED GENES

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Mechanotransduction in osteoblasts is a heterogeneous and incompletely understood process that transduces extracellular mechanical stimuli into cellular signaling processes which are essential for bone development and fracture healing. In order to characterize pathways and genes influenced by this process, we performed transcriptome analysis using the osteoblast-like cell line MC3T3-E1 examined over a time course of up to 8 hours after a 10-minute equi-biaxial dynamic mechanical stimulus applied at 2 Hz with an elongation of 5%, as compared to unstimulated cells. Following stimulation (control and 15, 30, 60, 120, 240, and 480 minutes, 2 to 4 replicates for each condition) RNA was extracted and hybridized to Affymetrix mouse 430 microarrays using standard procedures. Q-PCR was performed to confirm differential expression in MC3T3-E1 as well as primary calvaria osteoblasts using the ABI 7900HT Fast Real-Time PCR System.

Between 1 and 111 differentially expressed genes per time point were identified using pairwise comparisons between the control samples and each of the time points following stimulation. Analysis based on the expression profile over the entire time course revealed slightly over 1000 differentially expressed genes. Inspection of genes found to be differentially expressed in at least two time points revealed several immediate early-response genes such as Egr1, -2, and -3, Fos, Jun and a number of other genes including two genes encoding nuclear transcription factors, Nr4a1 and Nr4a3. These genes have previously been shown to play a role in the immediate-early response of neural cells to depolarization.

A number of genes differentially expressed at the 480 minute time point were related to cell cycle and microtubule organization, as revealed by Gene Ontology analysis, suggesting that the mechanical stimulus resulted in regulation of genes involved in increased proliferation.

We confirmed differential expression for a number of the genes using Q-PCR assays. These results concerning the depolarization-induced genes suggest that some of the same genes involved in neural cell response to depolarization are also involved in similar responses of osteoblasts to mechanical stimuli. We are currently conducting experiments involving inhibition of several of the pathways induced by calcium signals in order to investigate the impact on the induction of candidate polarization-induced genes.

Conflict of Interest: None declared

Tu-P229

EXCLUSION OF TWO FUNCTIONAL AND POSITIONAL CANDIDATE GENES (CLCN7 AND ATP6V0C) FOR THE OSTEOPETROSIS (OP) RATMODEL

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Osteopetrosis is a disease characterised by a generalized skeletal sclerosis resulting from a reduced osteoclast-mediated bone resorption. Several spontaneous mutations lead to osteopetrotic phenotypes in animals. Moutier et al. (1973) discovered the osteopetrotic (op) mutation in the rat as a spontaneous, lethal mutation. The affected mutants exhibit the typical radiographic features of osteopetrosis and can be cured by bone-marrow transplantation from normal littermates, which indicates that the primary problem lies within the osteoclast itself. In op rats, osteoclasts are significantly reduced in number but are larger and more vacuolated than in normal littermates. Despite their foamy appearance, mutant osteoclasts can form ruffled borders and clear zones, but their ability to resorb bone is greatly impaired. Dobbins et al. (2002) localised the disease causing gene to a 1,5 cM genetic interval on rat chromosome 10.

In this study we first confirmed the genomic localisation of the disease gene by outbreeding the op animals. Male LEW/SsN. +/op were crossed with normal BN/SsN rats because these strains differ for a high percentage of polymorphisms. We intercrossed the F1 generation and selected the affected F2 pups. Genomic DNA from the tails of the affected pups was purified by standard methods. Variation in simple sequence repeat length was used to identify the LEW and BN alleles of each SSLP marker in 15 affected rats. The results obtained, provided statistical evidence for a disease causing gene in a small region of rat chromosome 10. Two strong functional candidate genes are within the delineated region. Clcn7 was previously shown to underly different forms of osteopetrosis, both in human and mice. ATP6v0c is a subunit of the vacuolar H(+)-ATPase or proton-pump. Mutations in TCIRG1,

another subunit of the proton-pump, are known to cause a severe form of osteopetrosis. We sequenced the 25 exons of clcn7 and 3 exons of atp6v0c, but were not able to find any sequence difference between the op/op rat and the normal rats. Therefore, these candidate genes can be excluded for causing the osteopetrosis in these animals. Mutation analysis of other positional candidate genes should finally lead to the identification of a new osteopetrosis gene.

Moutier R. et al. Exp. Anim. 6:87–101.(1973)
Dobbins D.E. et al. JBMR. 17:1761–1767.(2002)

Conflict of Interest: None declared

Tu-P230

INFLUENCE OF LYS656ASN POLYMORPHISM OF LEPTIN RECEPTOR GENE ON THE CHANGES PRODUCED BY ATORVASTATIN IN BONE MINERAL DENSITY IN PATIENTS WITH ACUTE CORONARY SYNDROME

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Aims: To evaluate the effect of atorvastatin on bone mass and markers of bone remodeling in patients with acute coronary syndrome according to the lys656asn polymorphism of leptin receptor gene polymorphism.

Methods: Sixty-two patients with acute coronary syndrome (35 males and 27 females), average age 60 ± 10 years, were included. Patients were allocated low (10–20 mg) and high doses (40–80 mg) atorvastatin according to baseline levels of cholesterol and triglycerides and the index of vascular risk. Patients were studied during hospital admission (baseline) and at 12 months of follow up. Cholesterol, triglycerides, total calcium, phosphorus, magnesium, osteocalcin and urinary deoxypyridinoline were determined in all patients at baseline and at 12 months of follow up. Densitometric studies were conducted in the lumbar spine (L2–L4) and femoral neck and trochanter using an X-ray densitometer. The lys656asn polymorphism of leptin receptor gene was determined by PCR.

Results: Forty-two patients were lys/asn homozygotic (69%) and 20 lys/asn heterozygotic (31%). The prevalence of osteoporosis (T score < -2.5 in lumbar spine and/or hip) was 31% for the lys/lys genotype and 27% for the lys/asn genotype with no statistically significant differences between groups. There was a statistically significant increase in bone mineral density in the lumbar spine (1.117 ± 0.24 versus 1.135 ± 0.24, $p = 0.008$) in patients with lys/lys genotype. No changes were observed in patients with the lys/asn genotype.

Conclusions: In patients with acute coronary syndrome, atorvastatin increases lumbar spine bone mineral density solely in patients with the lys/lys genotype of the lys656asn polymorphism.

Conflict of Interest: None declared

Tu-P231

POLYMORPHISMS IN THE DKK1 GENE ARE ASSOCIATED WITH HIP AXIS LENGTH IN YOUNG ADULT MEN FROM THE ODENSE ANDROGEN STUDY BUT NOT WITH BMD AND BONE TURNOVER MARKERS

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Osteoporosis is a common disease characterized by low BMD and microarchitectural deterioration of bone leading to an increased fracture risk. LRP5, encoding a coreceptor of canonical Wnt signaling, was recently discovered as an important susceptibility gene for osteoporosis. Our objective was to evaluate the effect of DKK1 polymorphisms on BMD, bone geometry and turnover. DKK1 is a secreted protein that binds to LRP5/6 receptors and inhibits canonical Wnt signaling. Studies with transgenic mice repeatedly showed that changes in dkk1 expression have a marked effect on bone formation and BMD. Additional evidence comes from mutations in LRP5 that cause high bone mass through reduced interaction with DKK1 and abolished inhibition of canonical Wnt signaling.

By means of the HapMap, we selected 3 SNPs that cover most of the variation in a 13.53 kb region comprising DKK1. The study cohort was obtained from the Odense Androgen Study (OAS), a large prospective population-based observational study comprising 780 Caucasian men aged 20–29 years. BMD was measured at the femoral neck and the lumbar spine by DXA using a Hologic 4500 device. Total hip and whole body BMD were calculated. Hip structural analysis was obtained using APEX 2.0 software (Hologic inc). Serum levels of bone turnover markers (1-CTP, BALP and osteocalcin) were available and used as a secondary endpoint. Genotypes were generated by melting curve analysis or Taqman allelic discrimination PCR on the LightCycler 480. All genotype frequencies were compatible with HWE. ANOVA and tests for linear trend were used to compare the studied phenotypes across the three genotypes. Additional analyses were done according to the level of physical activity. P-values less than 0.05 were considered significant.

We were not able to find significant differences in BMD or markers of bone turnover between the different genotype groups. On the contrary, when considering an additive model a significant p-value of 0.034 was found for rs1569198 with hip axis length. Furthermore, when sedentary and non-sedentary men were analyzed separately, the association seemed to be driven by the non-sedentary subgroup ($p = 0.027$). Within this subpopulation also rs1991392 was highly associated ($p = 0.019$).

As almost all variance within the DKK1 gene was covered, we can conclude that common variation in this gene does not markedly influence BMD or bone turnover markers but has an effect on hip axis length at least in the OAS cohort.

Conflict of Interest: None declared

Tu-P232

GEOGRAPHIC VARIATION IN TYPE AND FREQUENCY OF SEQUESTOSOME 1 MUTATIONS IN PAGET'S DISEASE OF BONE

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Paget's disease of bone (PDB) is a common condition with a strong genetic component. Mutations affecting the Sequestosome 1 gene (SQSTM1) have now been described in many populations, raising the possibility that geographical differences in the prevalence of PDB might be related to differences in the frequency of SQSTM1 mutations in different populations. Here we studied the prevalence and type of SQSTM1 mutations in several regions within the UK and compared these results with those reported in other countries. We screened for SQSTM1 mutations by DNA sequencing of samples obtained from 676 participants of the PRISM study who were

attending 39 referral secondary care referral centres across the UK. The frequency of SQSTM1 mutations was compared in different regions and with those previously reported in other countries. Mutations of SQSTM1 were identified in 72/676 (10.6%) patients overall but the prevalence of mutations was much higher (45.8%) in 103 patients who had a positive family history of PDB. Mutations identified were P392L (72.2%); G425R (8.3%); I424T (6.9%); M404V (6.9%); E396X (1.3%); Q400X (1.3%); M404T (1.3%) and a I424T/G425R compound heterozygote (1.3%). Two of these mutations (Q400X and I424T) are novel and have not been previously described. The lowest prevalence of SQSTM1 mutations was in Wales, where they occurred in 5.9% of patients screened and the highest was in NE England where 20.8% carried mutations. Figures for other regions were; 12% in Scotland; 13.4% in SE England; 11% in NW England; 8.1% in the Midlands and 11.1% in SW England. The prevalence was lower in Wales compared with NE England ($p = 0.02$), but the difference between other regions was not significant. We compared these data with published data on the prevalence of SQSTM1 mutations in patients with "sporadic" PDB in other countries. The prevalence of mutations was not significantly different in UK (10.6%), Canada (8.9%); Belgium (5.4%); Italy (7.1%); France (6.8%) and Australia (7.3%), but in Holland the prevalence of SQSTM1 mutations (2.4%) was less than in the UK ($p = 0.01$). We conclude that SQSTM1 mutations are a commonly associated with PDB in patients attending secondary care referral centres in the UK but find that there are no major differences in the prevalence of SQSTM1 mutations in different regions within the UK or between the UK and most other countries.

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Tu-P233

SEVEN QTLS FOR BONE TRAITS ARE SHARED BETWEEN TWO REPLICATE CHICKEN INTERCROSSES

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Background: We have previously conducted QTL-analysis for femoral bone traits in an intercross between the red junglefowl (RJ), the wild ancestor of domestic chicken, and White Leghorn (WL) strain L13 (L13), a domestic breed heavily selected for maximal egg-production. We have now performed QTL-analyses in a replicate intercross where RJ was intercrossed to another strain of White Leghorn, the obese strain (OS). The two WL-strains have been reproductively separated for at least 50 years, during which genetic recombination has taken place within each strain. QTL-analyses in the OS/RJ-intercross may therefore considerably confine confidence intervals for shared QTLs.

Methods: Genotyping of 700 F2-individuals was performed for 359 SNP-markers on 29 autosomal chromosomes. Femurs from F2 individuals were subjected to phenotyping by three-point bending tests (554 individuals) and by peripheral quantitative computerized tomography (pQCT) scans (537 individuals) of the metaphysis and diaphysis. In total 27 femoral bone phenotypes were recorded. QTLs were mapped in the software QTL-express using forward selection for loci with significant marginal effects. For each phenotype analyzed, batch and sex were included as fixed effects in the QTL-model, whereas bodyweight was included as an additive covariate.

Results: 14 QTLs were identified as significant on the 5% genome-wide level. A QTL at 140 cM on chr. 3 affected various traits attributed to diaphyseal size and also affected biomechanical properties (LOD = 8.4 and LOD = 5.5 for endosteal circumference and energy to failure, respectively). Depending on trait, this QTL explained 7–16% of the phenotypic variation in the F2-population. Seven QTL-regions (on chr. 1, 2, 3 and 15) had confidence intervals that overlapped with QTLs in the intercross between RJ and L13.

Conclusions: We report the identification of 14 QTL-regions affecting bone traits in an intercross between domestic and wild-type chicken. Most QTLs are syntenic to regions where linkage to bone phenotypes has previously been reported in man or mouse. Seven identified loci overlap with QTLs identified in a separate chicken intercross, which could help confine QTL-intervals greatly in cases where the domestic strains share selective sweeps. Information derived from this study could be of importance for inferring the genetic basis of bone phenotypic variation, not only in chicken, but also in man.

Conflict of Interest: None declared

Tu-P234

ASSOCIATION OF THE CALCITONIN GENE DINUCLEOTIDE REPEAT (CA) POLYMORPHISM WITH BONE MINERAL DENSITY AND OSTEOPOROTIC FRACTURES IN WOMEN FROM VOLGA-URAL REGION OF RUSSIA

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Calcitonin (CT) is a hormone known to participate in calcium and phosphorus metabolism. Calcitonin suppresses resorption of bone by inhibiting the activity of osteoclasts and plays a role in the pathogenesis of osteoporosis. Recent studies have shown a relationship between dinucleotide (cytosine-adenine) repeat polymorphism of the calcitonin gene (CALCA) and bone mineral density (Miyao, 2000; Magana, 2006). The aims of the present study were to analyze the distribution of the (CA) polymorphism in the CALCA gene in the Russian population (N = 140) and to test an association of the (CA) polymorphism with bone mineral density (BMD) and osteoporotic fractures in Russian postmenopausal women (N = 327). Patients with chronic diseases and conditions which may potentially affect bone mass were excluded. BMD was measured at the lumbar spine and femoral neck using dual energy X-ray absorptiometry. Eight alleles were identified in Russian population containing 10, 11, 12, 15, 16, 17, 18 and 19 CA repeats. Three major (CA) alleles were present with a frequency greater than 5%: CALCA*17 (61%), CALCA*10 (26%), CALCA*16 (9.4%). Allele CALCA*10 was associated with BMD at the lumbar spine: subjects with two copies of the CALCA*10 allele showed higher values for lumbar BMD than those who possesses one CALCA*10 allele ($p = 0.029$) and those who do not present any CALCA*10 alleles ($p = 0.022$). The genotype CALCA*10*10 frequency was significantly lower in osteoporotic women and women with osteopenia than in non-osteoporotic women (3.6% vs 15.4%; $p = 0.03$ and 4.2% vs 15.4%; $p = 0.038$, respectively). No significant association was observed between allelic status and osteoporotic fractures. Our results inconsistent with other studies, reported an association between the CALCA*10 allele and lower BMD (Miyao, 2000, Magana, 2006), it may be explained by different ethnic background. Overall, our data suggest that the calcitonin gene dinucleotide

repeat (CA) polymorphism is associated with lumbar BMD in Russian postmenopausal women from Volga-Ural region of Russia.
Conflict of Interest: None declared

Tu-P235

THE IMPACT OF GENETICS ON LOW BONE MASS IN ADULTS

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Background: Low bone mass in adults is a major risk factor for low trauma fractures. It is considered of complex origin due to interaction of environmental and genetic factors each with modest effect. Our objective was four-fold: 1) To assess the relative impact of genetics and environment on low bone mass. 2) To estimate the relative risk ratio of low bone mass in first-degree relatives. 3) To translate the genetic effect into accumulated years of bone loss compared to individuals of same age. 4) To test the hypothesis that only one or a few genes play a significant and important role in low bone mass.

Methods and study group: Four-hundred and forty Icelandic nuclear families with 880 first-degree relatives of both sexes were created out of total group of 17.000 individuals who had undergone bone densitometry (DXA) at the University Hospital in Reykjavik 1998–2006. Index cases (male or female) had bone mineral density (BMD) in lumbar spine or hip < -1.5 SD, age and sex matched. At least two members of their family had undergone DXA-BMD. Heritability (h2) of BMD was estimated by maximum likelihood method and variance component analysis was used to partition the genetic and environmental effect. Risk ratio (RR) of low BMD in first-degree relatives was estimated and observed heritable decrement in BMD was calculated compared with normal reference group.

Results: RR of low BMD amongst relatives was as high as 2.3 and the prevalence increased the lower the BMD of the proband. Yield of screening first-degree relatives was as high as 36 percent. Heritability (h2) of hip BMD was 0.66 and 0.61 for lumbar spine BMD. The genetic influence was consistent with one gene (or a few genes) with a considerable effect, as opposed to the genetic effect being solely due to many genes each with a small effect. The genetic deficit in BMD was already present before the age of thirty-five and equals 8–15 years of bone loss after menopause.

Conclusion: We have confirmed that genetics are by far the most important factor for low bone mass in adults. Our modelling indicates a very few underlying genes with considerable effects. The prevalence of low bone mass amongst first-degree relatives is common suggesting that screening amongst them should be cost effective and informative in elucidating the underlying genetics.
Conflict of Interest: None declared

Tu-P236

MOLECULAR MECHANISMS UNDERLYING JOINT AND BONE DISEASE IN THE MUCOPOLYSACCHARIDOSES (MPS)

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The mucopolysaccaridoses (MPS) are a group of inherited disorders with severe bone manifestations. Most are caused by deficiencies in enzymes required to catabolize glycosaminoglycans (GAGs). MPS animal models have provided important insights into the causes of bone and joint pathology in these disorders. For example, by 6 months of age an abnormal cellular and molecular profile was seen in the bones and joints of rats with MPS type VI (Maroteaux-Lamy disease), with characteristic increases in cytokines, MMPs, and apoptotic cells. We proposed that GAG storage in the MPS disorders leads to inflammation and apoptosis within cartilage, most likely through activation of the lipopolysaccharide (LPS) signaling pathway (Simonaro et al., 2001, 2005). We have also recently performed gene and protein expression analysis on fibroblast-like synoviocytes (FLS) from MPS VI rats, which similarly revealed that numerous inflammatory molecules were elevated, including several molecules important for LPS signaling (e.g., toll-like receptor 4, MyD88 and lipoprotein binding protein). TLR4 reporter cells lines have been generated, and will be used to investigate whether GAGs directly activate LPS signaling in vitro. In addition, MPS VII/TLR4 double mutants are being generated to evaluate the in vivo effects of GAGs on this important signaling pathway. We have also found that treatment of normal FLS and chondrocytes with GAGs leads to proliferation and apoptosis, respectively. This correlated with the production of the "pro-survival" lipid, sphingosine-1-phosphate, in FLS, and the "pro-apoptotic" lipid, ceramide, in chondrocytes. Both lipids have been implicated in LPS signaling. These findings have important implications for the pathogenesis and treatment of MPS, and have defined a novel mechanism of GAG-stimulated disease that may be occurring in other common bone disorders.
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Tu-P237

A TRANSVERSION IN THE TUMOUR NECROSIS FACTOR RECEPTOR ASSOCIATED FACTOR 6 GENE PROMOTER INCREASES GENE EXPRESSION IN RAW264.7 CELLS

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TNF receptor-associated factor (TRAF)-6 plays a very important role in the activation and differentiation of osteoclasts following activation through the RANK/RANKL system. A rare variant in the promoter region of the TRAF6 gene was identified in three osteoporotic family members following a genome-wide scan performed in a Maltese family.

To study the functional role of this variant on gene expression, a luciferase reporter assay system was used. Three inserts of variable length were synthesised by PCR, one for each of the A and T alleles, and cloned into a pGL3E vector. The synthesised constructs were transfected into HeLa and RAW264.7 cells, with the latter being stimulated with M-CSF, with and without RANKL. Luciferase activity was measured following incubation and lysis of the cells, and t-tests used for pair way comparisons.

A decrease in luciferase activity was observed with the increasing length of the wild-type constructs, for both cell lines. A statistically significant decrease in reporter activity was observed for the shortest mutated construct when compared to the wild type allele ($p < 0.05$). Conversely, a marked increase in gene expression for the mutated allele was observed for the other two constructs, where statistical significance was reached for the longest construct ($p < 0.05$) in HeLa and RAW264.7 without RANKL. The difference in luciferase activity was not significant in RAW264.7 cells following the addition of

RANKL, although similar trends in un-stimulated cells were observed. Analysis using MatInspector 7.4.8 revealed that a consensus sequence for nuclear factor (NF)-Y might be present in the wild-type allele but not in the mutated one.

In this study, we showed that a rare variant in the TRAF6 gene promoter significantly affects gene expression. Better understanding of the TRAF6 gene regulation is important for the development of novel pharmacological treatments to control osteoclast activity.

Conflict of Interest: None declared

Tu-P238

THE RELATIONSHIP BETWEEN RECEPTOR ACTIVATOR OF NUCLEAR FACTOR-KAPPAB LIGAND GENE POLYMORPHISM AND AORTIC CALCIFICATION OR BONE METABOLISM IN KOREAN WOMEN

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Background: The receptor activator of nuclear factor kappaB ligand (RANKL) has been identified as a key mediator for osteoclastogenesis. The effects of RANKL are physiologically counterbalanced by osteoprotegerin (OPG). RANKL and OPG have been proposed as a link molecule between osteoporosis and vascular disease, but the relationship between RANKL gene and bone resorption or arterial calcification in human populations is unclear. Thus, the aim of this study was to investigate the relationship between RANKL gene polymorphisms and bone metabolism or aortic calcification in Korean women.

Methods: In 237 healthy Korean women (mean age 51.3 ± 6.9 years), anthropometric measurements were done, aortic calcification in thoracic and abdominal aorta was examined by simple radiological methods, lumbar spine and femoral neck BMD were examined by dual energy X-ray absorptiometry, and serum OPG levels and bone turnover markers, such as serum alkaline phosphatase (ALP) levels, urine deoxypyridinoline levels, and urine calcium excretion were measured. Genotyping of two polymorphisms, rs2277438 and rs9594782, in the RANKL gene was performed by allelic discrimination using the 5′ nuclease polymerase chain reaction assay.

Results: The subjects with CT/CC genotypes of the rs9594782 polymorphism had a 3.9 times higher risk of aortic calcification compared with TT genotype (95% CI, 1.57–9.59). This significance was persisted after adjustment for age, body mass index (BMI), blood pressure, fasting plasma glucose, serum low density lipoprotein cholesterol ($p = 0.001$). Mean levels of urine deoxypyridinoline were significantly higher in the subjects with AG/GG genotypes of the rs2277438 polymorphism compared with AA genotype (7.37 nMol/mMol vs. 6.62 nMol/mMol, $p = 0.043$). After adjustment for age and BMI, urine deoxypyridinoline was persistently significant ($p = 0.040$). There were no differences in mean values for age, BMI, serum OPG, serum ALP, urine calcium excretion, and BMDs among different genotypes of each polymorphism in the RANKL gene.

Conclusion: The RANKL gene rs9594782 polymorphism was identified as a genetic factor associated with aortic calcification in Korean women. Also, rs2277438 polymorphism has influence on the levels of urine deoxypyridinoline, a bone resorption marker. Thus, we can suggest its role in relation to vascular calcification and bone metabolism in humans.

Conflict of Interest: None declared

Su-P239

TRANSACTIVATION OF VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR-2 BY THE C-TERMINAL DOMAIN OF PARATHYROID HORMONE-RELATED PROTEIN INDUCES OSTEOBLAST SURVIVAL

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The N-terminal region of parathyroid hormone (PTH)-related protein (PTHrP) modulates bone formation by promoting osteoblast differentiation and survival. Recent data indicate that the C-terminal domain of PTHrP can stimulate osteoblast function by interacting with vascular endothelial growth factor (VEGF). We assessed whether PTHrP (107–139) might affect cell survival, and the putative implication of VEGF, in human osteoblastic MG-63 cells. Cell death was analyzed by trypan blue exclusion, propidium iodide staining, and caspase-3 activity. Apoptosis-related proteins were evaluated by Western blot. Phosphorylated VEGF receptor 2 (VEGFR2) was detected after immunoprecipitation using a VEGFR2 antibody and protein A-agarose, followed by Western blot with anti-phospho-Tyr antibody PY-20. [116Tyr]PTHrP (107–115) was 125I-labeled (125I-CtPTHrP) and then affinity cross-linked to its putative receptor in these cells. We found that pre-incubation with PTHrP (107–139) dose-dependently inhibited (maximal 50%, at 100 nM, within 1–24 h) dexamethasone- and etoposide-induced cell death in MG-63 cells. Moreover, PTHrP (107–139) rapidly stimulated (maximal, 2-3-fold vs basal, at 1–6 h) the Bcl2/Bax protein ratio in these cells. The anti-apoptotic effect of this PTHrP peptide was abolished by the VEGFR2 inhibitors SU5614 and SU1498, and also by either a neutralizing anti-VEGFR2 antibody (α-VEGFR2) or CBO-P11, an inhibitor of VEGF binding to its receptors; but not by a neutralizing anti-VEGF antibody (α-VEGF). Moreover, PTHrP (107–139) rapidly (2 min) and transiently (up to 1 h) induced VEGFR2 Tyr-phosphorylation in MG-63 cells. This effect was unaffected by either two metalloprotease inhibitors or α-VEGF, or the src kinase inhibitor PP1. Both extracellular signal-regulated kinase and phosphatidylinositol-3 phosphate kinase pathways are involved downstream of VEGFR2 transactivation and PTHrP (107–139)-induced MG-63 cell survival. Neither α-VEGFR2 or CBO-P11 affected 125I-CtPTHrP binding to MG-63 cells. On the other hand, immunoprecipitation assays showed that, upon binding to its receptor (approx. mol.wt. 60 kDa), 125I-CtPTHrP induces the formation of a complex with VEGFR2 in these cells.

Conclusions: Our results show that the C.-terminal domain of PTHrP exerts anti-apoptotic features in human osteoblastic cells. These findings also demonstrate that transactivation of VEGFR2 appears to be required for the effects of this domain on osteoblast survival.

Conflict of Interest: None declared

Su-P240

ESTROGEN INHIBITS DLK1/FA1/PREF-1 PRODUCTION: A POTENTIAL MECHANISM FOR ESTROGEN EFFECTS ON BONE AND CARTILAGE TURNOVER

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Background: Dlk1/FA1 (Delta-like 1/fetal antigen1), also known as Pref-1, (Pre-adipocyte factor 1), is a member of the Delta/Notch family, known to modulate differentiation of stem cells in bone marrow. Recently, Dlk1/FA1 has been identified as a novel endocrine inhibitor of human bone marrow stromal (hMSC) cell differentiation into osteoblasts and chondrocytes through pro-inflammatory effects in bone micro-environment. Since estrogen (E) is known to regulate bone and cartilage metabolism, we investigated the effects of estrogen-deprivation and substitution on serum levels (s) of Dlk1/FA1 and its correlation with bone and cartilage turnover markers.

Methods: s-Dlk1/FA1, as well as serum/urinary bone (serum CTx and Osteocalcin) and cartilage turnover markers (urinary (u) CTX-II), were measured in: 1) ovariectomized rats, where half were substituted with 17b-Estradiol ($n = 18$), and sham-operated controls ($n = 8$), 2) A group of pre- and postmenopausal women ($n = 100$), 3) A group of postmenopausal women, where half had received estrogen replacement therapy (ERT) ($n = 166$).

Results: Ovariectomized rats exhibited an elevated s-Dlk1/FA1 compared to E-treated ovariectomized rats and E-replete sham-operated rats ($P < 0.001$). A positive correlation was observed between s-Dlk1/FA1 and s-CTX-II ($r = 0.51, P < 0.01$). Similarly, s-Dlk1/FA1, u-CTX-II and s-CTx were elevated in postmenopausal E-deficient compared to premenopausal E-replete women (all; $P < 0.001$). s-Dlk1/FA1 was correlated with both u-CTX-II and s-CTx ($r = 0.30, P < 0.01$ for both). Finally, ERT in postmenopausal women decreased s-Dlk1/FA1, as well as s-CTx and s-Osteoclacin (all; $P < 0.0001$). Changes in s-Dlk1 were significantly correlated with those observed in s-CTx ($r = 0.18, P < 0.05$) and s-Osteocalcin ($r = 0.28, P < 0.001$).

Conclusion: Increased levels of Dlk1/FA1 in post-menopausal women are a potential mechanism for the negative effects of estrogen deficiency on bone and cartilage metabolism that can be abolished by estrogen substitution. Targeting DLK1/FA1 may thus be a novel approach to prevent postmenopausal bone and cartilage degeneration.
Conflict of Interest: None declared

Su-P241

VITAMIN D METABOLISM IN FAMILIAL HYPOCALCIURIC HYPERCALCAEMIA AND PRIMARY HYPERPARATHYROIDISM

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Introduction: Familiar Hypocalciuric Hypercalcaemia (FHH) and Primary Hyperparathyroidism (PHPT) are typically characterized by equilibrium hypercalcaemia and normal renal function. Plasma PTH is normal or elevated in FHH and high normal or elevated in PHPT. FHH is a lifelong, benign, inherited condition caused by inactivating mutation(s) in the Calcium Sensing Receptor (CaSR) gene, whereas PHPT is an acquired disease, curable by neck surgery. PHPT is associated with low levels of 25OHD and high levels of 1,25(OH)2D. Calcium levels and 1,25(OH)2D may influence dermal keratinocyte differentiation and function through CaSR and VDR and affect melanocyte apoptosis and melanin production through VDR. This could lead to decreased dermal Vitamin D synthesis in PHPT. Calcium also regulates PTH secretion through CaSR and affects renal 1,25(OH)2D production. Increased 1,25(OH)2D enhances renal degradation of 25OHD. Furthermore, the increased fat mass observed in PHPT may also enhance storage and degradation of 25OHD and 1,25(OH)2D. The above mentioned issues are not settled in FHH.

Objective: To compare Vitamin D metabolism in FHH and PHPT.
Design: Cross-sectional.

Materials: Sixty-six hypercalcaemic FHH-patients with clinically significant mutations in the CaSR gene and 147 hypercalcaemic patients with surgically verified PHPT, all with a plasma creatinine level < 140 pmol/l.

Methods: Plasma 25OHD was measured by enzyme-immunoassay (IDS) and 1,25(OH)2D by radioimmunoassay (IDS). In FHH, all protein coding exons in the CaSR were sequenced and aligned to GenBank reference sequence NM_000388.

Results: The PHPT and FHH patients had comparable levels of P-Ca2+ (PHPT 1.43(1.28–1.98) (median (range)) mmol/l v FHH 1.41 (1.32–1.70) mmol/l, $2p = 0.32$) and of 25OHD (60 (12–177) v 57(18–154) nmol/l, $2p = 0.69$). The PHPT patients had higher BMI (26.6 (17.6–43.9)kg/m2 than FHH patients (23.8 (18.2–39.3) kg/m2, $2p < 0.01$), higher P-PTH (11.2 (4.6–108.0) pmol/l v 5.3 (2.5–20.8) pmol/l, $2p < 0.01$), higher 1,25(OH)2D (159 (50–400) pmol/l v 126 (56–307) pmol/l, $2p < 0.01$) and higher 1,25(OH)2D/25OHD ratio (2.7 (0.7–9.2) v 2.0 (0.8–5.7), $2p < 0.01$). Plasma phosphate was slightly lower ($p < 0.04$) in PHPT compared with FHH. Adjustment for age, sex, BMI, season and plasma creatinine did not change results.

Conclusion: Vitamin D metabolism differs in PHPT and FHH with comparable Ca2+ levels. The higher 1,25(OH)2 levels could be explained by higher PTH and lower phosphate levels in PHPT.
Conflict of Interest: None declared.

Su-P242

VITAMIN D-METABOLISM AND PTH LEVELS SHOW A BIPHASIC SEASONAL PATTERN BUT ARE NOT CORRELATED TO OSTEOCALCIN LEVELS IN OUTPATIENTS FROM WESTERN NORWAY

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Background: The western coastal part of Norway is situated around 60°N. At this latitude the sufficient levels of the effective light wavelength of ultra-violet B (290–315 nanometers) to produce vitamin D in the skin is limited to the months from may to august. So far north food like fat fish, cod-liver oil and substitutions to food becomes an important source of Vitamin D.

Materials and methods: In order to map the seasonal shifts in the level of the vitamin D-metabolites 25(OH)vitD and 1,25(OH)2vitD, parathyroid hormone (iPTH) and the bone formation marker osteocalcin (Oc), the monthly patient mean for these markers measured at the Hormone laboratory, Haukeland University Hospital, Bergen, in the period from 2005–2007 were calculated. All samples obtained from patients hospitalized at Haukeland University hospital were excluded.

Results: The number (range) of samples measured per month for 25(OH)vitD, 1,25(OH)2vitD and iPTH were 93–205, 87–161 and 68–123 respectively. A marked peak of 25(OH)vitD and of 1,25(OH)2vitD was observed in the summer/early autumn months (July-September). In addition, a less pronounced peak appeared in the winter/early spring months (February–March). This pattern was repeated over the three years investigated, although with slight shifts from year to year. In 2006 significant differences between the 25(OH)vitD values in the month with the peak value (August, mean value 74 nmol/l) and the month with the lowest value (April, mean value 52.6 nmol/l) was found, $p < 0.05$. The monthly patient mean of iPTH also showed a biphasic, although apparently inverse seasonal pattern as compared to the vitamin D metabolites. Oc showed significant higher values during the summer months (mean value13.5 nmol/l in the peak month June) than in the

winter months (mean value 7.0 nmol/l in January), $p < 0.05$. However, no biphasic pattern of Oc and no apparent correlation between 25(OH)vitD, 1,25(OH)2vitD, iPTH and Oc was observed.

Conclusion: We observed a significant seasonal variation of 25(OH)vitD and iPTH. This is in accordance with previous studies. Seasonal variation was also observed for 1,25(OH)2vitD. We found an apparent covariation between the seasonal variations of 25(OH)vitD, 1,25(OH)2vitD and PTH. In contrast we observed no covariation between osteocalcin and 25(OH)vitD, 1,25(OH)2vitD or PTH. This may indicate that the variation in Oc does not reflect vitamin D status or the level of PTH in our material.

Conflict of Interest: None declared

Su-P243

BONE DENSITY (PHALANGEAL QUANTITATIVE ULTRASOUND (PQUS), SERUM 25-0H-D AND PTH LEVELS IN DIFFERENT BMI POPULATIONS

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Background: Evidences are increasing about relations between body weight and many factors involved in the regulatory mechanisms of calcium metabolism. Vitamin D deficiency is associated not only with skeletal diseases, but also to others conditions like weight gain and obesity. In this study we compared different BMI, belonged to four WHO classes, in relationship to bone density and levels of serum calciotropic hormones.

Methods: We took in consideration a randomized group of 200 subjects without clinical relevant affections, of both gender, with age above 50 years; they represent an homogeneous sample, coming from the same little town.

Amplitude dependent speed of sound (AD-SoS) at the hand phalanges was measured with DBM Sonic BP (IGEA, Italy).

Serum PTH and 25-OH D were assayed by chemiluminescent method.

Results: All subjects were divided in two groups: one (N° 130 people) with normal or moderate increasing of BMI (WHO classes 0–1). the other (N° 70 people) with medium or severe elevation of BMI (WHO classes 2–3)

In table 1 results of mean values of Ad-SoS, 25-0H-D and PTH serum levels are reported in the two populations, we considered according different BMI. Significative differences were found, higher for AD-SoS and PTH (< 0.005 and < 0.01 respectively), lesser for 25-0H-D (< 0.04).

Conclusions: Body weight seems to be correlated in negative way to phalangeal density, evaluated by PQUS, as well as to 25-0H-D levels; on the contrary the correlation is positive between body weight and PTH levels. The difference of serum PTH levels and bone phalangeal density between the two groups (obese and not obese) is highly significative, more than for 25-0H-D levels, suggesting an important role played by PTH increasing in the reduction of bone density in obese subjects, even at phalangeal site.

Table 1 QUS,25-0H-D and PTH in two different BMI groups

	BMI (WHO 0–1) Mean DS	BMI (WHO 2–3) Mean DS	P
AD SoS m/s	2002 (87)	1960 (59)	<0.005
T-score	−1.68 (1.26)	−2.30 (0.82)	<0.005
25-0H-D ng/ml	11.1 (8.4)	8.2 (7.2)	<0.04
PTH pg/ml	47.14 (20.9)	58.3 (33.8)	<0.01

Conflict of Interest: None declared

Su-P244

AROMATASE INHIBITION INCREASES LONGITUDINAL GROWTH AND CANCELLOUS BONE LOSS IN PREPUBERTAL FEMALE RATS

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Treatment with growth hormone in young patients suffering from idiopathic short stature does not always have the expected outcome. Therefore, aromatase inhibition, and hence reduction of estrogen levels, was proposed as a potential treatment for increasing longitudinal growth. As estrogens are essential for normal bone turnover, the aim of the present report was to study the possible effects of aromatase inhibition on growth and on bone in female rats.

Prepubertal 26-days old Wistar rats ($n = 36$) were either treated with the aromatase inhibitor exemestane or ovariectomized (OVX). Exemestane was administrated by intramuscular injections for 3 weeks (10, 30, 100 mg/kg/week). Growth was evaluated by weekly measurements of nose-anus length. To assess the presence of osteopenia, the right femurs were studied by high resolution X-ray microtomography (micro-CT) (Skyscan 1072, Kontich, Belgium). Quantitative image analysis of metafyseal and epifyseal cancellous bone resulted in bone parameters such as trabecular number and thickness, bone volume, calcium density, etc.

End point measurements of tibia length and growth plate width were recorded and morphology of ovaria and uterus were examined by histology.

Administration of the highest dose of exemestane resulted in an increased growth plate width and tibia length, gain in body weight and nose-anus length. However, these findings were less prominent than seen after OVX. Osteopenia was present in metaphyseal and epiphyseal trabecular bone since a reduction of calcified tissue was observed due to thinning and loss of trabeculae. Changes in bone volume and calcium density of the femur were not found. Histological examination revealed multiple cyst-like structures in the ovaria.

In conclusion, although aromatase inhibition increases longitudinal growth in all female rats, this treatment caused severe osteopenia, as a loss of cancellous bone was observed, as well as a PCOS-like (polycystic ovary syndrome) phenotype. These side effects are a serious drawback for application of aromatase inhibition therapies in patients.

Conflict of Interest: None declared

Su-P245

AORTIC STIFFNESS: A REVERSIBLE MARKER OF CARDIOVASCULAR RISK IN PRIMARY HYPERPARATHYROIDISM?

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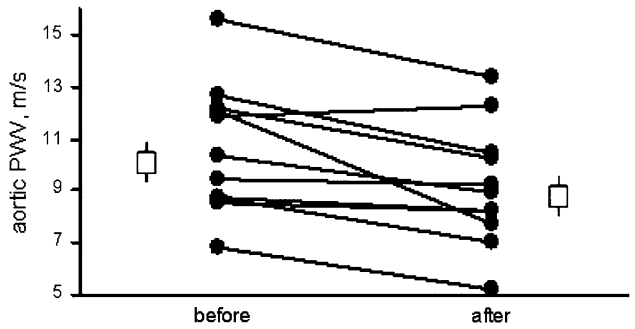
Background: Patients with primary hyperparathyroidism (PHPT) are at increased risk of cardiovascular death. We investigated whether aortic stiffness, an early marker of arteriosclerosis, (Study A) is increased in PHPT, and (Study B) improves after parathyroidectomy.

Methods and Results: Study A: 19 patients with PHPT (age 57 ± 11 years, BP 149/89 mmHg) and 38 age-, sex- and blood pressure-matched control subjects underwent aortic pulse wave velocity (PWV) determination (tonometry). Aortic PWV was significantly higher among PHTP patients (11.3 ± 2 vs 9.7 ± 2 m/s, $p < 0.01$).

Study B: 11 of the PHTP patients were reexamined 4 weeks after surgical parathyroidectomy. As expected, surgery was accompanied by a reduction in serum calcium (from 11.4 ± 1 to 8.9 ± 1 mg/dL, $p < 0.001$) and parathyroid hormone (from 215 ± 72 to 31 ± 27 pg/mL, $p < 0.001$). Aortic PWV decreased after surgery (from 10.6 ± 2 to 9.2 ± 2 m/s, $p = 0.004$), also after adjustment for changes in blood pressure.

Conclusions: Primary hyperparathyroidism is associated with increased aortic stiffness, which improves after parathyroidectomy. We demonstrate that aortic stiffness may improve upon removal of hyperparathyroidic stimuli.



Conflict of Interest: None declared

Su-P246

EFFICACY OF CHOLECALCIFEROL 800 IU SUPPLEMENTATION WITH OR WITHOUT CALCIUM SUPPLEMENTS IN PREVENTING FALLS OF THE ELDERLY PEOPLE

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To estimates the effect of cholecalciferol 800 IU supplementation with or without calcium supplements in preventing falls in postmenopausal women with evidence from randomized clinical trials. A systematic review of studies in MEDLINE and EMBASE up to June 2007 was performed. Clinical trials that assessed the vitamin D supplementation of 800 IU (cholecalciferol) oral daily with or without calcium supplementation in men and women aged 60 years and older were included. Findings from various studies were synthesized using Bayesian fixed and random-effects meta-analysis. Four trials were included in the meta-analysis. The additional effect of cholecalciferol 800 IU daily in preventing falls when calcium supplements were used as supplements were evaluated. In the fixed effect model, the pooled odds ratio (OR) in preventing falls was 0.59 [credential limits (CrL): 0.31–1.00, the probably of having better efficacy than placebo P = 97%]. In the random effect model, OR = 0.61 (CrL: 0.26–1.20; P = 94%). Two trials with 720 women 60 years and older were included in a sub-analysis for female subjects. When calcium supplementations were not used as background treatment, cholecalciferol 800 IU daily had a 75% (OR: 0.91; CrL: 0.66, 1.22) chance of being better than placebo in preventing falls in the fixed effect model and a 77% (OR: 0.96; CrL: 0.23, 2.14) chance of better than placebo in random effect model. Cholecalciferol 800 IU per day together with calcium supplemental is effective in preventing falls of elderly people. Cholecalciferol 800 IU even can provide additional benefits to calcium supplements in falls preventing among both male and female elderly. These results suggested the benefits of vitamin D3 supplement in reducing falls and relevant fractures among osteoporosis patients.

Conflict of Interest: SS Sen, Merck & Co., Inc., employee and Shareholders
T Fan, Merck & Co., Inc., employee and Shareholders

Su-P247

EFFICACY OF CHOLECALCIFEROL 800 IU SUPPLEMENTATION WITH OR WITHOUT CALCIUM SUPPLEMENTS IN PREVENTING NON-VERTEBRAL AND HIP FRACTURES FOR WOMEN 60 YEARS AND OLDER

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The purpose of this study was to estimates the effect of cholecalciferol 800 IU supplementation with or without calcium supplements in preventing non-vertebral and hip fractures in postmenopausal women with recently published evidence. A systematic literature review was performed to identify randomized controlled trials that assessed the vitamin D supplementation of 800 IU (cholecalciferol) oral daily in women aged 60 years and older. Findings from various studies were synthesized using Bayesian fixed and random-effects meta-analysis. There were 3,510 patients from 4 trials that compared vitamin D to placebo without calcium supplements were included in the assessment for non-vertebral fracture risk. For cholecalciferol 800 IU plus calcium supplementals versus placebo using a fixed effects model, the pooled odds ratio (OR) was for preventing non-vertebral fractures was 0.77 [CrL: 0.63–0.93]. There is a 99% probability that for cholecalciferol 800 IU plus calcium is a better treatment than placebo. Using a random effect model, the pooled OR was for preventing non-vertebral fractures was less favourable 0.95 [CrL: 0.28–2.54]. In a sub-analysis, using a fixed effects model, for cholecalciferol 800 IU versus placebo given calcium supplementation as background treatment, the pooled OR for preventing non-vertebral fractures was 0.81 [CrL: 0.11–2.89]. Using a random effect model, the pooled OR was for preventing non-vertebral fractures was 0.73 [CrL: 0.17–1.93]. There is 87% probability that cholecalciferol 800 IU plus calcium is a better treatment than calcium supplementation alone. There were 7,473 elderly women from 5 randomized trials were included in the meta-analysis for hip fractures. In the fixed effects model, the pooled OR for preventing hip fractures was 0.72 (CrL = 0.55–0.91; probability of being the better treatment was 100%). The random effect model showed a more conservative estimate (OR = 0.73; CrL = 0.42–1.19), and the probability of being the better treatment for Vitamin D relative to placebo was slightly lower (P = 94%). Cholecalciferol 800 IU per is effective in preventing hip fractures in elderly or postmenopausal women. Furthermore, Cholecalciferol 800 IU per day provides additional benefits than calcium supplements in preventing non-vertebral fractures of elderly women.

Conflict of Interest: SS Sen, Merck & Co., Inc., employee and Shareholders
T Fan, Merck & Co., Inc., employee and Shareholders

Su-P248

BONE METABOLISM IN MARES DURING LATE PREGNANCY AND EARLY LACTATION

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In the present study, the changes in the blood serum parathyroid hormone (PTH), total calcium (Ca), inorganic phosphorus (P), carboxy-terminal telopeptide of type I collagen (CTx), and estradiol (E) concentrations and the bone specific alkaline phosphatase (BALP) activity in mares were investigated with the aim of examining the influence of late pregnancy and lactation on bone metabolism parameters in mares. The research was conducted on mares of Croatian cold-blood horse breed ($n = 13$), between 3 and 18 years of age. Samples were taken 60 ± 10 days and 20 ± 10 days before foaling, and 20 ± 10 days and 60 ± 10 days after foaling (1st, 2nd, 3rd and 4th period, respectively). Significant changes in the concentration of Ca ($F = 5.26$, $p < 0.005$), P ($F = 7.18$, $p < 0.001$), E ($F = 50.17$, $p < 0.0001$) and in the activity of BALP ($F = 3.27$, $p < 0.05$) were observed. Significant changes in the concentration of CTx and PTH were not established. Ca was higher in the 3rd ($p < 0.05$) and 4th ($p < 0.01$) in comparison with the 1st period. P was higher in the 3rd in comparison with the 1st and 2nd period ($p < 0.01$). The activity of BALP was higher in the 4th in comparison with the 1st period ($p < 0.05$). E was lower in the 2nd, 3rd and 4th in comparison with the 1st period, and in the 3rd and 4th in comparison with the 2nd period ($p < 0.0005$). A positive correlation between Ca and P ($r = 0.42$, $p < 0.005$), and between Ca and BALP ($r = 0.55$, $p < 0.0001$) and P and BALP ($r = 0.29$, $p < 0.05$) was found. A negative correlation between E and Ca ($r = -0.61$, $p < 0.0001$), E and P ($r = -0.49$, $p < 0.001$), E and BALP ($r = -0.46$, $p < 0.005$), age and P ($r = -0.41$, $p < 0.005$) and age and BALP ($r = -0.30$, $p < 0.05$) was found. Low concentrations of Ca in the 1st period show that demands for minerals in mares were the greatest in late pregnancy, during the fastest growth and mineralization of the foetal skeleton. The lowest activity of BALP in the same period shows that those demands were, at least partially, satisfied by decreased bone synthesis, while bone resorption was not elevated. A negative correlation between E and Ca, P and BALP are in agreement with the known effects of estrogens on decreased bone remodelling. A negative correlation between BALP and age could indicate a decrease in osteoblast activity in older mares.

Conflict of Interest: None declared

Su-P249

SERUM CONCENTRATION OF CALCIFICATION INHIBITOR, FETUIN A, IS NOT ASSSOCIATED WITH IMT OR AOPWV IN PATIENTS WITH ESSENTIAL HYPERTENSION - PRELIMINARY RESULTS

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Introduction: It was shown that serum concentration of calcification inhibitor, fetuin A, is associated with subclinical atherosclerosis, arterial stiffness, vascular calcifications and cardiovascular outcomes in chronic kidney disease patients. The present study aimed to assess a relationship between fetuin A and intima-media thickness (IMT), left ventricular mass and aortic stiffness in patients with essential hypertension (EH).

Methods: To answer this question, 61 EH patients were examined (mean age 50.4 ± 5.2 y, BMI 29.5 ± 4.5 kg/m2). In each patient serum concentration of fetuin A was estimated, echocardiography was performed and LVMI were calculated. IMT was measured Aortic pulse wave velocity was assesed using AtCor device. For univariate regression analysis Pearson linear correlation coefficient was used. Multiple regression model was also constructed.

Results: Mean LVMI was 152.1 ± 36.8 g/cm^2, AoPWV 7.99 ± 1.17 m/s and IMT 0.80 ± 0.72 mm. Serum fetuin A concentration was 758 ± 204 pg/ml. No correlations between serum fetuin A concentration and above mentioned parameters were shown in univariate as well as multivariate analysis.

Conclusion: In a relatively small group of patients with essential hypertension no association between fetuin A and aortic pulse wave velocity, LVMI or IMT could be shown.

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Conflict of Interest: None declared

Su-P250

SERUM ESTRADIOL IS A PREDICTOR OF BMD IN ELDERLY MEN—RESULTS FROM THE ODENSE ANDROGEN STUDY

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Estrogen has been suggested to be the critical hormone for bone homeostasis in men. This has been very well demonstrated by studies on men lacking the estrogen receptor gene. Testosterone is supposed to play a minor role compared to estrogen, but severe hypogonadism in men will also result in low BMD.

Aim: We hypothesise that body weight, bio-available estradiol (bE2) and bio-available testosterone (bT) correlate with BMD in elderly men.

Materials and Methods: The Odense Androgen study of the Elderly is a population based cross sectional study on men aged 60–75. A total of4975 men were randomly drawn from the Danish civil registration system, 3743 responded, 1845 were invited to participate in more elaborate examinations and tests, 864 were interviewed, 803 proved eligible, and 600 men were included.

BMD in spine, hip, and whole-body was measured using a Hologic-4500a densitometer. Testosterone, estrogen and SHBG were measured using RIA and bio-available testosterone and estradiol calculated (Van den Beld et al 2000).

Results: Table 1 shows the results of the correlation analysis. bT was inversely correlated with age ($r = -0.13$, $p < 0.01$). No correlation was seen between age and bE2. bE2 was positively correlated with BMD in the hip, spine and whole body (see Table 1) and bT was not significantly correlated with BMD.* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$

Conclusion: BMD correlates positively with body weight and bE2, but not with bT in elderly men.

Table 1 Data are shown as correlation coefficients (R)

	Weight	bE2	bT	BMD hip	BMD spi	BMD wb
Age	NS	NS	−0.13*	NS	0.11**	NS
Weight	–	0.12**	−0.23**	0.40***	0.32***	0.23***
bE2	–	–	0.30***	0.08*	0.10*	0.08*
bT	–	–	–	NS	NS	NS

* = p<0.05, ** = p<0.01, *** = p<0.001

Conflict of Interest: None declared

Su-P251

RELATIONSHIPS OF GHRELIN WITH BONE MINERAL DENSITY AND BONE TURNOVER MARKERS IN ELDERLY MEN

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Background and Aims: Some in vitro studies have reported that ghrelin, a recently discovered orexigenic peptide mainly secreted by the stomach, is able to stimulate bone formation. No clear evidence is present in literature about the effects of ghrelin on bone turnover and bone mineral density (BMD) in humans. This study aimed to investigate for any associations between ghrelin levels, bone turnover markers and BMD in elderly men.

Methods: We studied 137 men aged 55 years and older (67.4 ± 5.5 yrs) who were participating in an epidemiological study. In all subjects we evaluated ghrelin (Ghrelin RIA, Linco Research), adiponectin, parathyroid hormone, 25-hydroxyvitamin D, bone alkaline phosphatase (B-ALP) and the carboxy-terminal telopeptide of type I collagen (CTX). BMD was assessed at lumbar spine (BMD-LS), at femoral neck (BMD-FN) and at total femur (BMD-T); whole body mineral content (BMC) was also evaluated. Body composition (fat mass and lean mass) was assessed by using a DXA device (Prodigy, Lunar GE). A Food Frequency Questionnaire was used for calculation of dietary calcium intake.

Results: The values of ghrelin were lower in osteoporotic men than in osteopenic and normal men but the difference did not reach the statistical significance (737.5 ± 82.4; 825.3 ± 112.5 and 853.6 ± 136.8 pg/ml, respectively). A significant correlation was found between ghrelin and lean mass ($r = 0.19$; $p < 0.05$) but not between ghrelin and fat mass. Ghrelin showed positive correlations with BMD-FN and with BMD-T which remained significant after adjustment for BMI and calcium intake ($r = 0.23$; $p < 0.05$ and $r = 0.21$; $p < 0.05$, respectively); whereas the relationship between ghrelin and BMD-LS did not reach the statistical significance. A significant correlation ($r = 0.25$; $p < 0.01$) between BMC and ghrelin was found. No significant associations were found between ghrelin and bone turnover markers (B-ALP and CTX). Multiple regression analysis showed that ghrelin serum levels were significantly influenced by calcium intake and BMI.

Conclusion: Our study suggests a positive association of ghrelin with femoral BMD and whole body BMC in elderly men. However ghrelin does not seem to influence bone turnover markers.

Conflict of Interest: None declared

Su-P252

LACK OF VITAMIN D PROMOTES DISEASE PROGRESSION IN A GUINEA PIG MODEL OF OSTEOARTHRITIS

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Introduction: Osteoarthritis (OA) affects all tissues of the joint. The Framingham study has shown that low serum levels of vitamin D is associated with an increase risk of OA progression in the knee. The aim of this study was to determine whether vitamin D deficiency will accelerate the progression of OA in a spontaneous guinea pig OA model.

Methods: Male Dunkin-Hartley guinea pigs (2 months old) received a diet that was either vitamin D deficient (no Vitamin D) or a normal diet (153 IU of vitamin D/day) ($n = 8$ in each group). At 3 months a left medial menisectomy was performed (menisecctomy creates an accelerated and predictable model) and the guinea pigs were sacrificed 1, 3 and 6 months later. Ca, P, 25(OH) Vitamin D and alkaline phosphatase (ALP) serum levels were measured. BMD of the distal femur was measured by DEXA. Safranin-O stained sections of the tibial plateau were graded for OA using a modified Mankin scale. Undecalcified sections from distal femurs were used for bone histomorphometry. Subchondral bone mineralization was determined by backscattered electron (qBSE) imaging and the same blocks were used for microhardness.

Results: There was a decrease ($p < 0.05$) with time in serum P and an increase in ALP was seen in the no vitamin D group. With time an increase ($p < 0.05$) in the Mankin score was seen in the no vitamin D animals and the tidemark progressed towards the articular cartilage ($p < 0.05$) surface, while the subchondral bone plate increased ($p < 0.05$) with time. The BMD of the no vitamin D group was lower ($p < 0.05$) than the control group at 6 months. An increase ($p < 0.05$) in bone volume was seen up to 3 months post menisectomy, while a decrease ($p < 0.05$) in bone volume in the no vitamin D group after 3 months was noted. No changes in osteoid were seen in the no vitamin D group, while a decrease ($p < 0.05$) in osteoid with time was seen in the controls. A decreased mineralization and microhardness for the no vitamin D group ($p < 0.05$) was seen with time.

Discussion: The vitamin D deficient animals developed more severe OA changes when compared to the animals with a normal diet. The advancing tidemark suggests a decrease in cartilage thickness and an increase in calcified cartilage. Vitamin D deficiency may have caused an increase in PTH to maintain calcium homeostasis by stimulating osteoclastic bone resorption, inducing a decreased bone volume and connectivity in this group. The impaired mineralization, microhardness and bone quality of the vitamin D deficient bone may predispose the joint to further OA progression.

Conflict of Interest: None declared

Su-P253

PARATHYROID HORMONE REGULATION AND EXERCISE

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The serum ionized calcium concentrations are recognized as the major determinant of parathyroid hormone (PTH) secretion and a sigmoidal inverse relationship between them has been described. Nevertheless, in many studies dealing with the effects of intensive endurance exercise such a relationship was not observed. The present study was designed to determine whether taking into account the variations in blood hematocrit i.e. variations in plasma volume and their effects on total amount of ionized calcium calculated according to van Beaumont et al. could explain the exercise-induced stimulation of PTH secretion.

Twelve triathletes (mean ± SD age: 30.7 ± 4.2 year) performed a one hour (from 09.30 to 10.30) intensive (at a 80% VO2 max) endurance exercise on ergometric bicycle. Blood samples were collected from 08.30 to 11.30 at 30 min intervals and thereafter at 12.30. Serum concentrations of intact PTH and of ionized calcium were measured and blood hematocrit as well. This assay was repeated

twice: one assay with prior intake of one 1 g calcium load and the another one without any calcium load.

A significant (ANOVA $P < 0.0001$) rise in serum PTH occurred during exercise and this rise was only partially suppressed by the prior intake of calcium. In the same time, the levels of serum ionized calcium remained constant, if no calcium load, while they progressively rose after calcium load. By contrast, the changes in the total amount of serum ionized calcium showed a significant (ANOVA $P < 0.0001$) decrease simultaneously with the rise in PTH. These two patterns (with and without calcium load) were significantly (ANOVA $P = 0.0011$) different as were the respective peaks in PTH (ANOVA $P = 0.002$). A significant ($r = 0.974$; $P = 0.0001$) polynomial regression relationship between PTH and the total amount of serum ionized calcium was obtained.

The present study shows that during intense endurance exercise serum ionized calcium as expressed as a total amount, which takes into account the variations in plasma volume, represents a valid determinant of PTH secretion.

Conflict of Interest: None declared

Mo-P254

GPR30 ESTROGEN RECEPTOR EXPRESSION IN BONE CELLS DURING PUBERTAL DEVELOPMENT

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Background and aim: Estrogen (17β-estradiol) has a significant impact on bone mineral metabolism. Estrogen mediates its effects via the classical estrogen receptors (ERα and ERβ). In addition, a transmembrane G-protein-coupled receptor GPR30 was recently demonstrated to act as an estrogen receptor and has been proposed to act as a mediator of the non-genomic effects of estrogen. We aimed to study the GPR30 expression in bone cells and the correlation between the expression level and pubertal stage.

Methods: Biopsies were collected from tibial growth plates and underlying metaphyseal bone of 14 boys and 6 girls at different stages of puberty. Paraffin-embedded sections were used to study GPR30 protein expression by immunohistochemistry. The percentages of GPR30 positive osteocytes and osteoblasts were quantified and linear regression analysis was applied.

Results: GPR30 expression was detected in osteoblasts, osteocytes and osteoclasts. The staining was cytoplasmic in all cells. The expression level of GPR30 was significantly higher in osteocytes than in osteoblasts ($58\% \pm 4\%$ vs. $46\% \pm 3\%$ positive cells, respectively, $p < 0.05$). The levels of GPR30 expression varied during pubertal development. Detailed analysis demonstrated that GPR30 expression significantly declined in a linear manner in osteocytes ($R = -0.56$, $p < 0.01$) but not in osteoblasts ($R = -0.31$, $p = 0.191$). No gender difference in the osteoblastic and osteocytic GPR30 expression was observed. Furthermore, the decline in GPR30 expression did not correlate with either chronological or bone age.

Conclusion: This is the first report on the expression of a novel estrogen receptor GPR30 in human bone cells. Moreover, the level of GPR30 expression in osteocytes was dependent on pubertal stage, suggesting that non-genomic estrogen signaling via GPR30 might play a role in bone mass accrual during puberty. However, more mechanistic and functional studies are needed to confirm this.

Conflict of Interest: None declared

Mo-P255

THE EFFICACY AND SAFETY OF SHORT TERM TREATMENT WITH INCREASING DOSES OF CHOLECALCIFEROL ON 25-HYDROXY VITAMIN D LEVELS IN HEALTHY POSTMENOPAUSAL WOMEN

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The study was performed on the baseline data from a cohort of 344 ambulatory postmenopausal females (age 60 to 85 y) screened between March and November 2007 for participation in an osteoporosis study. Main inclusion criteria was a DEXA-scanning demonstrating osteoporosis as defined by a T-score below—2.5 SD or between—1.0 and—2.5 SD and at least one osteoporotic fracture. An amendment included a new FDA guideline to change the lower acceptable limit of 25-hydroxy vitamin D (25(OH)D) for inclusion to 60 nmol/l. Subjects with a 25(OH)D <60 nmol/l were allowed to be substituted with vitamin D and the biochemistry retested. This study reports on the efficacy and safety of oral treatment with 25 μg ($N = 21$; mean age: 70.6 y), 50 μg ($N = 19$; mean age: 66.7 y), 75 μg ($N = 19$; mean age: 68.7 y) or 100 μg ($N = 35$; mean age: 70.2 y) of cholecalciferol daily for 10 days. In the group of subjects treated with 25 μg of cholecalciferol daily 25(OH)D increased significantly from 32.4 ± 2.7 nmol/l (mean ± SEM) to 50.8 ± 2.9 nmol/l. In the groups treated with 50 μg, 75 μg and 100 μg daily 25(OH)D increased significantly from 46.7 ± 2.8 nmol/l to 65.8 ± 2.6 nmol/l, from 41.6 ± 2.7 nmol/l to 67.4 ± 2.9 nmol/l and from 46.7 ± 1.4 nmol/l to 64.4 ± 2.2 nmol/l respectively ($P < 0.001$ for all groups). Seventy-one, 37, 37 and 40 %, respectively, had a 25(OH)D level below 60 nmol/l in response to the four different doses of cholecalciferol. In the 25 μg and 100 μg group s-calcium increased within the reference range ($P < 0.006$) whereas PTH only decreased in the 25 μg group ($P = 0.004$). Apart from a significant increase in alkaline phosphatase (AP) in the 50 μg group ($P = 0.003$) no change in AP or creatinine was observed in any of the other groups ($P > 0.1$). No adverse events were observed during treatment with any dose of vitamin D. In conclusion, 10 days of treatment with 25 μg, 50 μg, 75 μg or 100 μg of vitamin D3 was safe and increased 25(OH)D levels significantly in all groups although responses were variable. Doses of cholecalciferol above 50 μg daily for ten days did not increase the number of subjects responding with 25(OH)D levels above 60 nmol/l. Future strategies for correction of vitamin D insufficiency on a short term basis should take this information in to consideration.

Conflict of Interest: H.C. Hoeck: Employed by CCBR a company engaged in contract research B. Li: Employed by Nordic Bioscience P. Qvist: Employed by Nordic Bioscience. The Vitamin D assay is developed by Nordic Bioscience for non-commercial purpose

Mo-P256

THE CHANGES OF MINERAL BONE DENSITY AMONG WOMEN WHO HAVE HYPERPROLACTINEMIA DEPENDENT ON PATHOGENETIC SPECIFICS OF THE DISEASE

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During the hyperprolactineamia (GPRL) the hypogonadism and the direct influence of high level of plolactin (PRL) in the blood's whey play an important role in the disorder of remodeling of bone. Hypoestrogenia is a starting factor in the development of a complex of interrelated metabolic disorders.

Patients and methods. We have examined 78 women at the age from the age 24 to 47 years old with functional GPRL. The level of gonadotropic hormones (GG) in the blood were determined -PRL, lutenizing hormone (LG), follicle-stimulating hormone (FSH) and estrogens (E). The skull was examined by the computer tomography. The examination of bone mineral density (BMD) was implemented by the ultrasonic densitometry. The bone resorption marker was determined - B-CrossLaps (CTx).

Results: All the patients had the ovarian hypofunction; clinically it showed up amenorea and oligomenorea. 4 types of gonadotropic hormone's secretion were found: 1. LG and FSH lower than basal level; 2. LG is high leveled, FSH is lower than the basal level; 3. LG is lower than basal level, the FSH is high leveled; 4. LG and FSH are high leveled. 82% of women with the 1st type secretion of GG had normal MBD, 18% of them had osteopenia; however, increased resorption of bone was found in 38% of women. 15% of the patients with the 2nd type of secretion of GG had osteopenia, 85% of them had normal BMD results, CTx was high-leveled in 32% of women. 28% of women with the 3rd type had osteopenia, the normal results were in 72% of patients, high-leveled CTx was found in 46% of patients. 57% of patients with the 4th type had normal BMD, 38% of them had osteopenia, 5% of them had osteoporosis, the increased resorption of bone was found in 64% of women.

Conclusion. Received results show the dependency of frequency of BMD's decreasing and increased resorption of bone among the patients with GPRL on pathogenetic specifics of this pathology. It is necessary to take it into consideration and well-timely appoint the antiresorptive therapy.

Conflict of Interest: None declared

Mo-P257

SERUM 25(OH)D AND PARATHYROID HORMONE ACCORDING TO AGE IN KOREAN WOMEN

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Objectives: Definition of vitamin D insufficiency in Asian people could be different from that of Caucasian because of the racial differences. We planned to analyze serum 25(OH)D and PTH levels according to age in Korean women who visited one of the general hospital in Seoul.

Methods: The subjects were classified to four groups, 40–49, 50–59, 60–69, 70–79 by ages and evaluated age, BMI, Lipid profile, bone turnover markers, serum 25(OH)D, and PTH in each groups.

Results: Serum 25(OH)D level was increased from 40–49 to 70–79, especially between 60–69 and 70–79 groups by statistical significances. And the results showed decline of serum PTH from 40–49 to 70–79, especially among 50–59, 60–69, and 70–79 groups by statistical significances. In 70–80 percentile of the subjects, serum 25(OH)D level pointed at 24 ng/ml.

Conclusion: The average serum 25(OH)D level in Korean women was lower than that of Caucasian people. And higher dose of vitamin D could be considered to the elderly Korean women.

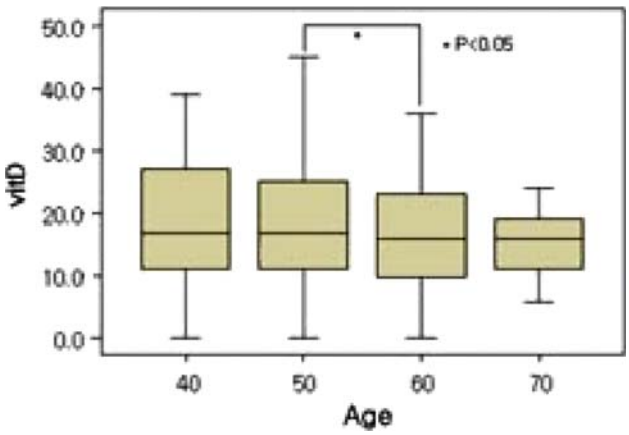

Fig. 1 Serum 25(OH)D level according to age

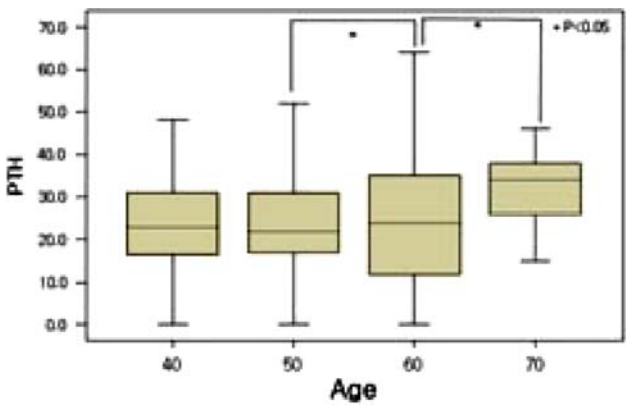

Fig. 2 Serum PTH level according to age

Conflict of Interest: None declared

Mo-P258

LACK OF ASSOCIATION BETWEEN INSULIN RESISTANCE AND VITAMIN D STATUS IN FIRST TRIMESTER OF PREGNANCY IN NORTH INDIAN SUBJECTS

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India has the highest number of cases of type 2 diabetes in the world. This prevalence is progressively increasing as also seen in other developing countries. On other hand, very high prevalence of vitamin D deficiency has also been reported in many papers from the Indian population. Few studies have shown association of vitamin D deficiency with insulin resistance but this correlation is not well studied. We planned a study to assess this correlation in Indian population. We are presenting early data from our ongoing project.

Material and methods: This prospective study included 129 consecutive non diabetic pregnant women in first trimester of gestation ($\leq$12 weeks). Those subjects who were known case of diabetes,

history of impaired fasting glucose or impaired glucose tolerance or GDM in previous pregnancy, subjects taking steroids, metformin or immunosuppressive drugs, multiple fetuses or subjects with known pancreatic disease were excluded from the study. Blood samples for serum calcium, phosphates, alkaline phosphatase, renal, liver function tests, plasma insulin, blood glucose and vitamin D subjects were collected in fasting state (minimum 8 hours). Blood glucose was measured by glucose oxidase peroxidase colorimetric method, plasma insulin by electrochemiluminescence immunoassay (ECLIA) and vitamin D by radioimmunoassay. Different indices of insulin resistance/sensitivity were calculated by homeostasis model assessment (HOMA).

Results: We evaluated 131 pregnant subjects in first trimester of pregnancy. Vitamin D deficiency (serum 25 hydroxy vitamin D level less than 20 ng/ml) was seen in 96.4% subjects. Vitamin D levels less than 5 ng/ml was seen in 45.4% and less than 10 ng/ml in 79.2% subjects. We could not found any correlation between insulin resistance and vitamin D levels (r 0.086, p 0.336), even if controlled for BMI (r −0.37, p 0.712). HOMA-IR showed significant correlation with fasting blood glucose (r 0.98, p 0.000) and fasting plasma insulin (r 0.595, p 0.000). HOMA-IR showed positive correlation with BMI (r 0.447, p 0.000). BMI showed positive correlation with fasting plasma insulin (r 0.481, p 0.000).

Conclusion: Our data did not show any significant correlation between insulin resistance and vitamin D status in pregnancy.

Conflict of Interest: None declared

Mo-P259

LOW FETUIN AHSH CONCENTRATION IN SERUM IS MARKER OF CALCIFICATION WITH THE PATIENTS ON HAEMODIALYSIS AND PREDICATOR OF CORONARY VESSELS CALCIFICATION

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Introduction and aims: Systemic metastasis calcifications are only one of the many complications of complex pathogenic mechanism of chronic renal insufficiency. Deposit of calcium salt in vascular apparatus of myocardium structures specially in passing systems, leads to very early cardial dysfunction. Many factors as hypertension, dyslipoproteinaemia, high level of homocystein, pathologic mechanism Ca and PHO4, are main factors of calcification appearance. H. Schmidt glycoprotein AHSH is important circular inhibitor for calcification. It is synthesized in liver and is found in serum in high concentration. It is specific calcium regulator protein and important factor in pathogeneses of uremic extraosseuous calcification as well as in cardial dysfunction appearance.

Methods: In our research 148 examiners were included, divided in three groups:

I. patients on haemodialysis without proved calcifications 67/ 35 men, 32 female, between age of 28 to 69 ;
II. patients with proved calcification / CT heart, Echo heart/ 50 patients, 25 m. 25 f. between age of 30 to 70 ;
III. control group were 31 patients, 17 m. 14 f. between ages of 40 to 65.

By all the examiners fetuin A and Ca/P was determined.

Fetuin is determined by Elise method.

Results: I and II group compared with III have higher proportion of Ca/ P 4.43/:2.6 and lower fetuin A X 23 ng/ml concentration with X 38 ng/ml.$P < 0.001$, what is statistically important. Also II group compared with the I, has significantly lower fetuin A on the level $p < 0.001$.d. Difference in calcium and phosphor proportion are not statistically important for I and II group.

Conclusion: Our results point out to significantly lower fetuin A serum concentration with the patients on haemodialysis. It is important to stress lower level of Fetuin A in II group in regard to the I. Low fetuin A level proves its connection with vascular calcification and calciphylaxis and accelerated atherosclerosis process and development of patients on haemodialysis cardial dysfunction.

Conflict of Interest: Not declared

Mo-P260

THE RELATIONSHIP BETWEEN SEX STEROIDS AND BONE DENSITY IN MEN WITH DOWN SYNDROME

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Background: Sex steroids have consequential influence on the regulation of bone metabolism. Several abnormalities in sexual development are described in people with Down syndrome. Various reports of increased incidence of gonadal dysfunction were published, that is in contrary to findings of normal sexual characteristics and the intact pituitary-gonadal axis and normal testosteron levels in other reports.

Aims: The aim of our study was to examine the possible role of testosterone (T), 17-beta-estradiol (E) and DHEA-S in bone metabolism in men with Down syndrome.

Methods: 46 men with Down syndrome (DS; age:18–55 y) and 39 healthy controls (C; age:18–64 y) were enrolled into the study. Probands were tested for serum Ca, Mg, P, ALP, 25(OH)vitamin D, CTx, DHEA-S, T and E concentrations. BMD for lumbar spine and right femoral neck were measured with dual energy X-ray absorptiometry.

Results: The mean 25(OH)vitamin D levels were 38.8 ± 14.5 ng/ml in C and 20.9 ± 9.3 ng/ml in DS ($p < 0.001$). Hypovitaminosis of vitamin D was noted in 25.6% of C and in 82.6% of DS. Serum Ca, Mg and P were in normal range and did not differ in both groups. ALP was also in normal range in both groups, but significantly higher in men with DS ($p < 0.001$). CTx concentrations were significantly above in upper limit of normal in DS (0.92 ± 0.44 ng/ml) and in normal range in C (0.28 ± 0.12 ng/ml). There were no differences between E as well as T levels comparing both groups, but DHEA-S concentrations in DS were significantly lower then in C ($p < 0.001$). Bone mass density (BMD) and Z-score for lumbar spine and femoral neck were significantly lowered in DS group ($p < 0.001$). Positive correlation between femoral neck BMD and DHEA-S concentrations was found ($r = 0.3261$, $p < 0.01$).

Conclusions: Results of this study demonstrate: a) high prevalence of vitamin D hypovitaminosis in DS group, 2) significantly lower femoral neck and spine BMD in DS group, 3) increased bone turnover in DS, 4) increased marker of bone resorption (CTx) and marker of bone formation (ALP) in DS and 5) decreased DHEA-S levels in DS. This data indicate the risk of elevated bone resorption in men with Down syndrome. Further research is needed for better understanding of the relative roles of androgens and estrogens in the male skeleton.

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Conflict of Interest: None declared

Mo-P261

ORAL TREATMENT WITH THE CALCIUM RECEPTOR ANTAGONIST SB-423557 CAUSES PTH RELEASE IN MULTIPLE SPECIES AND POSITIVE BONE FORMING EFFECTS IN RATS

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Antagonists of the parathyroid calcium receptor (calcilytics) stimulate the secretion of PTH. Previously, we demonstrated the ability of an orally active calcilytic compound to cause sustained increases in circulating levels of endogenous PTH and stimulate bone formation and resorption (without a net increase in bone formation) in the ovariectomized (OVX) rat. In the present study, a prodrug approach has been used to preserve oral bioavailability and yield a calcilytic with a shorter half-life *in vivo*. SB-423557 is the ethyl ester prodrug of SB-423562 that when administered orally to rats, dogs or monkeys, caused a dose-dependent, transient increase in circulating levels of endogenous PTH. To examine the bone forming effect of SB-423557, 6-month-old female rats received OVX or sham surgery and were untreated for 6 weeks to allow osteopenia to develop and then treated orally daily with either vehicle, SB-423557 (50 mg/kg), or with rat PTH(1–34) (5 μg/kg SC) for 12 weeks. Plasma levels of PTH peaked at 10–60 min following oral administration of 50 mg/kg SB-423557 (3-fold, Cmax 40–60 pM) and returned to baseline by 2–3 hours. SC administration of rat PTH(1–34) resulted in a systemic Cmax 127–240 pM at 10 min post-injection. SB-423557 significantly and completely prevented additional OVX-induced loss of BMD in the lumbar spine and partially prevented trabecular BMD loss in the proximal tibia by 39% (ns) compared to OVX controls. Histomorphometric analysis indicated greater trabecular bone area (36%, ns) in the spine and increased cortical area (72%) and endocortical bone formation rate (220%) with no effect on the eroded perimeter of the distal tibia in the SB-423557 treated rats compared to vehicle-treated OVX animals. Serum osteocalcin increased (ns) with SB-423557 treatment with no effect on urinary deoxypyridinoline levels. Treatment with SB-423557 also resulted in greater ultimate strength (ns), toughness, and elastic modulus of a lumbar vertebral body and at the femoral diaphysis compared to OVX controls. Treatment with PTH(1–34) also completely prevented the OVX-induced loss in bone mass, BMD and strength. These data provide proof of principle for stimulation of bone formation following daily brief antagonism of the calcium receptor in the OVX rat and support the potential use of these agents to treat disorders of bone metabolism such as osteoporosis.

Conflict of Interest: S Kumar, X Liang, J Vasko, G Stroup, S Hoffman, V Vaden, H Haley, A Lago, J Callahan, P Bhatnager, W Huffman and M Gowen are employees of GSK. J Fox and E Nemeth are employees of NPS Pharmaceuticals.

Mo-P262

FRACTURE INCIDENCE AND CHANGES IN QUALITY OF LIFE AND BACK PAIN IN WOMEN WITH OSTEOPOROSIS TREATED WITH RHPTH(1–34) (TERIPARATIDE): 18 MONTH RESULTS FROM THE EUROPEAN FORSTEO OBSERVATIONAL STUDY (EFOS)

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Aim: To describe the clinical fracture outcomes, health related quality of life (HRQoL) and back pain (BP) of postmenopausal women with osteoporosis treated with teriparatide for 18 months in the European Forsteo® Observational Study (EFOS).

Patients and Methods: Prospective observational study in female patients with osteoporosis from 8 countries. Treatment was for up to 18 months with an additional 18-month follow-up period. Clinical vertebral and non-vertebral fragility fractures were collected at follow-up visits. HRQoL was measured using EQ-5D. BP was measured using a 100 mm Visual Analogue Scale (VAS) ranging from no BP (0) to worst possible BP (100), and a BP questionnaire.

Results: 1648 patients were enrolled for a total 1924.7 women-years follow-up. Mean (SD) age was 71.5 (8.4) years. At baseline, 69.7% of patients had 2 or more preexisting fractures. 9.2% of the study participants were osteoporosis-treatment naïve. 138 women (8.8%) sustained a total of 168 incident fractures (38.7% clinical vertebral, 61.3% non-vertebral) (821 fractures /10,000 women-years) during follow-up. Mean total EQ-5D Health State Values increased from baseline to 18 months ($p = < 0.001$). The largest improvement was reported in the EQ-5D sub-domains of usual activities and pain/discomfort. A reduction of BP was observed after 18 months ($p < 0.001$). At baseline, in the 12 months prior to study entry, 95.5% of patients experienced BP. At 6 and 18 months, respectively 91.1% and 83.6% experienced BP in the month prior to observation. A 47% decrease in the odds of fracture in last 6-month period vs first 6-month period was observed ($p = 0.004$). A summary of the EQ-5D and BP results are shown in the Table 1.

Conclusion: Patients being prescribed teriparatide in the EFOS cohort suffer from severe osteoporosis. Treatment with teriparatide resulted in a reduction of back pain and fractures over time, and a clinical significant improvement in health related quality of life.

Table 1

Mean ± SD	Baseline	6 months	18 months
EQ-5D HSV	0.41 ± 0.38	0.64 ± 0.28	0.67 ± 0.29
EQ-5D VAS (mm)	51.7 ± 22.0	61.8 ± 20.4	68.1 ± 21.7
Every day or almst every day BP*	65.8%	34.8%	29.9%
Moderate and severe BP*	89.2%	65.8%	57.2%
BP VAS (mm)*	57.7 ± 26.6	38.2 ± 25.4	31.6 ± 25.6

* in the last month in pts with BP

Conflict of Interest: None declared

Mo-P263

PRE-OPERATIVE SERUM ALKALINE PHOSPHATASE PREDICTS HUNGRY BONE SYNDROME AFTER PARATHYROIDECTOMY FOR PRIMARY HYPERPARATHYROIDISM

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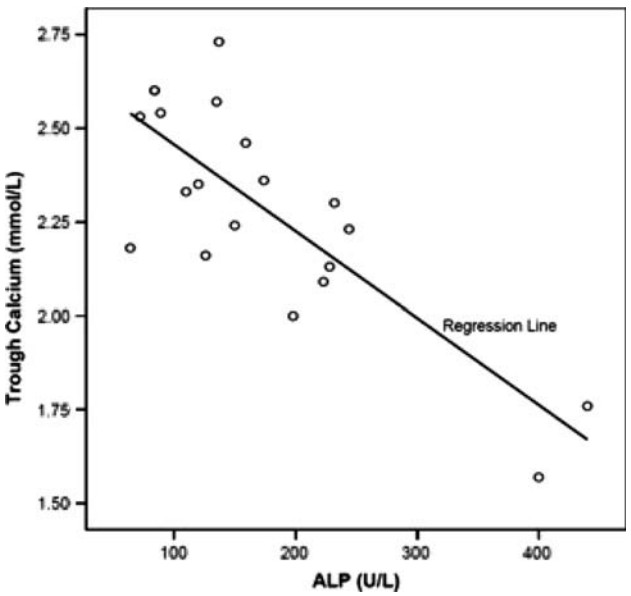
Parathyroidectomy for primary hyperparathyroidism is often complicated by post-operative hypocalcaemia. This may occur in several forms, of which the 'Hungry Bone Syndrome' (HBS) is one of the most severe. The study aims to investigate the relationship between HBS and a known risk factor, as well as establish a simple method to predict the risk of HBS occurring.

Data was collected retrospectively for patients who underwent parathyroidectomy. Primary analysis was done using linear regression between pre-operative serum alkaline phosphatase (ALP) and post-operative trough serum calcium (TSC). Secondary analysis was done by estimating the ALP threshold which corresponds to the 90% lower prediction limit for a TSC of 2.00 mmol/L (8.0 mg/dL). A comparison was done between patients who received bisphosphonate pre-treatment and those who did not using an Independent Samples T-Test.

The primary analysis shows a strong inverse correlation ($r = -0.805$, $p < 0.0001$) between pre-operative ALP and post-operative TSC. The secondary analysis shows that a pre-operative ALP of 158.5 U/L gives rise to a post-operative TSC less than 2.00 mmol/L (8.0 mg/dL) only 5% of the time. The T-Test did not show a significant difference between bisphosphonate pre-treatment groups.

The study shows that pre-operative ALP is a good predictor of post-operative TSC, and can reliably predict HBS.



Conflict of Interest: Grant/Research Support: Research University Grant Scheme (RUGS), Universiti Putra Malaysia

Mo-P264

EFFICACY OF AN INTRAVENOUS CALCIUM GLUCONATE INFUSION IN CONTROLLING SERUM CALCIUM AFTER PARATHYROIDECTOMY FOR TERTIARY HYPERPARATHYROIDISM

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Tertiary hyperparathyroidism (THP) is often seen in chronic renal insufficiency, and the treatment of choice is surgical removal of 3½ parathyroid glands. Surgery however can be complicated by severe post-operative hypocalcaemia, and to avoid this, we routinely start patients on an intravenous 10% calcium gluconate infusion according to a sliding scale immediately after surgery.

This study aims to assess the efficacy of this regimen in controlling serum calcium within the target range of 2–3 mmol/L during the immediate post-surgical period. Data was collected retrospectively for patients who underwent parathyroidectomy for THP. Variables looked at were total calcium infused (mmol), total treatment duration (days), trough and peak serum calcium (mmol/L), and excursions outside the target calcium range as a percentage of treatment duration (%).

Records for 63 cases were looked at, of which 38 had sufficient documentation for analysis. Mean calcium infused was 94.8 ± 10.6 mmol, average treatment duration was 2.92 ± 0.28 days, trough calcium was 2.06 ± 0.05 mmol/L, and peak calcium was 3.11 ± 0.06 mmol/L. Low excursions (< 2 mmol/L) occurred in 6.9 ± 1.8%, high excursions (> 3 mmol/L) occurred in 9.1 ± 2.5%, and excursions outside the target range (2–3 mmol/L) occurred in 16.0 ± 2.7% of total treatment duration. Of the 8 patients with a trough calcium < 1.8 mmol/L, 4 were due to premature discontinuation of the calcium infusion. Of the 9 patients with peak calcium > 3.2 mmol/L, only 2 were due to non-compliance with the infusion scale.

The results suggest that while the scale controls post-operative calcium reasonably, two problems were identified. First is that the infusion should not be stopped until the calcium level has stabilised. Second is that there is a tendency to over-replacement and hypercalcaemia, which can be corrected by adjusting the scale downwards.

Conflict of Interest: Grant/Research Support: Research University Grant Scheme (RUGS), Universiti Putra Malaysia

Mo-P265

EFFECTS OF EXTRACELLULAR INORGANIC PHOSPHATE ON FIBROBLAST GROWTH FACTOR 23 SIGNALING IN RENAL TUBULE CELLS

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It has been recently established that extracellular inorganic phosphate (Pi) exerts signals and results in altered gene expression in mineralizing bone cells such as osteoblasts and mature chondrocytes. Since renal proximal tubule cell plays a central role in Pi homeostasis and is exposed to varied extracellular Pi, we hypothesized that alteration in extracellular Pi concentration might trigger the signal transduction in renal tubular cells as well as bone cells. To address this issue, we examined the effects of altered extracellular Pi on signal transduction and gene expression, using HEK293 human embryonic kidney cells and LLC-PK1 porcine proximal tubular cells. Increased extracellular Pi induced phosphorylation of ERK1/2 within 15 minutes in a dose-dependent manner. Phosphorylation of c-Raf at Ser 338 was also observed, indicating that increased extracellular Pi activates c-Raf/MEK/ERK pathway. When the cells were treated with recombinant FGF23[R179Q] that is a constitutively active form of FGF23, phosphorylation of ERK1/2 and the expression of early growth response-1 (Egr-1) gene were induced. The effects of FGF23[R179Q] were facilitated by exogenous expression of klotho, which was consistent with previous reports. Interestingly, increase in extracellular Pi induced the expression of Egr-1, the target gene of FGF23 signaling. The treatment with phosphonoformic acid (PFA), an inhibitor of Na^+/Pi co-transporter, abolished the effects of increased extracellular Pi on phosphorylation of ERK1/2 and induction of Egr-1. In addition, exogenous expression of type IIa Na^+/Pi co-transporter enhanced the responsiveness of the cells to low concentration of extracellular Pi, suggesting that Na^+/Pi co-transporter might be involved in the responsiveness of the cells to extracellular Pi. We then examined the

effects of simultaneous treatment of the cells with increased extracellular Pi and recombinant FGF23[R179Q], and found additive effects in terms of phosphorylation of ERK1/2 and induction of Egr-1 expression. These results suggest that signaling induced by increased extracellular Pi shares the downstream signaling network with FGF23, and that the concentration of extracellular Pi might influence the responsiveness of the renal tubular cells to FGF23.
Conflict of Interest: None declared.

Mo-P266

DEVELOPMENT OF AN ASSAY FOR 25-HYDROXY VITAMIN D ON THE NEW AUTOMATED 3X3 SYSTEM

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Background and Objective: The 3x3 analyser is an innovative platform that integrates measurement modules for the simultaneous analysis of three different types of diagnostic tests; immunochemistry, biochemistry and coagulation. The purpose of this study is to develop and evaluate an immunochemistry assay for the determination of 25-Hydroxy Vitamin D (25-OH D). As the 3X3 has integrated modules for both biochemistry and immunochemistry, the system enables laboratories to determine 25-OH D, PTH, Calcium and Phosphorus simultaneously from single specimen tube. 25-OH D tests are performed to determine a patient•s nutritional Vitamin D status. Vitamin D levels are associated with a number of medical conditions including bone diseases, muscle function, diabetes, immune disorders, heart and circulatory disease, cancer and nervous system disorders.

Method: The 3 × 3 25-OH D assay is a chemiluminescent immunoassay for the measurement of 25-OH D in serum and plasma. Briefly, the method involves competition between sample and 25-OH D coated magnetic particles for an acridinium labelled anti 25-OH D antibody. Following a wash step the signal is determined using a luminometer. The signal is relative to the amount of acridinium present which is inversely proportional to the amount of analyte present. Time to first result is less than 40 minutes.

Results: The 3 × 25-OH Vitamin D assay has a reportable range of 0–160 ng/mL. The Analytical Sensitivity for this assay was reported as 2.2 ng/mL (95% Confidence method). Correlation to RIA ($n = 30$) gave an r value of 0.93. 25-OH D spiked into samples gave a mean recovery of 106% and linearity performed by diluting high and low samples gave a mean of 88.9% observed/expected.

Conclusions: This assay provides a rapid, and accurate automated measurement for 25-OH D on the 3 × 3 platform, with excellent correlation to existing manual assays. This will form an essential part of a comprehensive bone panel for use in the clinical setting.
Conflict of Interest: None Declared

Mo-P267

HYPOGONADISM AND BONE IN MEN: IMPACT OF BODY WEIGHT IN A FOLLOW-UP OVER 4 YEARS

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Background: Hypogonadism is one of the major causes of male osteoporosis. We followed hypogonadal and eugonadal men for hormonal and anthropometric parameters and bone mineral density (BMD) in a long-term follow-up for 4 years.

Methods: Based on an initial survey of 278 men, we invited patients with either normal or clearly reduced testosterone levels for a follow-up including clinical examination, hypogonadism and calcium intake questionnaires, hormonal and metabolic laboratory parameters as well as lumbar and femoral bone mineral density (BMD).

Results: Patients had a mean age of 59 ± 10 years and a median follow-up time of 4 ± 0.3 years. Hypogonadal men were significantly heavier than eugonadal men (7+/− 2 kg, $p = 0.03$). Osteoporosis as defined by decreased BMD was found in 17% of hypogonadal vs. 3% of eugonadal men. After 4 years, lumbar BMD measurements were stable in hypogonadal patients, whereas mean hip BMD was slightly decreased.

Interestingly, testosterone levels were found to have increased in 53% of our patients without overt supplementation ($p = 0.002$). Questionnaires of hypogonadism symptoms and parameters of bone metabolism did not correlate to androgen levels. Calcium intake in hypogonadal patients was significantly higher ($p = 0.007$) than in eugonadal men.

Multivariate regression analysis showed that only changes in body weight (ß = 0.58, $p = 0.004$) and calcium intake (ß = 0.42, $p = 0.03$) were relevant for changes in hip BMD, whereas lumbar BMD was mainly dependent on age (ß = 0.52, $p = 0.02$).

Conclusion: Hypogonadism is clearly relevant for osteoporosis in men. However, clinical questionnaires did not correlate with testosterone levels and should therefore be complemented by serum androgen measurements. Body weight and calcium intake were found to be independent factors of long-term hip BMD modulation and might therefore influence hip fracture risk in hypogonadal patients.
Conflict of Interest: None declared

Mo-P268

VITAMIN D RECEPTOR POLYMORPHISMS IN HYPOCALCEMIC VITAMIN D-RESISTANT RICKETS CARRIERS

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Hypocalcemic vitamin D-resistant rickets (HVDRR) is a rare autosomal recessive disorder characterized by severe rickets, hypocalcemia, secondary hyperparathyroidism, elevated levels of 1,25(OH)2D3 and occasionally, alopecia. In most cases the disease is associated with mutations in the gene of the Vitamin D receptor (VDR), the mediator of 1,25(OH)2D3 action. According to our previous work, the apparently healthy HVDRR heterozygotes differed from their respective controls, originated from the same population, in 1,25(OH)2D3 and PTH levels, as well as in the distribution of two common VDR polymorphisms -BsmI and TaqI. In order to eliminate the effect of the close population we expanded our study in a larger healthy population. We investigated the distributions of four common VDR polymorphisms -BsmI, TaqI, ApaI and FokI- in 67 relatives of 2 HVDRR patients, all members of an extended Greek kindred, and in 185 healthy individuals from another geographic region (Athens). VDR allelic polymorphisms were assessed by RFLPs after specific PCR amplification. The bb, TT and aa genotypes were less frequent in HVDRR carriers than in the healthy population in a statistically significant manner ($p = 0.008$, <0.001 and 0.052 respectively). No

difference was detected between the healthy Athenian population and the non affected individuals of the kindred studied regarding the distributions the same VDR polymorphisms. Our findings showed that the apparently healthy HVDRR carriers population present different genetic profile vis-à-vis the VDR polymorphisms when compared with non affected individuals suggesting that further investigation of HVDRR carrier population may elucidate the implication of VDR alleles in VDR function.

Conflict of Interest: None declared

Tu-P269

TOTAL KNEE ARTHROPLASTY MODIFIES THE SERUM LEVEL OF INTACT-PARATHYROID HORMONE IN POSTMENOPAUSAL WOMEN SUFFERING FROM END-STAGE KNEE OSTEOARTHRITIS

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Background/Aims: There is emerging evidence that intermittent treatment with Parathyroid Hormone (PTH) enhances the early fixation of orthopaedic implants, whereas continuously elevated levels of PTH may potentially play a negative role in the implants' incorporation process. Aim of this study was the evaluation of the impact of Total Knee Replacement (TKA) on the serum level of Intact-PTH (I-PTH).

Methods: During a period of 29 months, 119 postmenopausal women suffering from end-stage idiopathic knee osteoarthritis, scheduled to undergo TKA, were enrolled in this prospective study. Their mean age was 69.8 (±6.01) years. The serum levels of I-PTH, Calcium, Phosphorus & Creatinine were evaluated and the clearance of creatinine was calculated 1 day pre-operatively and on the 7th post-operative day. Patients with abnormal preoperative values, suffering from any endocrine disorder, rheumatoid or any other secondary arthritis, osteoporosis or any other disease that could interfere with their bone homeostasis or receiving medication affecting bone metabolism, were excluded. None had suffered a fracture or underwent any orthopaedic surgical operation during the 36 months prior to their enrollment.

Results: Sixteen patients (13.4%) had abnormally elevated post-operative I-PTH values. However, statistical analysis revealed a statistically significant trend towards decrease in post-operative I-PTH values ($p = 0.018$). The weight ($p = 0.763$), age ($p = 0.776$), serum creatinine level ($p = 0.922$) and creatinine clearance of the patients ($p = 0.963$) did not have a statistically significant impact on the observed alteration of I-PTH values after TKA.

Conclusion. The serum levels of I-PTH seem to decrease following a TKA, as immediately after implantation, bone cells adjacent to the implant are likely to be dead due to necrosis or apoptosis. The latter is a strong stimulus for bone resorption that probably leads to increased serum calcium concentrations that may well decrease the endogenous PTH production. Another possible explanation could be the temporary immobilization of the patients undergoing TKA. However, a substantial number of women had abnormally elevated post-operative I-PTH values. Regardless of what actually caused that increase, the potentially negative impact of continuously elevated PTH on bone formation, may interfere with the implant's incorporation procedure, hence the evaluation of serum I-PTH before and after TKA is strongly recommended.

Conflict of Interest: None declared

Tu-P270

AGE-RELATED CHANGES IN SERUM TESTOSTERONE AND SEX HORMONE BINDING GLOBULIN IN UKRAINIAN MEN

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The purpose of this study was to assess sex steroid levels in cross-sectional cohort of men.

Object. 160 men in age from 30 to 79 years ((M ± m): age–57,6 ± 1,2 years; height −1,74 ± 0,06 m; weight −83,6 ± 1,2 kg) were examined and divided into the following age-dependent groups: 20–29, 30–39, 40–49, 50–59, 60–69, and 70–79 years old.

Methods: Levels of testosterone and sex-hormone binding globulin were determined by the method of immunoadherence technique, free and bioavailable testosterone were calculated using action equations (A. Vermeulen et alt., 1999).

Results: The indexes of hormonal status in men depending on age are represented in table.

We found the positive correlation between age and level of SHGB in serum ($r = 0.22$, $p = 0.029$). Also we established negative correlation between age and level of testosterone and its fractions: testosterone ($r = -0.32$, $p = 0.001$), free testosterone ($r = -0.43$, $p = 0.000007$), bioavailable testosterone ($r = -0.32$, $p = 0.001$).

Conclusion. Age significant affect on the indexes of hormonal status in men. Ageing connect with decline the level of testosterone and its fractions and increase the level of SHGB.

Notes: results are represented as M ± m, BMI - body mass index, SHBG - sex-hormone binding globulin, T - testosterone, FT - free testosterone, BT - bioavailable testosterone.

Table 1 The indexes of hormonal status in men depending on age

Parameters Age, years	30–39 y(n17) 36.0 ± 0.5	40–49 y(n34) 45.5 ± 0.4	50–59 y(n33) 55.1 ± 0.5	60–69 y(n41) 65.1 ± 0.5	70–79 y(n35) 72.9 ± 0.5
Height, m	1.79 ± 0.02	1.76 ± 0.01	1.74 ± 0.009	1.72 ± 0.01	1.71 ± 0.01
Weight, kg	85.8 ± 4.6	86.1 ± 2.8	86.7 ± 2.4	82.4 ± 2.3	78.6 ± 2.2
BMI, kg/m2	26.5 ± 0.8	27.6 ± 0.9	28.4 ± 0.6	27.9 ± 0.7	27.0 ± 0.6
SHBG, nmol/L	35.9 ± 4.7	38.4 ± 3.8	37.8 ± 2.4	43.7 ± 4.4	55.3 ± 3.8
T, nmol/L	21.9 ± 1.6	15.1 ± 0.8	17.1 ± 1.0	14.6 ± 1.0	14.2 ± 1.0
FT, nmol/L	0.51 ± 0.05	0.30 ± 0.02	0.35 ± 0.02	0.28 ± 0.02	0.23 ± 0.02
FT, %	2.23 ± 0.14	2.07 ± 0.11	2.02 ± 0.08	1.93 ± 0.10	1.55 ± 0.07
BT, nmol/L	11.9 ± 1.2	7.1 ± 0.4	8.2 ± 0.6	6.5 ± 0.5	5.3 ± 0.5
BT, %	52.4 ± 3.3	48.5 ± 2.6	47.5 ± 1.9	45.2 ± 2.2	36.5 ± 1.7

M ± m

Conflict of Interest: None declared

Tu-P271

VITAMIN D AND CALCIUM SUPPLEMENTATION IMPROVES MUSCLE STRENGTH IN OLDER WOMEN WITH VITAMIN D INSUFFICIENCY

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The mechanism of the effect vitamin D to reduce falls is uncertain although some authors have identified effects on muscle strength

others have not. As part of an RCT which has shown that calcium and vitamin D reduces falls by 20% compared to calcium alone explanatory data on muscle function were studied.

302 elderly women (age 77.2 ± 4.6 years) with serum 25OHD concentrations less than 60 nmol/L participated in a 1 year randomised, double-blind, placebo controlled trial. Subjects received 1000 mg calcium citrate per day with 1000 IU ergocalciferol (vitamin D) or identical placebo (control). The main outcome measures were lower limb muscle strength measured by voluntary maximal contraction and mobility as assessed by Timed Up and Go (TUAG) a test whereby the time taken to stand from sitting walk three meters turn and return to the chair is measured.

At baseline 25OHD was 44.7 ± 12.6 nmol/L, this increased in the vitamin D group but not the control group after 1 year (59.8 ± 13.8 vs 45.0 ± 13.3 nmol/L, $P < 0.001$). Irrespective of treatment group the change in 25OHD concentration correlated with the change in knee and hip flexor strength ($R = 0.13$, $P = 0.034$; $R = 0.14$, $P = 0.026$) respectively. The effect was most prominent in those who received vitamin D supplementation, (knee flexor $R = 0.21$ $P = 0.018$; hip flexor $R = 0.18$; $P = 0.046$ control NS).

In those with baseline hip muscle strength values in the lowest tertile, vitamin D improved muscle strength compared to calcium alone (hip extensors 22.6 ± 9.5%; hip adductors 13.5 ± 6.75% $P < 0.05$ cf control). Vitamin D treatment also significantly reduced TUAG (−17.5 ± 7.6%, $P < 0.05$ cf control) in those with baseline values in the highest (slowest) tertile.

Vitamin D and calcium, but not calcium alone, improved hip muscle strength and mobility in those with low baseline values. Vitamin D should be added to those with insufficiency or deficiency to improve muscle function.

Conflict of Interest: None declared

Tu-P272

WOMEN WITH SEVERE VITAMIN D DEFICIENCY: WHICH DOSE OF CHOLECALCIFEROL TO RAPIDLY INCREASE 25(OH)D3 SERUM LEVEL OVER 75 NMOL/L ?

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Aim: According to current recommendations, vitamin D deficiency should be treated before introducing any antiresorptive agent. The aim of our study was to define the dose of cholecalciferol allowing to reach rapidly correct level of 25(OH)D3 (>75 nmol/L) among women with osteoporosis and severe vitamin D deficiency, in order to start as soon as possible bisphosphonate or other specific therapy.

Patients and methods: We included, from february to november 2007, 40 women (>65 years of age), living in the general community (91%) or in nursing homes (9%) with severe vitamin D deficiency (25(OH)D < 25 nmol/L). Women were randomly assigned into one of the three groups to receive 100.000 UI (group 1, n = 10), 200.000 UI (group 2, n = 15) or 300.000 UI (group 3, n = 15) of cholecalciferol. Serum levels of calcium, phosphate, 25(0H)D3, and creatinine were determinate at baseline, after one week (D8) and one month (D30).

Results: In the group 1, 2 women reached the level of 75 nmol/L at D8 (20%), 4 in the group 2 (26,7%) and 13 in the group 3 (86,7%), which shows a significant difference between the group 3 and the two others ($p < 0,05$). The mean serum level raised from 19,5 to 52,5 nmol/L in the group 1, from 20,25 to 67,5 nmol/L in the group 2 and from 22,75 to 95,25 nmol/L in the group 3. No excessive serum level of calcium or 25(OH)D3 was observed at D30.

CONCLUSION : In order to introduce rapidly (less than 8 days) an antiresorptive therapy in good conditions, women with osteoporosis and severe deficiency of vitamin D should be received 300.000 UI of cholecalciferol. Vitamin D supplementation can then be continued with 100.000 UI every two or three months.

Conflict of Interest: None declared

Tu-P273

CALCITONIN BLOCKS THE ACUTE EFFECT OF TERIPARATIDE ON BONE RESORPTION MARKER BETA-CTX IN HEALTHY MEN

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Background: A single-dose teriparatide injection in healthy men resulted in an acute increase of the serum cross-linked C-telopeptide of type I collagen (beta-CTX) (Zikan and Stepan, Biomarker Insights, in press). Beta-CTX is released into the circulation during osteoclastic resorption of bone. Experimental data indicated that active osteoclasts are required within one hour after PTH administration to achieve the full anabolic effect of PTH (Gooi et al.; ASBMR Annual Meeting 2006). The aim of this study was to test the hypothesis that salmon calcitonin (sCT), a specific inhibitor of osteoclast activity, is able to modify the acute effect of teriparatide on the bone resorption. Methods: Six healthy men (age range 28–35) were each studied twice. The tests were started after overnight fast, 3 h after a standard calcium load. Blood samples were obtained before (baseline) and 30, 60, 90, 120, and 180 min after a single subcutaneous injection of teriparatide (20 ug) with and without a simultaneous administration of nasal sCT (200 IU). Results: The teriparatide alone resulted in a significant increase in serum beta-CTX with maximum mean increase of 87 % at 180 min after teriparatide administration. When sCT was administered simultaneously with teriparatide, a significant decrease in serum beta-CTX was observed (maximum mean decrease by 63 % at 120 min with a subsequent increase near to baseline value). Conclusions: The present data in healthy men indicate that the acute effect of teriparatide on bone resorption marker beta-CTX can be blocked by co-administration of sCT. Thus, the acute effect of PTH on bone involves active osteoclasts. However, whether active osteoclasts are needed to the anabolic effect of PTH in humans requires further study.

Conflict of Interest: None declared

Tu-P274

WINTER HOLIDAYS IN THE SUN IMPROVES VITAMIN D STATUS

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In northern Europe, vitamin D insufficiency occurs with a high frequency during wintertime. Only at latitudes south of app. 38° N, vitamin D is synthesised in the skin in response to sun-exposure during wintertime in northern Europe.

Aim: To study effects on vitamin D status of going on a winter holiday in the sun.

Design: In a cross-sectional study including 2,624 women, we identified women who had been on a winter holiday during October to February at a location south of 38° N, and in whom blood samples were obtained within 4 months after returning home. For each holiday-seeking woman, we identified 4 age-matched women who had not been on a winter holiday and in whom blood samples were obtained at the same time (± 2 weeks) as those who had been on a winter holiday.

Results: In the group of 2,624 women, P-25OHD showed the well known circannual rhythm with highest levels in August (median 95 (range: 22–204) nmol/l) and lowest levels in Marts (60 (9–149) nmol/l). Median duration of winter holidays was 8 (4–37) days. Median time from returning from a winter holiday until blood samples were drawn was 73 (18–109) days. Median P-25OHD was significantly higher ($p < 0.001$) in women who had been on a winter holyday (85 (42–142) nmol/l) than in those who had not been on a holiday (65 (4–145) nmol/l). Use of vitamin D supplements did not differ between groups ($p = 0.48$)

Conclusion: Going on a winter holiday in the sun increases P-25OHD to summer vitamin D levels. The effect seems to last for several months after returning home.

Conflict of Interest: None declared

Tu-P275

SHORT- AND LONG-TERM VARIATIONS OF SERUM CALCIOTROPHIC HORMONES AFTER A SINGLE MASSIVE DOSE OF ERGOCALCIFEROL OR CHOLECALCIFEROL

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The potencies of vitamins D2 and D3 were evaluated after a single dose of 300,000 IU of the respective calciferols either by os or by intramuscular (im) route to 4 groups of 8 elderly, female nursing home patients (D3 os: age = 78.5 ± 7.5 yrs mean ± SD; D3 im: 80.6 ± 5.0; D2 os: 80 ± 10.1; D2 im = 79.3 ± 4.6). The time course of serum calcidiol [25(OH)D], calcitriol [1,25(OH)2D], Ca++ and PTH was followed at 0 and at 3, 7, 30 and 60 days. Serum calcidiol and calcitriol were determined by RIA and PTH levels using IRMA (N-tact PTHSP) (DiaSorin Inc., Stillwater, MN, USA).

Basal calcidiol levels were: D3 os: 13.3 ± 9.9 ng/ml; D3 im: 12.9 ± 9.8; D2 os: 8.35 ± 3.6; D2 im: 7.3 ± 2.6. We observed a brisk increase in 25(OH)D levels at day 3 only when vitamins were given orally. The 30 day-basal difference in serum calcidiol was significantly greater after D3 oral administration (47.8 ± 7.3 ng/ml) compared with other forms (D3 im: 15.9 ± 11.3; D2 os:17.3 ± 4.7; D2 im: 5 ± 4.4; all $p < 0.001$). The 60 day-basal difference of 25(OH)D was significantly lower for D2 compared with D3, independently of the route of administration ($p < 0.01$). Furthermore, the greater potency of D3, particularly when given orally, was shown by AUC of the serum 25(OH)D against time. In fact, AUC_{60} was: D3 os: 3193 ± 759 ng"d/mL vs D2 os: 1820 ± 512,$p < 0.001$; D3 im: 1361 ± 492 vs D2 im 728 ± 195, $p < 0.01$.

The 3 day-basal difference in serum levels of 1,25(OH)2D showed a significant brisk increase only after D2 oral administration (83 ± 19 pg/mL) compared with other forms (D3 os: 40.1 ± 39.8 pg/ml, $p < 0.05$; D3 im: 7.6 ± 10.2 and D2 im: −1.12 ± 8, $p < 0.001$). However, no differences were found between groups as far as the AUC_{60} of the serum 1,25(OH)2D was concerned.

The influence of calcidiol on PTH levels has been studied on the residual part of the variation which is not explained by Ca++ using a stepwise procedure. Calcidiol played a significant role in influencing PTH levels at 3 ($p < 0.03$), 7 ($p < 0.01$), 30 ($p < 0.01$) and 60 days ($p < 0.05$). Moreover, at 60 days, the form of vitamin (D3), but not its way of administration, significantly lowers PTH levels ($p = 0.037$). Our data demonstrate 1) that, based on our 60 day values, vitamin D3, after a single massive dose, is almost twice as potent as vitamin D2 in raising serum calcidiol, when administered either by mouth or im; 2) a role of calcidiol in modulating serum PTH, possibly via a residential parathyroid 1-α-hydroxylase.

Conflict of Interest: None declared

Tu-P276

EFFECTS OF ALPHA-KETOGLUTARATE (AKG) AND BETA-HYDROXY-BETA-METHYLBUTYRATE (HMB) ON CALCIUM AND PHOSPHORUS CONTENT IN FEMUR AND BONE-SPECIFIC ALKALINE PHOSPHATASE ACTIVITY, INSULIN-LIKE GROWTH FACTOR-1 AND LEPTIN CONCENTRATIONS IN BLOOD PLASMA OF FUNDECTOM

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The stomach has a variety of physiological functions. Recent studies have shown that fundectomy-evoked macroelement malabsorption contribute to mechanism responsible for development of osteopenia. The aim of this study was to determine the effects of long-term administration with alpha-ketoglutarate (AKG) combined with calcium salt of beta-hydroxy-beta-methylbutyrate (CaHMB) to fundectomized pigs on calcium and phosphorus content in femur. Furthermore, plasma concentrations of insulin-like growth factor-1 (IGF-1), leptin, and bone-specific alkaline phosphatase (BAP) activity were evaluated. Forty day old animals were divided into five groups ($n = 6$ per group). Four groups of animals were fundectomized and orally administered with placebo (FX group), AKG (AKG group), CaHMB (HMB group) or AKG with CaHMB (AH group), respectively, while the fifth group underwent sham operation (SHO group). Placebo (CaCO3) and CaHMB were administered at the dosage of 0.05 g/kg of BWday-1 while AKG of 0.4 g/kg of BWday-1. Femur and blood plasma were obtained from 9 month old animals. Fundectomy significantly decreased the content of calcium and phosporus in femur, and blood plasma concentrations of IGF-1 and leptin compared with the sham operated animals ($P < 0.01$). Administered AKG with CaHMB significantly increased the content of calcium and phosphorus in femur, blood plasma concentration of leptin and plasma BAP activity compared with the placebo-treated controls ($P < 0.05$). Treatment with AKG increased IGF-1 concentration in plasma compared with the FX group ($P = 0.04$). In conclusion, oral administration of AKG and CaHMB induced anabolic effect on bone tissue in fundectomized pigs and this effect was associated with increased blood plasma concentration of leptin.

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Conflict of Interest: None declared

Tu-P277

CHANGES IN MARKERS OF BONE METABOLISM AFTER PRENATAL DEXAMETHASONE TREATMENT IN MALE AND FEMALE PIGLETS

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The most important factor for normal growth is growth hormone (GH) which influences the proliferation and function of osteoblasts. Bone mineralization depends on bone specific alkaline phosphatase (BALP) and osteocalcin (OC) - a noncollagenous protein correlating with osteoblast activity. Maternal glucocorticoid treatment affects foetal bone development. The aim was to quantify the bone formation in piglets by measurement of blood serum concentrations of GH, BALP, OC after maternal administration of dexamethasone at the dosage of 3 mg/sow/48h from approximately day 70 up to the parturition of a 115–116 days pregnancy. Blood samples were collected from control group (males $n = 15$, females $n = 15$) and maternal dexamethasone treated piglets (dex group, males $n = 15$, females $n = 15$). Sampling made just after the birth and in the 35th day of postnatal life. OC and BALP showed a progressive decrease in the concentration with advancing postnatal age in the control males and females. Maternal dexamethasone treatment resulted in 2 fold decrease of GH and BALP concentration in female newborns compared with their control. OC and BALP decreased approximately 30% in male, and OC also in female newborns of dex group compared with their controls. In the 35th day of postnatal life all the markers were significantly higher in females from dex group compared with the control females. In conclusion, maternal dexamethasone treatment caused suppression of GH, OC and BALP in blood serum. The negative maternal effect of dexamethasone on metabolic bone markers is transient, and serum levels of GH, BALP and OC promptly return to control levels in 35 day old piglets.

Conflict of Interest: None declared

Tu-P278

EARLY PREVALENCE OF OSTEOPOROSIS IN DOWN SYNDROME

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Background: Down syndrome (DS), phenotypic expression of trisomy 21 is associated with mental retardation, immune disorders, congenital diseases and in adult age with accelerating ageing, Alzheimer disease and osteoporosis. Up to now there is inadequate information dealing with bone mineral density in people with DS. In addition, the most data are related to woman and information on BMD in males is rare. Health conditions, thyroid dysfunction, abnormalities of sexual development, anticonvulsant medication, insufficient diet and other factors may contribute to the development of osteoporosis.

Methods: Dual-energy X-ray absorptiometry of the lumbal spine and femoral neck, biochemical examination.

Results: We examined 90 adults with DS (45 women, 45 men), mean age 25.71 years. In both group we found low level of 25-(OH) vitamin D (belong 20 ng/ml. The significant differences between both groups showed followed results: decreased 25-(OH) vitamin D in women ($p < 0.005$), decreased level of osteocalcin in women ($p < 0.05$), but decreased bone density in men – NF neck ($p < 0.05$), NF Z-score ($p < 0.0001$), lumbal BMD, Z-score ($p < 0.003$). We did not find the differences in levels of minerals in serum and urine.

Conclusions: We conclude that the osteoporosis is most frequent in men with DS and is connecting with sexual immature, the 25-(OH) vitamin D deficiency is evident in both group.

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Conflict of Interest: None declared

Tu-P279

FUNCTIONAL GENOMICS STUDY ON HUMAN OSTEOBLASTS FROM OSTEOPOROTIC PATIENTS

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Osteoblasts represent potential cellular target for the development of novel anabolic therapeutic agents. In order to identify genes involved in osteoblasts response to the anabolic stimulus, we performed functional genomics analysis by HG-U133 Plus 2.0 GeneChip® microarrays (Affymetrix®) in human primary osteoblast-like cells upon treatment with vitamin D3. Osteoblast-like cell cultures were obtained from trabecular bone samples of 21 female donors undergoing orthopaedic surgical procedures for hip replacement (14 subjects had surgery for femur fracture; 7 subjects had surgery for arthrosis). Complete patient phenotyping, thus including DXA bone mineral density assessment (femur, Hologic QDR 4500) blood and urinary biochemical parameters testing, was collected for 18 of the patients. Gene expression profiles were analyzed and statistically validated by means of R\Bioconductor. Pathway analysis was performed by Ingenuity® IPA5.5. Supervised analysis was performed to identify genes modulated in treated versus control cells and verify the presence of expression patterns correlating with bone quantitative parameters in osteoporotic versus control patients. Functional annotation of the identified genes allowed the identification of molecular pathways that might play a role in osteoblasts response to anabolic stimuli including a number of "druggable genes" that may thus represent potential therapeutic targets.

Conflict of Interest: None declared

Tu-P280

AGE AND GENDER DISTRIBUTION OF PRIMARY HYPERPARATHYROIDISM IN A EUROPEAN COUNTRY WITH A PARTICULARLY HIGH LIFE EXPECTANCY

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Background: Primary hyperparathyroidism (PHPT) is a frequent endocrine disease in the elderly, but little is known about its epidemiology in the aging European population. We thus investigated the age- and sex-dependence of PHPT in Switzerland, a country with a particularly high life expectancy.

Methods: A population-based retrospective study was performed using data from the Swiss Federal Statistical Office covering 2000 to 2004. Anonymized hospital discharge codes for PHPT and

parathyroidectomy (PTX) were analysed and the hospitalization rate for PHPT and incidence of PTX, in-hospital prevalence of PHPT, and the proportion of surgically-treated PHPT patients were calculated. We also investigated 117 patients undergoing PTX in one university hospital between 1999 and 2006 to describe the clinical characteristics.

Results: The mean annual hospitalization rate of patients with PHPT was 8.3/100,000 inhabitants with an age-dependent increase. The rate was approximately three times higher in women with the highest estimate found in women ≥80 years (63.7/100,000). In-hospital prevalence of PHPT was 43.8/100,000 hospitalizations, also rising with age. The population-adjusted incidence of PTX was higher in individuals ≥50 years than in younger persons (8.7/100,000 vs 1.3/100,000). In a sample of 117 PHPT patients undergoing PTX, 42% were symptomatic.

Conclusion: In a European population with high life expectancy, the hospitalization rate and in-hospital prevalence of PHPT are higher in women and increase continuously with age. Symptomatic disease is frequent among surgically-treated patients. These findings underscore the need for further research on the impact of population aging on the epidemiology of PHPT.

Conflict of Interest: None declared

Tu-P281

ALCOHOL CONSUMPTION AND BONE METABOLISM IN PHYSICALLY ACTIVE MALE SOLDIERS: POSSIBLE ROLE OF SEX STEROIDS

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Background: Previous studies indicate that moderate alcohol consumption is associated with higher BMD; however the underlying mechanism is not clear. We investigated the relation between alcohol consumption and bone mineral density (BMD), sex steroids, calcitropic hormones and bone turnover markers in a cohort of males with well-defined lifestyle conditions that minimizes the effect of confounding variables.

Materials and Methods: 330 men from Indian armed forces ($n = 330$) having uniform and defined routine were enrolled for the study. BMD at spine and hip was measured by dual-energy X-ray absorptiometry (DXA). The association between the alcohol consumption with BMD, sex steroids and bone turnover markers were assessed by ANCOVA. Multiple regression models were used to assess the independent influence of alcohol on BMD.

Results: Subjects consuming alcohol up to 4drinks/week had significantly higher BMD at femur compared with non-alcohol consumers ($p < 0.05$) and a linear increase in mean femoral BMD over increasing categories of alcohol intake ($p < 0.05$) was observed. In stepwise multiple regression analysis, age and alcohol consumption were independent predictors of femoral BMD, together explained 13% of the variance. At the lumbar spine, age and BMI were independent predictors, explaining 9% variance in BMD. There was a marked decrease in SHBG ($p = 0.0178$) and PTH ($p < 0.0002$) levels in alcohol consumers compared to non-consumers, whereas no change was observed in mean levels of total testosterone as well free and bioavailable forms. However, the mean levels of total estradiol ($p = 0.043$), free estradiol and bioavailable estradiol ($p < 0.001$) were increased significantly in alcohol consumers compared to non-consumers. The bone remodeling was increased in alcohol consumers as reflected by elevated mean levels of Osteocalcin and CTx.

Conclusions: In physically active men with well-defined lifestyle conditions, alcohol consumption was associated with higher BMD and the beneficial effect of alcohol is complex and is probably mediated through concurrent decrease in SHBG and PTH levels with resultant increase in free and bioavailable estradiol levels thereby protecting the bone mass.

Conflict of Interest: None declared

Tu-P282

WINTERTIME VITAMIN D SUPPLEMENTATION INHIBITS SEASONAL VARIATION OF CALCITROPIC HORMONES AND MAINTAINS BONE TURNOVER IN HEALTHY MEN

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Introduction: Vitamin D is suggested to have a role in the coupling of bone remodelling. The association between bone mineral density (BMD) and vitamin D status is evident among men. Compared with women, men are believed to have more stable bone remodelling, and thus, are considered less susceptible to the seasonal variation of calcitropic hormones.

Aims: We examinated whether seasonal variation exists in calcitropic hormones, bone remodelling markers and BMD in healthy men. Furthermore, we determined adequate vitamin D intake to prevent this variation.

Subjects and methods: Subjects ($N = 54$) comprised healthy Caucasian men aged 21–49 years with a mean (SD) habitual dietary intake of vitamin D of 6.6 (5.1) μg/d. This was a double-blinded vitamin D intervention study, in which subjects were allocated to three groups of 20 μg (800 IU), 10 μg (400 IU), or placebo. The trial lasted from November to April, altogether 26 weeks. Fasting blood samplings were collected six times for analyses of serum 25-OHD, iPTH, BALP and TRACP. Radial volumetric BMD was measured at the beginning and end of the study with peripheral QCT. Statistical analyses were performed with repeated measures ANCOVA.

Results: Seasonal variation was noted in S-25-OHD, S-iPTH and S-TRACP ($p < 0.001$, $p = 0.012$ and $p < 0.05$, respectively), but not in the bone formation marker or vBMD in the placebo group. Until the 20-week time point, vitamin D supplementation increased the S-25-OHD concentration dose-dependently, the mean dose-response being 1.55 (1.24) nmol/l/μg. Supplementation inhibited the winter elevation of PTH ($p = 0.035$), decreased the S-BALP concentration ($p = 0.044$), but had no effect on the S-TRACP.

Conclusion: Healthy men are exposed to seasonal variation in vitamin D status that impacts PTH concentration. Vitamin D supplementation improved vitamin D status and inhibited the winter elevation of PTH, but also decreased BALP concentration. Bone remodelling seemed to stay coupled in supplemented groups, but not in the placebo group. Although the results concerning peripheral radial BMD show only a tendency towards benefit, our study supports the prevailing data of the benefits of vitamin D. A total intake of 17 μg of vitamin D is required to prevent season-related changes in bone metabolism among healthy men.

Conflict of Interest: None declared

Tu-P283

VITAMIN D DEFICIENCY AMONG NON-WESTERN IMMIGRANTS: RANDOMIZED CT OF SUNSHINE EXPOSURE AND SUPPLEMENTATION

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Background: Vitamin D deficiency (25-hydroxyvitamin D [25-OHD]< 25 nmol/l) is common among non-western-immigrants. Vitamin D deficiency can be treated with supplementation and sunlight.

Objective: The aim of this study was to determine whether the effects of supplementation with vitamin D3 (daily 800 IU or 3 monthly 100,000 IU) or sunshine exposure are similar with regard to serum 25-OHD and PTH concentrations, and clinical outcomes.

Design: This randomized clinical trial was conducted in 12 general practices in The Netherlands. Non-western immigrants, aged 18–65 years (n = 232), who were vitamin D deficient (25-OHD < 25 nmol/l) were randomly assigned to receive supplementation or advice for sunshine exposure for a period of 6 months (March-September). Data and blood samples were collected during treatment (baseline, 3 months, 6 months) and follow-up (12 months). Both intention to treat and per protocol analysis were performed with multilevel regression modelling (MLwiN).

Results: Significant higher serum 25-OHD concentrations were observed in both supplementation-groups (800 IU and 100,000 IU) compared to the sunshine-group (adjusted for sex, age, and BMI ($\chi 2 = 79.45, 6df, p < 0.001$). Serum PTH concentrations were lower in both supplementation-groups compared to the sunshine-group (adjusted for baseline 25-OHD, sex, age, and BMI ($\chi 2 = 14.64$, 6 df, p = 0.023). Results of clinical outcomes (physical performance, functional limitations, and pain) showed no relevant changes over time.

Conclusion: Vitamin D supplementation is much more effective than sunshine exposure for treating vitamin D deficiency in non-western immigrants.

Conflict of Interest: None declared

Su-P284

A COST-UTILITY ANALYSIS OF FULL-LENGTH PARATHYROID HORMONE PTH (1–84)

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Background: A cost-utility analysis was carried out to estimate the health economic impact of full-length parathyroid hormone PTH (1–84) compared to placebo in postmenopausal women at high risk of fracture.

Methods: A randomised controlled trial[1] compared 18 months treatment with full-length parathyroid hormone PTH (1–84) 100 μg daily and placebo in postmenopausal women with a BMD T-score <−2.5. 70% of patients were treatment-naïve. Results showed a 61% reduction in the risk of a new vertebral fracture with full-length parathyroid hormone PTH (1–84) compared to placebo (95% CI 31%–78%). A Markov model was constructed, based on published epidemiological sources, in order to describe the likely fracture and mortality outcomes for a population of patients with prior vertebral fractures. Using efficacy estimates from the study above, quality adjusted life years (QALYs) gained and associated health care costs were estimated over a 10 year time frame following 18 months treatment with full-length parathyroid hormone PTH (1–84) vs placebo. Both variables were discounted at 3.5% per year.

Results: Central estimates of treatment benefit across the age range 60–85 years demonstrated QALY differences ranging from 0.12 to 0.20 and cost differences ranging from £4,177 and £4,599. This yields incremental cost effectiveness ratios (ICER) ranging from £21,204/QALY to £34,826/QALY. Sensitivity analysis across the full range of age cohorts and treatment benefit for full-length parathyroid hormone PTH (1–84) were conducted. For any given age cohort there is an approximate 2.5-fold variation in results across the efficacy range, with the central estimate being skewed towards the lower figure. For patients at higher risk (3 or more prior fractures) the ICER is reduced to £16,485–£27,222 versus placebo.

Conclusions: Full-length parathyroid hormone PTH (1–84) is effective in the reduction of osteoporotic vertebral fracture. The results of this cost-utility analysis demonstrate that, when considered from the perspective of clinical vertebral fracture, conventionally acceptable levels of cost effectiveness (<£30,000/QALY) were achieved for most age groups in women with severe osteoporosis. For postmenopausal women with three or more prior fractures, these figures fell below £20,000/QALY for all but the oldest patients.

Reference: 1. Greenspan SL et al. Ann Intern Med 2007;146: 326–39.

Conflict of Interest: Dr. J Belsey, Nycomed, consultation

Su-P285

FEMUR ULTRASOUND (FEMUS)—A NEW METHOD FOR THE ESTIMATION OF OSTEOPOROTIC FRACTURE RISK?

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Although calcaneus Quantitative Ultrasound (QUS) has similar power as DXA for the prediction of the osteoporotic fracture risk, the highest risk gradient is found between femur DXA and hip fracture risk. We developed FemUS, a QUS scanner for measurements directly at the proximal femur. In two independent studies in Kiel and Odense we investigated the ability of the FemUS scanner to discriminate between women with and without recent hip fractures in comparison with femoral DXA and calcaneal ultrasound.

In Kiel, 20 subjects (10 fractured, age 78 ± 6, and 10 controls, age 72 ± 10 years) and in Odense 42 subjects (20 fractured, age 68 ± 7, and 22 controls, age 62 ± 4 years) were included in the study. All subjects were measured on a DXA-scanner, the FemUS-scanner and a calcaneus QUS device (Achilles InSight). Evaluated variables were BMD of the total femur measured using DXA (Femur-BMD), SOS and BUA measured using the FemUS scanner (Femur-SOS, Femur-BUA), and SOS, BUA and stiffness measured using the InSight (Calc.-SOS, Calc.-BUA, Calc.-STI). Femur-SOS was adjusted for leg

width using ultrasound echoes reflected from the skin of the leg. Age-adjusted odds ratios were calculated to characterize the power of fracture discrimination.

In Kiel only Femur-BMD and Femur-SOS showed significant differences between the groups at $p < 0.05$. In Odense all variables discriminated the groups to a similar degree. Age-adjusted odds ratios and the corresponding confidence intervals are depicted in the table 1.

In both studies Femur-SOS and DXA-BMD discriminated significantly and equally well between women with and without osteoporotic fractures. Performance of the other variables was inconsistent. Our first measurements indicate that femoral QUS might become a new method for the estimation of osteoporotic fracture risk.

Table 1

	Odds ratios (Kiel)	Odds ratios (Odense)
Femur-BMD	9.8 (1.1–86.1)	4.0 (1.5–10.8)
Femur-SOS	10.7 (1.2–93.9)	3.5 (1.5–7.9)
Femur-BUA	2.2 (0.7–6.3)	3.2 (1.5–6.9)
Calc.-SOS	1.4 (0.6–2.9)	3.7 (1.5–9.6)
Calc.-BUA	3.4 (0.9–12.6)	4.2 (1.6–10.8)
Calc.-STI	2.6 (0.8–8.4)	4.3 (1.7–11.2)

Hip fracture discrimination: age-adjusted odds ratios

Conflict of Interest: None declared

Su-P286

BONE MINERAL DENSITY AND BODY COMPOSITION MEASURED BY DXA HALF SCAN PREDICTS TOTAL BODY VALUES

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DXA technology, a preferred method to measure bone mineral density (BMD) and body composition, has become a valuable diagnostic tool in the clinical management of patients with obesity and other metabolic conditions. Change in fat mass is a principal parameter used in following the obese patient. However, measurement accuracy may be affected by artefacts or in patients with supine body width larger than the DXA scan window. An alternative solution to the total body scan may be to scan half the body and double the values, assuming left-right symmetry in composition of tissues and organs.

Objective: To evaluate the predictive value of half scans for total body BMD and body composition, and to determine the degree of left-right asymmetry due to organ location.

Methods: We used 1027 DXA total body scans (GE Lunar Prodigy model): 656 women, 371 men, age range 20–89 years old. We measured fat mass (FM), lean mass (LM) and bone mineral density (BMD) of the total body, right half body and left half body.

Statistics: We studied the strength of association of the values using a linear regression model. We measured the half scan correlation with total body results by Pearson's test.

Results: There were very strong and equivalent correlations between each half-scan and the respective total body measurement: BMD $R^2 = 0.963$ and SEE = 0.02 for right half, $R^2 = 0.965$ and SEE = 0.02 for left half; FM $R^2 = 0.996$ and SEE = 707.6 for right half, $R^2 = 0.996$ and SEE = 702.1 for left half; LM $R^2 = 0.987$ and SEE = 1224.5 for right half, $R^2 = 0.987$ and SEE = 1232.9 for left half. The average asymmetry of the two halves was 0.142% for BMD, with non-significant slightly higher values in left body half, while FM and LM asymmetry values were 0.521% and 0.311% respectively, with similar non-significant higher values on the right side.

Conclusion: Measurements from half total body scans may be used in cases where the total body cannot be scanned due to extraordinary large body width or artefacts, with a good quality and accuracy values, especially for fat mass and lean mass parameters. Either the left or right half may be used to predict total body values.

Conflict of Interest: None declared

Su-P287

DOES WRITTEN STANDARDIZED INFORMATION IMPROVE PATIENT UNDERSTANDING OF DXA-RESULTS?

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Self-reported DXA-results reflect patient understanding of physicians' information and may influence persistence with osteoporosis therapy and subsequent fracture prevention. Few studies have addressed this issue and showed at best moderate agreement between self-reported and actual DXA-results. In our out-patient clinic a strategy to improve patient perception of DXA-results was developed, relying on written standardized information following DXA-scanning.

We evaluated patient understanding of DXA-results one year after it was performed. Information on diagnosis and treatment recommendations was mailed to 1000 consecutive patients and their GPs. Patients were encouraged to discuss the information with their GP. To assess self-reported DXA-results, all patients received a mailed questionnaire one year later addressing perceived results. 717 patients responded (72%; mean age = 63.5 yrs, SD = 10.3). Results were compared against the DXA reports registered in the clinic (Table 1). Overall agreement between self-reported and actual DXA-results was excellent (q.wt.kappa = 0.83). Differences in results of self-report was not related to severity of diagnosis (Chi2-test, $p = 0.4$). Confusion seems to exist on the subject of osteopenia, with 18% of osteoporotic patients perceiving to be osteopenic, and vice versa 9%. Of all patients 4% were completely unaware of DXA-results. Conclusion: Communicating results of DXA-scanning in writing to both patient and GP diminishes risk of misconception in theory. Our findings suggest that standardized written information can provide most patients with the basic understanding of DXA-results. Efforts to improve understanding of osteopenia should be put forward, as these patients form a group of potentially preventable osteoporotic patients.

Table 1 Self-report compared against registered DXA-results

Registered/ Self-report	Osteoporosis	Osteopenia	Normal BMD	Total
Osteoporosis	168 (77%)	28 (9%)	3 (1%)	199
Osteopenia	40 (18%)	228 (77%)	24 (12%)	292
Normal BMD	1	29 (10%)	165 (82%)	195
Other	8 (4%)	13 (4%)	10 (5%)	31
Total	217	298	202	717

Conflict of Interest: None declared

Su-P288

REFERENCE VALUES FOR HIP STRUCTURE ANALYSIS (HSA) IN HEALTHY YOUNG MEN - RESULTS FROM THE ODENSE ANDROGEN STUDY

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Bone mineral density (BMD) is closely related to fracture risk (1), however, several studies have suggested that hip geometry or hip structural analysis (HSA) (2,3) may also predict fracture risk or even improve such prediction. Normative data regarding HSA, however, are scarce. Aim: To establish reference values for HSA in young men. Subjects and methods: The Odense Androgen Study is a population-based, prospective, observational study on the inter-relationship between endocrine status, body composition, muscle function, and bone metabolism in young men including 783 males aged 20–30 years in Funen County, Denmark. DXA and hip structure analysis of the hip was performed using a Hologic-4500a densitometer and the recently developed APEX V2.0 software. Results: The LMS method (4) was used to generate the reference values because the data was not normally distributed. Results are shown in table 1. Conclusion: Reference data were established regarding HSA in young health males.

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Table 1 Data are shown as median ± SD (L)

	Narrow Neck	Inter trochanteric	Femoral shaft
CSA cm2	4.01 ± 0.62 (0.13)	6.59 ± 1.08 (0.28)	5.47 ± 0.71 (0.40)
CSMI (cm2)2	4.70 ± 1.08 (−0.17)	21.0 ± 4.90 (0.09)	5.37 ± 1.17 (−0.13)
Section modulus cm3	2.38 ± 0.46 (−0.05)	6.09 ± 1.21 (0.30)	3.18 ± 0.51 (0.06)
Buckling ratio	9.16 ± 1.78 (0.09)	7.00 ± 1.28 (−0.21)	2.46 ± 0.52 (−0.09)
Hip axis length mm	124 ± 6 (2)		
Neck-shaft angle degrees	131 ± 5 (4)		

Conflict of Interest: Kim Brixen, technical support: Hologic Inc

Su-P289

DEGRADATION OF BONE BIOMECHANICAL PROPERTIES SECONDARY TO QUANTITATIVE AND QUALITATIVE BONE DISTURBANCES INDUCED BY ARTHRITIS IN SKG MICE

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Background: Rheumatoid arthritis (RA) increases the fracture risk caused by low energy trauma and such fact seems to be partially explained by the reduced bone mass. Nevertheless, bone strength is dependent not only on its mineral density, but also on its microstructural quality.

The objective of this study was to evaluate bone biomechanical properties and bone structural defects due to arthritis.

Methods: The study was performed using an animal model of arthritis, the SKG mouse, which develops a chronic autoimmune polyarthritis, resembling human RA. BALB/c mice were used as controls. Three point bending tests were performed in mice femoral bones, while vertebrae were submitted to compressive tests. Density measurements were made using a water pycnometer. Samples were observed and chemically analysed by scanning electron microscope (SEM) associated with energy dispersive X-ray spectroscopy (EDS). Collagen structure was evaluated using multiphoton microscopy and second harmonic generation (MPM /SHG).

Results: Arthritic bones showed disturbances in their biomechanical properties (affecting stiffness, ductility and bone strength), in comparison to control femurs and vertebrae. Regarding density measurements, arthritic female mice have less bone mass than healthy ones. SEM images indicated the existence of less trabeculae in vertebral arthritic bone, as compared to controls. Although, in arthritic mice collagen appeared quantitatively normal using MPM/SHG.

Conclusion: These results clearly showed that inflammation induces a reduction in bone density, affecting also qualitatively bone organization, which is reflected in a degradation of bone biomechanical properties, namely stiffness, ductility and bone strength.

Conflict of Interest: None declared

Su-P290

ARE THERE DIFFERENCES AMONG WOMEN WITH POSTMENOPAUSAL OSTEOPOROSIS DEPENDING ON THE PRESENCE OF VERTEBRAL FRACTURES?

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Vertebral fractures are usually asymptomatic and they are a major risk factor for developing further fractures. Thus, it is important to assess if there are differences in the clinical and laboratory profile between patients with and without vertebral fractures.

Objectives: To analyse the clinical characteristics in postmenopausal women with osteoporosis (OP) and vertebral fractures and to compare them with those without fractures.

Methods: This cross-sectional prospective study included 152 postmenopausal women with OP with a mean age 64,2 ± 9,7 years (44–88) who were referred to an outpatient rheumatology department to evaluate treatment of OP. None had an evident secondary cause of OP. A clinical history was obtained with special reference to risk

factors for OP. Bone mass assessment (BMD), spine X-rays, laboratory tests including complete blood count, chemistry profile, PTH, 25(OH) vitamin D (25OHD), thyroid hormones, urinary NTX and 24-h urinary calcium and cortisol were performed on all patients before treatment.

Results: 26% had previous vertebral fractures, 36% had non-vertebral fractures and 33% had family history of fractures. Comparing women with and without vertebral fractures, those with fractures were older (61.9 ± 8.5 vs 70.1 ± 10 years, $p < 0.001$), had a larger postmenopausal period (13.9 ± 9.4 vs 22.8 ± 11.9 years, $p < 0.001$), had lower stature (151.6 ± 7.9 vs 155.6 ± 6.3 cm, $p = 0.007$), and lower femoral BMD (Z score −1.9 ± 0.8 vs −2.2 ± 0.8, $p = 0.036$). Among the laboratory parameters women with OP and vertebral fractures showed lower serum levels of albumin (43 ± 2.6 vs 44.9 ± 2.5 g/L, $p = 0.01$), urinary calcium excretion (142.1 ± 124 vs 197.6 ± 128 mg/24 h, $p = 0.02$) and glomerular filtration rate (55.4 ± 16 vs 65.6 ± 16 ml/min, $p = 0.013$). There were no significant differences between groups in the other analysed variables. A negative correlation was noted between age and glomerular filtration rate (r −0.57, $p < 0.001$), urinary calcium excretion (r −0.28, $p < 0.001$), albumin levels (r −0.30, $p = 0.003$) and femoral neck BMD.

Conclusions: Postmenopausal women with osteoporosis and vertebral fractures were older, had lower stature and lower femoral neck BMD than osteoporotic women without fractures. Moreover, they had lower levels of albumin, lower glomerular filtration rate and lower urinary calcium excretion, abnormalities related to ageing.

Conflict of Interest: None declared

Su-P291

BONE MINERAL DENSITY AND MARKERS OF BONE TURNOVER IN MEN WITH IDIOPATHIC OR HYPOGONADAL OSTEOPOROSIS

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Histomorphometric studies indicate that idiopathic male osteoporosis is characterized by low turnover (Bilezikian. CTI. 69:248). The purpose of this post-hoc analysis was to compare baseline characteristics of men with idiopathic (n = 223) or hypogonadal (n = 213) osteoporosis enrolled in a clinical trial (Orwoll et al. 2003 J Bone Miner Res). Men aged 30–85 years were ambulatory and had spine or hip BMD at least 2 SD below the average for young healthy men. Men with causes of osteoporosis other than idiopathic or hypogonadism were excluded. Sixteen men with hypogonadal osteoporosis on stable doses of androgen or other anabolic steroids for at least 6 months continued this therapy. Assessments included DXA BMD of spine and hip and biochemical markers of bone turnover serum procollagen I carboxy-terminal (PICP), serum bone alkaline phosphatase (bone ALP), urinary free deoxypyridinoline (fDPD), and urinary N-telopeptide (NTX). Histomorphometry was not performed. Men with idiopathic osteoporosis were significantly older than those with hypogonadal osteoporosis (mean years ± SE, 61.1 ± 0.8 vs. 54.8 ± 0.9, $p < 0.001$). There was no difference between the proportion of men with prevalent nonvertebral fractures (58% idiopathic vs. 60% hypogonadal, $p = 0.616$). BMD, adjusted for group, age, country, and therapy was not significantly different between groups (mean ± SE, table). Median concentrations of turnover markers in the idiopathic and hypogonadal groups, respectively, were PICP, 116 vs.123 ng/L (normal range N/A); bone ALP, 10.3 vs. 11.7 ng/L (3.7–20.9); fDPD/Cr, 4.4 vs. 4.3 nM/mM (2.0–5.0); NTX/Cr, 31.1 vs. 35.4 nM/mM (0–85). Marker point estimates in the idiopathic compared with hypogonadal males ranged from 2% higher to 12% lower, with no statistically significant differences. Men with idiopathic versus hypogonadal osteoporosis were older, but there were no significant baseline differences in history of nonvertebral fracture, BMD, or markers of bone turnover.

This study was supported by Eli Lilly and Company.

Table 1 Bone mineral density

BMD (g/cm2)	n	Idiopathic	n	Hypogonadal
L spine	223	0.86 ± 0.01	213	0.87 ± 0.0
Total hip	221	0.83 ± 0.01	213	0.83 ± 0.01
Fem neck	213	0.71 ± 0.01	200	0.71 ± 0.01

Mean ± SE, adjusted for group, age, country, treatment

Conflict of Interest: JH Krege, K See, GP Dalsky, H Oertel are full-time employees of Eli Lilly and Company.

Su-P292

PERI-TROCHANTERIC FAT CUSHION EVALUATED BY DXA AS HIP FRACTURE PROTECTOR

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Decreased bone mass and micro-architecture degradation are well-established predictors of hip fracture. However, falls and associated issues such as type and energy of the fall could also play a significant role in hip fracture risk, especially in elderly subjects. The fat tissue surrounding the hip joint may help to absorb the energy generated by a fall. Fat mass surrounding the proximal femur can be measured by dual-energy X-ray absorptiometry (DXA) technology as part of conventional bone densitometry scans. In this study we evaluated in a case-control manner the regional fat mass in the gluteus and peri-trochanteric area measured by DXA as a factor in hip fracture risk.

Subjects: 109 male and female patients were divided into two groups. Cases group: 42 hip fracture patients (35 women and 7 men, mean age 63.2 years, mean BMI 78.5) with 29 cervical and 17 trochanter fractures) were selected by a history of low-energy hip fracture in the previous six months (mean 3,4 ± 2,5 month). Control group: 67 patients (54 women and 13 men, mean age 61.9 years, mean BMI 84.1) without hip fracture or history of any condition associated with soft-tissue asymmetry in the lower extremities.

Methods: Femur and total body DXA scans were performed with a GE Lunar Prodigy model on all patients. Bone mineral density (g/cm2) and "R" value were evaluated for every femur scan. The 'R' value, the ratio between low and high-energy absorptiometry coefficients, has a linear relationship with fat mass. To validate these results, the fat mass derived from the femur scan was compared to the fat mass measured in the same region of interest from the total body scan for each patient.

Statistics: Mean and standard deviation of all variables from both groups were compared by T-test. The results were divided in quartiles and c2 and odds ratios were obtained from Crosstab. An ANOVA test was also performed.

Results: Fat mass around the proximal femur did show a protective effect against trochanteric hip fractures, especially in patients with a lower bone density in the trochanter (ANOVA $p = 0.027$). Regional fat mass did not have a significant protective effect in femur neck fracture. The odds ratios in the lowest quartile for predicting any hip

fracture were: regional fat mass 3.01, total femur BMD 2.67, femoral neck BMD 3.46 and trochanter BMD 2.40.

Conclusion: We found higher amounts of fat surrounding the proximal femur have a protective effect against trochanteric hip fracture risk in this study.

Conflict of Interest: None declared

Su-P293

HIP-FRACTURE TYPE AND FUNCTIONAL OUTCOME IN ELDERLY WOMEN

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Aim: Many observations support the view that there are significant differences between the women who sustain trochanteric fracture and those with cervical fracture of the hip. Apart from the well established differences in the pathogenesis of the two types of hip fracture, an unfavourable outcome has been attributed to trochanteric fractures, although studies on this topic showed inconsistent results. Our aim was to investigate differences in the functional recovery between women sustaining cervical or trochanteric fractures of the hip.

Methods: We studied 684 of 736 women admitted consecutively to a rehabilitation hospital in Italy because of their first hip fracture. Functional recovery was assessed by using Barthel index scores. Fractures were classified as either cervical ($n = 335$) or trochanteric ($n = 349$) on the basis of surgical and radiographic findings.

Results: After acute in-patient rehabilitation, women with trochanteric fracture had a significantly lower Barthel index score than women with cervical fracture (median values were 85 and 90 respectively, interquartile ranges were 25 and 30 respectively, $p = 0.001$). Length of stay in hospital was significantly longer in women with trochanteric fractures (median was 37 days versus 36 days; interquartile range was 10 days versus 8 days, $p = 0.018$). However, the differences between the two groups were no longer significant after adjustment for eight variables that affect functional ability in the same population (i.e., age, pressure ulcers, cognitive impairment, neurologic impairment, infections during the length of stay, bone mineral density, body mass index, and Barthel index scores assessed before rehabilitation). Furthermore, we found no significant differences in the change of Barthel index scores during rehabilitation and in Barthel index efficiency (change in the Barthel index score after rehabilitation divided by the length of stay in hospital) between the two groups of women.

Conclusion: After adjustment for several confounders, we did not show significant differences in the functional outcome between women with cervical or trochanteric fracture of the hip.

Conflict of Interest: None declared

Su-P294

THE QUALITY OF LIFE ASSESSMENT IN A GROUP OF PATIENTS WITH OSTEOPOROSIS IN FELIX SPA ROMANIA USING QUALEFFO 41 QUESTIONNAIRE

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Aim of the study: to assess the quality of life of a group of patients with osteoporosis in Felix Spa, Romania.

Matherial and method: Our group of study consisted in 79 women with osteoporosis(DEXA Method), the mean age was 61.25, standard deviation 4.65 years. The patients were included in a specific physical exercise program, twice a week for one year. They were assessed with Quallefo-41 Questionnaire at the begining of the study, at 6 month and at 12 month.

Results: It was noticed a favourable evolution of all the domains of the Quallefo 41 Questionnaire, and also of the general Quallefo score at 6 month, and more evident at 12 month. It proved a beneficial effect of regular specific exercise program in improving quality of life of the patients in our study.

Conclusions: The regular physical exercise has a beneficial effect in osteoporosis. The Quallefo 41 Questionnaire is a useful tool in assessment the evolution of the quality of life in patients with osteoporosis.

Conflict of Interest: Non declared

Su-P295

ADVANCED IMAGING ASSESSMENT OF GLUCOCORTICOID-INDUCED OSTEOPOROSIS (GIO)

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Advanced bone imaging techniques provide structural information, beyond bone mineral density (BMD), and evidence indicates that BMD only partially explains bone strength and fracture resistance. In assessing GIO, especially, the documentation of glucocorticoid (GC) impact on trabecular and cortical bone, and on macro and micro structure is important.

Advanced methods for assessing macrostructure of bone include volumetric quantitative computed tomography (vQCT), high resolution computed tomography (hrCT) at 100–400 μ, and high resolution magnetic resonance imaging (hrMR) at 100–300 μ. The methods for assessing bone microstructure include micro computed tomography (μCT) at 1–100 μ, and micro magnetic resonance imaging (μMR) at 20–100 μ (1).

A number of advanced imaging techniques have been used in vitro and in vivo to examine structural effects of GIO in animals and in humans.

Chappard (2) reported micro architectural changes in male GIO, using histomorphometry and μCT; and found that trabecular plate perforations could be observed by μCT, mainly in GIO. Lill (3) used vQCT and μCT to examine osteoporosis induced by ovariectomy, malnutrition and GC in sheep; and found excellent correlations between vQCT density, μCT structural parameters, and biomechanical properties. Akahoshi (4) studied the modulation of bone turnover by alfacalcidol and alendronate in preventing GIO in minipigs, using vQCT, μCT and histomorphometry; and found that GC reduced age-dependent bone growth, reduced bone formation rate and activation frequency, and that treatment did not maintain skeletal mass and structure. Rehman (5) examined PM women with GIO and HRT to determine the best measure of BMD to predict vertebral fractures; and found that spinal BMD by vQCT, but not by DXA, was independently predictive of fracture. Lian (6) compared GC-treated and GC-naive PM women for differences in hip BMD by vQCT and in strength by finite element analysis; and found GC treatment caused decreased hip BMD and reduced hip strength, through losses of both trabecular and cortical bone.

These applications, illustrating advanced imaging in GIO, are still in early development; but their novelty is compelling and their utility is promising.

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KE: Consultant for Merck, Amgen, GSK. Novartis; Synarc stockholder

HKG: Consultant for Merck, Amgen, GSK, Novartis, Roche, Lilly, BMS, Sevier, Wyeth, GE, Hologic; Synarc stockholder

Su-P296

CLINICAL EVALUATION OF A NOVEL FRAGMENTS ABSORBED IMMUNOCAPTURE ENZYMATIC ASSAY (FAICEA) FOR TRACP 5B

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Until recently, tartrate-resistant acid phosphatase (TRACP) measurements have lacked specificity and not performed as other markers of bone resorption. We evaluated a novel fragments absorbed immunocapture enzymatic assay (FAICEA) for TRACP 5b. The effect of a freeze thaw cycle was determined using paired samples one subjected to a single freeze thaw cycle and one not previously thawed. Between-day variability and the effect of food intake were assessed in 14 healthy premenopausal women studied over 10 days. Subjects were either fasted or fed on alternate days prior to sample collection. The response to bisphosphonate treatment was assessed in 23 women with postmenopausal osteoporosis, mean age 64 years, who were randomized to receive 10 mg alendronate plus 500 mg calcium carbonate daily (n = 16) or 500 mg calcium carbonate alone (n = 7) for 24 weeks. Serum samples were collected, after an overnight fast, at baseline, 4, 12 and 24 weeks. Samples were assayed using the Nittobo TRACP 5b FAICEA (Nitto Boseki Co Ltd) and serum CTX was assayed by Elecys automated assay (Roche Diagnostic).

The intra-assay CV of the TRACP 5b assay was 1% and the interassay CV 2–4%. After one freeze thaw cycle there was a mean decrease of 53% (SEM 2%) compared to unthawed samples (n = 8). The mean TRACP 5b in the fasting state was not significantly different from in the fed state 2.19 IU/L (SE 0.21) vs, 2.14 (SE 0.22). Within subject variability was higher in fed subjects compared to fasting subjects (11% vs 8%, F statistic 1.89, $P < 0.05$). The response to alendronate is shown in the table 1.

In conclusion FAICEA performed well although freeze thaw cycles should be avoided. The higher variability in samples collected in the fed state this is unlikely to be of clinical significance. The response of TRACP 5b measured by FAICEA to alendronate treatment is similar to that for sCTX which suggests that it is a potentially useful marker in measuring response to antiresorptive treatment.

Table 1 Mean % Change (SEM) TRACP5b and sCTX

Week	TRACP 5b			sCTX		
	Alen+Ca	Ca alone	P	Alen+Ca	Ca alone	P
4	−51 (4)	−18 (4)	<0.005	−59 (7)	−7 (10)	<0.001
12	−59 (3)	−22 (4)	<0.0001	−70 (10)	−12 (11)	<0.05
24	−57 (5)	−13 (5)	<0.0001	−75 (8)	−7 (10)	<0.001

Conflict of Interest: TRACP 5b FAICEA kits supplied free of charge by Nitto Boseki Co Ltd Japan

Mo-P297

ESTIMATES OF VOLUMETRIC BONE MINERAL DENSITY FROM SCANS UTILIZED BY IDXA

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Background/Aims: Volumetric bone mineral density (VBMD) yields more accurate assessment of bone strength and quality, therefore is a better predictor of fracture than areal bone mineral density (ABMD) typically obtained by DXA technology. QCT technique gives the most accurate volumetric density measures, but it involves higher radiation and is not readily available for mass screening. Our objective was to estimate VBMD from lateral and anteroposterior (AP) spine measurements performed by iDXA and assess its association with ABMD and other parameters obtained by iDXA as well as with anthropometric variables. Methods: To ascertain for a normal vertebral shapes, the lateral vertebral assessment (LVA) was performed as well. LVA yields the posterior (P), mid (M) and anterior (A) vertebral heights which ratios are used to assess the degree of vertebral deformities. Only the subjects with normal-shaped vertebrae were evaluated. The subjects were 80 overweight, generally healthy, postmenopausal women whose bones and body composition were measured by iDXA in all available skeletal sites. The VBMD for L2–L4 was derived from the respective lateral BMC values divided by lateral area and AP width. Results: The average VBMD was 0.161 ± 0.04 g/cm^3 (mean ± SD) comparable with the data obtained from other studies in postemopausal women. The M/P ratios for L2, L3 and L4 were $91.4 \pm 4.7\%$, $92.9 \pm 4.7\%$, and $95.2 \pm 5.9\%$, respectively. The A/P ratios for L2, L3 and L4 were $102.1 \pm 7.1\%$, $105.9 \pm 7.5\%$, and $108.4 \pm 7.4\%$, respectively. Both M/P and A/P values indicated normal vertebral shapes. VBMD was best correlated with L2–L4 T-score, BMD, and BMC (with r of 0.6, 0.5, and 0.4, respectively, all $p < 0.05$). Age, weight, height, and gynoid fat (derived from total body scan) contributed to 31% variance of VBMD, with age (t-ratio = −1.86, $p = 0.066$), height (t-ratio = −3.28, $p = 0.002$), and gynoid fat (t-ratio = −3.34, $p = 0.001$) being negative and weight (t-ratio = 4.20, $p = < 0.001$) being positive predictors. The same variables contributed only to 7% variance of ABMD and only weight and gynoid fat were statistically significant. Conclusions: Based on this preliminary data, the VBMD estimated from lateral and AP iDXA scans appears to be more sensitive and responsive to anthropometric measures than ABMD. The next step would be to compare the iDXA derived VBMD with that obtained from QCT scans.

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Conflict of Interest: None declared

Mo-P298

FAMILY PHYSICIANS' X-RAY ORDERING PATTERNS IMPROVE IN HIGH RISK PATIENTS FOLLOWING THE CANADIAN QUALITY CIRCLE (CQC) NATIONAL PROJECT

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It is important that Family physicians' (FPs) order X-rays in patients that are likely to have vertebral fractures to confirm their existence. However, FPs X-ray ordering in high risk patients remains sub-optimal and not always consistent with Osteoporosis Canada (OC) guidelines (2002). Thus, the CQC Project was designed to improve FPs adherence with the OC guidelines. The project consists of five phases: baseline (BASE) data collection, 1st educational intervention, follow-up I (FOL-I) data collection, 2nd educational intervention, and follow-up II (FOL-II) data collection. This analysis examined the rate that FPs order X-rays in high risk patients. The OC guidelines recommends that all patients with prospective height loss ($>$ 2 cm), historical height loss ($>$ 6 cm) or kyphosis should have an X-ray to rule out a vertebral fracture. A total of 340, 301 and 162 FPs formed 34, 34 and 28 QCs, during BASE, FOL-I, FOL-II, respectively. For each phase, FPs gathered data from different patients via chart reviews and a standardized collection form. A total of 8376 (BASE), 7354 (FOL-I) and 3673 (FOL-II) patient records were selected at random and analyzed. All patients were women 55 years and older. The generalized estimating equations (GEE) approach was used to evaluate changes in X-ray ordering patterns in high risk patients. The cluster variable for the GEE model was physician. An exchangeable correlation matrix was used for the analysis. Odds ratios (OR) and 95% confidence intervals (CI) were calculated. According to the guidelines, 30.1% (2521/8376), 25.6% (1879/7354) and 26.8% (983/3673) of patients in our sample should have received an X-ray during BASE, FOL-I, and FOL-II, respectively. Of these high risk patients, 47.5% (1193/2514) at BASE, 60.9% (1143/1878) at FOL-I and 70.9% (695/981) at FOL-II received an X-ray. Compared with baseline measurements, the likelihood of a physician ordering X-rays increased during FOL-I (OR: 1.6; 95% CI: 1.4, 1.9) and FOL-II (OR: 2.4; 95% CI: 2.0, 3.0). In conclusion, more women with kyphosis or height loss had X-rays ordered at the end of the study. Appropriate X-ray ordering may result in a greater number of vertebral fractures detected. Once a vertebral fracture is identified, appropriate management can be initiated.

Sponsored by: Alliance for Better Bone Health, and Ontario College of Family Physicians.

Conflict of Interest: honoraria or consultancies- The Alliance for Better Bone Health (Procter & Gamble Pharmaceuticals and sanofi-aventis)

Mo-P299

FAMILY PHYSICIANS' UTILIZATION OF APPROPRIATE BONE MINERAL DENSITY TESTING IMPROVED FOLLOWING THE NATIONAL CANADIAN QUALITY CIRCLE (CQC) PROJECT

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It has been well documented that even with the publication of Osteoporosis guidelines, there is still inadequate evaluation of the disease. Thus, the Quality Circles (QCs) study was used to improve family physicians' (FPs) adherence with the Osteoporosis Canada (OC) 2002 Guidelines. The study consists of five phases: baseline (BASE) data collection, 1st educational intervention, follow-up I (FOL-I) data collection, 2nd educational intervention, and follow-up II (FOL-II) data collection. During the educational interventions, QC's met to discuss how they managed osteoporosis and to participate in an osteoporosis workshop. The OC guidelines recommends that only high-risk patients should be administered a bone mineral density (BMD) test and that high risk is defined as one or more major or two or more minor risk factors. A total of 340, 301 and 162 FPs formed 34, 34 and 28 QCs, during BASE, FOL-I, FOL-II, respectively. For each phase, FPs gathered data from different patients via chart reviews and a standardized collection form. All patient charts examined were women 55 years of age or older. A total of 8376 (BASE), 7354 (FOL-I) and 3673 (FOL-II) patient records were selected at random and analyzed. To adjust for possible clustering within a physician, generalized estimating equations (GEE) approach assuming an exchangeable correlation structure was used to evaluate differences in appropriate BMD testing. Odds ratios (OR) and 95% confidence intervals (CI) were calculated. A total of 62.0% (5192/8371), 68.6% (5026/7328) and 74.4% (2719/3657) patients appropriately received a BMD test during BASE, FOL-I, FOL-II, respectively. Compared with baseline values, the odds of a physician ordering appropriate BMD testing increased during FOL-I (OR: 1.3; 95% CI: 1.2, 1.4) and FOL-II (OR: 1.5; 95% CI: 1.4, 1.7). In conclusion, the use of QCs is an effective approach that improves physicians' management of osteoporosis by increasing the appropriate use of BMD testing. Patients with low BMD may benefit with treatment.

Sponsored by: Alliance for Better Bone Health, and Ontario College of Family Physicians.

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Mo-P300

THE CRACK-GROWTH RESISTANCE OF BONE AND THE INFLUENCE OF ORIENTATION AND DRUG TREATMENTS ON THE TOUGHENING MECHANISMS IN BONE

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Human bone has evolved to be more difficult to break than split. However, appropriate fracture-toughness measurements to break bone (in the transverse direction) are rare. Most measurements focus on crack initiation, whereas human bone principally derives its fracture resistance during crack growth; moreover, the few crack-growth toughness (R-curve) measurements are for longitudinal "splitting" orientations. Here we use nonlinear-elastic fracture mechanics to determine crack-resistance R-curves for both orientations in human cortical bone, using in-situ testing within an environmental scanning-electron microscope to simultaneously examine the salient damage/toughening mechanisms. We find that stress-intensities up to 5 MPa$\sqrt{m}$ are required to propagate large cracks along the bone long axis, whereas to propagate a crack only 500 microns in transverse directions requires stress-intensities five times higher. Such toughnesses are far larger than previously thought, yet represent a truer depiction of conditions to break, rather than split, bone. Mechanistically, this behavior results from microcracking at osteon/interstitial interfaces, which promotes gross crack deflections for transverse cracking and crack bridging for longitudinal orientations. This framework developed using "healthy" human bone was then applied to animal models used to evaluate the effect of bisphosphonates for the treatment of osteoporosis. It was found that the bisphosphonates

could affect the crack profile in bone and as a consequence, the extrinsic toughening mechanisms and toughness of bone.
Conflict of Interest: None declared

Mo-P301

THE IMPORTANCE OF BONE MINERAL DENSITY MEASUREMENT AT MULTIPLE SKELETON SITES

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Site-discordance in bone mineral density (BMD) assessment is common and affects patient categorization. The effect of site-discordance on patient categorization has been previously discussed. There is a need to define the effect of BMD measurement at the contralateral femur on osteoporosis diagnosis.

Aim. The aim of the study was to investigate the effect of BMD measurement at the contralateral femur on osteoporosis diagnosis.

Methods: BMD of the lumbar spine and both femurs was measured by dual energy X-ray absorptiometry in 124 consecutive Caucasian women aged $\geq$50 years (mean 62.7, SD $\pm$ 8.5, range 50–82) with a body mass index of 29.6 $\pm$ 4.6 (range 20.8–45.2). Using the T score from each site, measurements were classified according to the WHO criteria for the diagnosis of osteoporosis as normal, if T score > -1, osteopenia, if Tscore < -1 and osteoporosis, if T score < -2.5. The results from the three skeleton sites were compared to each other using the statistical package SPSS and the effect of the inclusion of the contralateral femur on the diagnosis of osteoporosis was determined.

Results: BMD did not differ between the two femurs, T score -1.83 ± 0.08 (mean $\pm$ SEM) and -1.77 ± 0.09, BMD (g/cm2) 0.819 ± 0.01 and 0.825 ± 0.01 of the right and left femur, respectively ($p > 0.05$, paired Student's t test). In 101 (81.4%) the classification of the women would be the same if either two or three sites were measured. In 23 (18.6%) of the women the classification would be affected by the measurement of the contralateral femur. In 12 (9.7%) of them T score of the lumbar spine was equal or lower than that of both femurs, thus lumbar spine measurement determined the diagnosis. In the remaining 11 (8.9%), classification depended on the choice of the femur measured and would be different if only the right or left femur was measured.

Conclusions: BMD measurement was not different between the femurs. Although the number of cases included is small, it appears that determining BMD at the lumbar spine and one femur is enough for a correct evaluation and diagnosis in the majority of the patients. Nevertheless, in a small number of patients the choice of the femur measured may affect patient diagnosis and classification.
Conflict of Interest: None declared

Mo-P302

BODY COMPOSITION AND BONE MASS IN HEALTHY CHILDREN OF EXTREMADURA (SPAIN): CORRELATION WITH ANTHROPOMETRICS AND NUTRITIONAL FACTORS

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Background: Several studies in children have shown relationships between lean mass, fat mass and bone mass. The objective of the study was to examine the association between body composition and bone mass in healthy children and its correlation with anthropometrics and nutritional factors.

Methods: Two hundred and twenty-one prepubertal participants (110 females and 111 males), aged between 4.0 and 14.0 years, were studied. Relationships between anthropometrics (height, weight and body mass index) and nutritional factors (daily intakes of calcium, carbohydrates, fats, and proteins) with ultrasound bone parameter and body composition (lean mass and fat mass) were analyzed. Body composition was analyzed using mono-frequency tetrapolar bioimpedance analyser (Holtain BC). Quantitative ultrasound measurements of the heel (nondominant side) were performed using McCue CUBA Clinical System sonometer and included speed of sound (SOS) and broad-band ultrasound attenuation (BUA). Dietary data (daily intakes of calcium, carbohydrates, fats and proteins) were assessed using a prospective 7-day diet survey.

Results: Quantitative ultrasound bone measurements, body composition and anthropometrics factors were similar in boys and girls. Nutrition intake was higher in boys ($p < 0.05$ in all). The percentage of boys and girls who consumed more than 1,000 mg of calcium per day was 92.79% and 81.81%, respectively. BUA and SOS correlated positively with lean mass ($p < 0.0001$ respectively) and with fat mass only BUA in females ($p = 0.02$). BUA ($p < 0.0001$ in all cases) and SOS ($p < 0.05$ in all cases) correlated positively with all anthropometrics factors, but not with nutrients intake.

Conclusion: In this study, bone status in children is determined by lean mass and anthropometrics factors rather than nutrients intake.
Conflict of Interest: None declared

Mo-P303

BODY COMPOSITION AND BONE MASS IN HEALTHY SPANISH WOMEN: RELATIONSHIP WITH GONADAL STATUS

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Background: There are some discrepancies about the correlation between the different measurements of bone mass and the body composition. We have investigated the contribution of body lean mass (LM) and body fat mass (FM) of the arms, legs, and trunk on bone mass in healthy Spanish women and their dependence on gonadal status.

Methods: The study was a cross-sectional analysis in 462 women of age 18–82 yr and mean body mass index 26.6 kg/m^2 (4.5 kg/m^2 SD). Bone mass measures were assessed using peripheral quantitative computed tomography (pQCT) of the nondominant distal forearm, phalangeal bone ultrasound, as the amplitude-dependent speed of bone ultrasound (Ad-SoS) and dual energy X-ray absorptiometry (DXA) (lumbar spine and hip), and the body composition by bioelectrical impedance (Tanita BC-418 equipment).

Results: Simple and multiple linear regression analyses showed significant positive relations between LM and FM of the arms, legs, and trunk and DXA measures of BM in pre and postmenopausal ($p < 0.05$, in all), and negative relations with Ad-SoS ($p < 0.0001$ in all). There were positive relations between FM of the arms and pQCT measurements of the total, cortical+subcortical and trabecular bone density, but only in premenopausal. No relationship was

observed between pQCT measurements and LM and FM of the legs and trunk.

Conclusion: The present study in healthy women, indicates that some discordances in the values of measured bone parameters between different peripheral (phalangeal bone ultrasound and pQCT) and central techniques (DXA) are related with body composition and gonadal status.

Conflict of Interest: None declared

Mo-P304

BONE TURNOVER MARKERS ASSESSED AT BASELINE CORRELATES WITH BASELINE AND 5-YEAR PROSPECTIVE CHANGES OF QUANTITATIVE ULTRASOUND (QUS) OF CALCANEUS

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Aims: There is increasing evidence that measurement of quantitative ultrasound (QUS) of the calcaneus could be a useful, non-invasive method to assess qualitative and quantitative properties of the bone. Bone turnover markers have been shown to correlate with bone mineral density (BMD), changes in BMD and fracture risk, but knowledge on the association of markers to QUS is more limited.

Methods: The association between baseline levels of eight bone turnover markers and baseline QUS variables, as well the 5-year changes of QUS, were studied in a population-based, random sample of 482, 75-year old women free of bone-active medications, as a part of the Malmö Osteoporosis Prospective Risk Assessment (OPRA) study. QUS measurements [speed of sound (SoS), broadband ultrasound attenuation (BUA) and stiffness index] were performed at baseline and after 5 years.

Bone turnover was assessed by serum bone-specific alkaline phosphatase (S-Bone ALP), three different assays for osteocalcin (S-OC), tartrate-resistant acid phosphatase 5b (S-TRACP5b), carboxy terminal telopeptides of type I collagen (S-CTX-I) and, urinary deoxypyridinoline (U-DPD) and mid fragments of osteocalcin (U-MidOC). The associations between QUS and bone turnover markers were evaluated by using standardized regression coefficients (Betastd).

Results: Baseline SoS and stiffness index showed similar associations to bone markers. SoS and stiffness correlated to all markers, except S-Bone ALP and U-DPD (significant Betastd results from -0.16 to -0.21 ($p < 0.001$)). BUA also correlated to all markers, except to S-Bone ALP, U-DPD and S-CTX-I (significant Betastd values from -0.12 to -0.17 ($p < 0.05$)).

When the correlations between the annual changes in SoS and baseline bone markers were evaluated, the results for all three S-OCs, S-TRACP5b and U-DPD were significant (Betastd from -0.10 ($p = 0.03$) to -0.15 ($p < 0.001$)). Annual changes of stiffness were correlated to all three S-OCs and S-CTX-I (Betastd $= -0.10$ to -0.14 ($p < 0.05$)) and changes in BUA to two S-OCs and S-CTX-I (Betastd from -0.10 to -0.12 $p < 0.05$).

Conclusion: Bone turnover markers correlate with QUS and prospective change of QUS of the calcaneus in elderly women. The correlations were most consistent for S-OCs, S-TRACP5b and S-CTX-I. Correlations between QUS and markers were of similar magnitude to, what we have been reported in earlier publications, between BMD and bone turnover markers.

Conflict of Interest: None declared

Mo-P305

THE ROUTINE USE OF VERTEBRAL FRACTURE ASSESSMENT IS MORE EFFECTIVE THAN TARGETED SCREENING

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Background/aims: Two thirds of women with vertebral fractures are unaware of them leading to an underestimation of fracture risk by BMD alone. Vertebral Fracture Assessment (VFA) is a potential tool for vertebral fracture screening. We compare the results of routine and targeted VFA screening.

Method: Our Centre initially only performed VFA on women with reasons to suspect a vertebral fracture (targeted screening group). Since 2005 all women over 65 years undergo VFA (routine screening group). The routine screening group was used to determine the prevalence of vertebral fractures in our population. Using this data, the number of women with vertebral fractures that remained undetected by targeted screening was estimated.

Results: 8564 women over the age of 65 underwent VFA: 6388 targeted and 2176 routinely. The routine VFA group were older (74.3 vs. 72.5, $p < 0.01$) and had a slightly lower hip BMD (0.776 vs. 0.784, $p < 0.02$). In the routine screening group vertebral fractures were identified in 420 (20%) women. The prevalence of vertebral fractures was 10.3% in women with normal BMD, 19.9% in osteopenic women and 33.2% in those with osteoporosis. 300 of the 420 (71.4%) women with prevalent vertebral fractures did not have BMD compatible with osteoporosis. In the targeted group 332 (5.2%) of the 6388 women underwent VFA. Targeted screening resulted in a higher detection rate per VFA performed (36.7%) however, only 122 women with fractures were detected. If it is assumed that the overall vertebral fracture prevalence rate was similar between the 2 groups then 1277 women in the targeted group would have been expected to have one or more prevalent vertebral fractures on VFA. Only 122 (9.6%) of these women with fractures were detected by targeted screening leaving undetected vertebral fractures in 1,155 women, 18.1% of the population attending for DXA.

Conclusions: Over 95% of women are willing and able to undergo VFA. Overall, 1 in 5 women over 65 in our local population has vertebral fractures and this increases to 1 in 3 in osteoporotic women. Routine VFA detects vertebral fractures in 1 in 5 osteopenic women, potentially influencing the need for bone protective treatment in these women. The use of targeted VFA detects only a small percentage of women with vertebral fractures. Routine VFA screening for vertebral fractures is more effective than targeted screening.

Conflict of Interest: None declared

Mo-P306

BONE TURNOVER MARKERS DECLINE WITH ADVANCING AGE IN HEALTHY PREMENOPAUSAL WOMEN

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Background: In healthy adults bone is continuously remodelled. Specific assays for bone turnover markers (BTMs) reflecting resorption or formation rates are widely used in metabolic bone diseases, such as osteoporosis. BTMs are associated with rate of bone loss and fracture risk in postmenopausal women. The lower half of the premenopausal reference range is considered an ideal target for antiresorptive treatment. Therefore, defining appropriate reference ranges is important. Most ranges provided by commercial laboratories are based on a limited number of observations ($\sim$100–200), and assume that BTMs do not vary according to age, BMD, exercise, calcium intake, family history of osteoporosis, etc. Here we report BTM levels in healthy, premenopausal women evaluated for known osteoporosis risk factors.

Methods: Serum BTMs (C-telopeptide of type 1 collagen [CTX], osteocalcin [OC] and N-terminal propeptide of type 1 procollagen [PINP]) were measured in 638 healthy premenopausal women aged 20–50 years, who received a DXA scan and completed a questionnaire on medical history and activities known to affect bone health.

Results: 83 women were on the contraceptive pill (CP) and differed from other subjects in: younger age, fewer pregnancies, greater physical activity. Levels of BTMs adjusted for confounding factors were 14–26% lower than other subjects ($p < 0.005$). In 18 perimenopausal women (serum FSH > 30IU/mL), despite regular menses, BTMs were significantly higher than age-matched women ($p < 0.05$). This group and those on the CP were excluded from further analysis. All BTMs significantly decreased with age ($p < 0.001$). BMD decreased with age with a statistically significant trend for hip BMD ($R = -0.180$; $p < 0.001$). Age and weight-adjusted BTMs were negatively related to BMD ($p = 0.1-0.005$). Weight was also negatively correlated with some BTMs ($p = 0.1$–0.005). In 89 women with a family history of osteoporotic fracture, age and weight-adjusted BTM levels were significantly lower than other subjects ($p = 0.03- < 0.005$).

Conclusions: BTM levels decline with age in premenopausal women, reaching a nadir in the fourth decade of life. Oral contraceptive use is associated with significantly lower BTM values. An increase in BTMs can be seen in perimenopausal women. Women with a family history of osteoporotic fracture have low BTM values. These data indicate a need to redefine the 'reference premenopausal range'.

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Conflict of Interest: None declared

Mo-P307

ABSOLUTE RISK OF FRACTURES IN MIDDLE-AGED AND OLDER MEN AND WOMEN: THE EUROPEAN PROSPECTIVE INVESTIGATION INTO CANCER-NORFOLK STUDY

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While estimates of relative risks associated with risk factors such as age and bone mineral density (BMD) may be of interest for etiologic and comparative purposes, clinical questions such as who might benefit most from preventive interventions or BMD monitoring depend on estimates of absolute fracture risk, which are influenced by a variety of risk factors including age, sex, past history of fracture and bone density. We aimed to estimate these absolute fracture risks in an older European population. Data from the European Prospective Investigation into Cancer-Norfolk (EPIC-Norfolk) study were used for the analyses. The original cohort comprises 25,639 participants (11,607 men) aged 40–79 years in 1993–1997. In 1997–2000, 14,514 men and women returned for a repeat health examination with heel quantitative ultrasound (QUS) measures. All participants were followed up for fracture outcomes up to July 2007. 10-year and 5-year absolute risk of fractures for any participant in first and second health checks were calculated using the baseline hazard function of multivariate Cox proportional-hazard models adjusting for age, sex, history of fractures, height, body mass index, smoking, and alcohol consumption. For women without history of previous fracture, the absolute risk of any fracture raised from 0.9% in the age of 40–44 years to 9.4% in the age of 75–79 years. The corresponding numbers for women with previous history of fracture were 1.8% and 17.8%, respectively. For men, the absolute risk raised from 1.1% to 2.9% in the youngest and oldest age groups without history of fracture and from 1.8% to 4.5% in those with history of fracture. Women aged $\geq$65 years with previous history of fracture had a 5-year risk of more than 5% in the second health check. 5-year risk of fracture was lower than 4% for all women without history of fracture and for all men. Adjustment for broadband ultrasound attenuation (BUA) and other risk factors further smoothed the risk charts. Comparison of sex-specific and sex-stratified models showed a marked difference in absolute risk of fractures in different age groups and confirmed an interaction between sex and age. This indicates that studies aiming to quantify absolute risk of fractures in older men cannot generalize the results of studies on women and need to rely on direct evidence from studies in men. The produced charts in this study can be used in development of fracture risk assessment tools for the elderly.

Conflict of Interest: None declared

Mo-P308

GENDER DIFFERENCES IN SPEED OF SOUND AS ASSESSED BY SUNLIGHT OMNISENSE MULTISITE QUANTITATIVE ULTRASOUND: THE CANADIAN MULTICENTRE OSTEOPOROSIS STUDY

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The objective of this investigation was to assess whether speed of sound (SOS), as measured by Sunlight Omnisense Multisite Quantitative Ultrasound (QUS), significantly differs between adult men and women from a large cohort of individuals from the Canadian Multicentre Osteoporosis Study (CaMOS). Since it is well accepted that women have a higher fracture incidence than men, confirmation of a higher SOS measurement in men would be essential in order to further validate the use of Sunlight QUS for fracture prediction. In year 5 of CaMOS, a randomly-selected population-based cohort of over 9,000 participants, 4124 men and women were assessed by QUS in the sites within CaMOS that were equipped with the Sunlight QUS. SOS (m/s) was assessed at the distal radius (DR), tibia (TIB) and phalanx (PX) sites. A two-sample equal-tailed t-test with a 95% confidence interval (95%CI) was performed between men and women for measures of SOS at each of the three sites investigated to elucidate any significant differences between the groups. In this subset of CaMOS data there were 2948 women and 1176 men included with a mean (SD) age of 66.5 (11.49) and 63.7 (13.04) years, respectively, and a range of 30–96 years of age. At the DR site the men had a significantly ($p < 0.0001$) greater SOS (mean $\pm$ SD = 4072 $\pm$ 131; 95%CI = 4065,4080) than the women (mean $\pm$ SD = 4026 $\pm$ 159; 95%CI = 4020,4033). At the

TIB site the women had significantly lower ($p < 0.0001$) SOS measurements (mean ± SD = 3834 ± 151; 95%CI = 3828,3840) as compared to men (mean ± SD = 3934 ± 122; 95%CI = 3926,3941). Lastly, men also had a significantly greater ($p < 0.0001$) SOS measurement at the PX site (mean ± SD = 3885 ± 192; 95%CI = 3974,3896) as compared to the women (mean ± SD = 3787 ± 226; 95%CI = 3778,3795). In this analysis, a consistent finding was that men had a greater SOS measurement than did the women. These findings are supported by the fact that many epidemiological trials have reported higher fracture rates among women than men. Further prospective research needs to be conducted into the utility of Sunlight QUS in assessing fracture risk in men and women.
Conflict of Interest: KSD, Consultant, Servier; WPO, sanofi-aventis, Procter & Gamble, Merck Frosst, Eli Lilly, Novartis, Amgen, Research support and/or consultant.

Mo-P309

DIAGNOSIS AND SECONDARY PREVENTION OF OSTEOPOROSIS. NATIONAL STUDY OF OSTEOPOROTIC FRACTURES IN OUTPATIENT TRAUMA CARE

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Study degree of correlation between final diagnosis (osteoporotic fracture) and emergency department discharge diagnosis. Evaluate degree of diagnosis and implementation of minimum measures for secondary prevention of osteoporosis.

Prospective, observational, multicenter national study collected data for five months in 358 specialty centers on all osteoporotic fractures (wrist, proximal humerus, vertebra and rib) treated for a period of 30 consecutive days in postmenopausal women aged +50 years. Fractures were classified as osteoporotic if they were due to low-energy trauma. Pathological fractures and those requiring surgery were exclude. The number of patients with a diagnosis of osteoporosis prior to the current fracture and if they were receiving any treatment for this disease was assessed. Inclusion of a diagnosis of fragility fracture in emergency department discharge reports and agreement with subsequent diagnosis in the specialty center were also assessed.

Of total women studied,2125 (41%) had suffered 2390 fractures prior to current fracture. Most common prior fracture site was distal radius. 1865 women (36%) had prior diagnosis of osteoporosis and 1641 (32%) had been prescribed treatment for their disease. Most common treatments were vitamin D and calcium supplements (50%) followed by biphosphonates (39%). Total number of current fractures recorded was 5317,and most common fracture site was distal radius (2364 fractures, 46%). Risk of suffering a new fracture was consistently greater in the site of the previous fracture for all sites studied. Only 40% of fragility fractures were identified as such in the emergency department report,with the lowest percentages for wrist and humerus fractures (37% and 36%,respectively). There was a significant difference in the number of fragility fractures diagnosed in the trauma clinic (94% identified). Prevention of new fragility fracture was recommended in 91% women. Biphosphonates were the treatment chosen in over 86% cases, followed by vitamin D and calcium supplements in 45%.

Fragility fractures are an increasing health problem, and patients may have various fractures over their lifetime if available preventive measures are not applied. A diagnosis of osteoporotic fracture at discharge is lacking in 60% of patients treated in the emergency department. Only 45% of patients who were administered treatment for osteoporosis were prescribed vitamin D and calcium supplements.
Conflict of Interest: F.E.Osorio-Picone, full-time MSD employee

Mo-P310

BONE MINERAL DENSITY IN FEMALE IMMIGRANTS FROM POLAND TO GERMANY

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Background: In the retrospective study was verified the thesis that women migration may affect the results of bone densitometry. To achieve this aim, data obtained in females from Germany and from Poland, and in Polish women who immigrated to Germany were compared.

Methods: A total of 152 women including 84% postmenopausal (99 Polish, 24 Polish who immigrated and 29 German) underwent lumbar spine and proximal femur bone densitometry measurements.

Results: In the whole group T-score ≤ -2.5 was present at spine, femoral neck and total hip in 20.4%, 8.5%, 5.3% subjects, respectively. Greater duration of stay in Germany, greater percentage of life in Germany and lower immigration age improved significantly spine and femoral neck bone mineral density in Polish women after immigration. Regression equations in Polish postmenopausal women ($n = 19$) are given below:

* spine BMD = 1.36 (g/cm^2)− 0.55 × immigration age (y.)
  $r = 0.55$, SEE = 0.14, $p < 0.05$
* femoral neck BMD = 1.37 (g/cm^2) − 0.37 × age (y.)
  − 0.33 × immigration age (y) $r = 0.64$, SEE = 0.08 $p < 0.05$
* femoral neck BMD = 1.31 (g/cm^2) − 0.54 × age (y.)
  + 0.24 × % of life (y.) $r = 0.64$, SEE = 0.08, $p < 0.05$
* femoral neck BMD = 1.37 (g/cm^2) − 0.59 × age (y.)
  + 0.24 × stay in Germany (y)$r = 0.64$, SEE = 0.08, $p < 0.01$

Conclusion. The study results indicate that women migration may positively influence on spine and femoral neck BMD results.
Conflict of Interest: None declared

Tu-P311

TBS OF THE AP SPINE AS ASSESSED BY DXA IS CORRELATED WITH 3D BONE MICROARCHITECTURE PARAMETERS: AN EXPERIMENTAL STUDY BASED ON HUMAN CADAVER VERTEBRAE

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Trabecular Bone Score (TBS) is a new grey-level texture measurement. TBS permits to accurately differentiate between two 3D microarchitectures that exhibit the same amount of bone, but different

trabecular bone characteristics. Significant correlations between TBS and 3D bone microarchitecture parameters have been already demonstrated (BONE, in press). The aim of this study was to evaluate the correlations between TBS as assessed by DXA image processing and 3D bone microarchitecture parameters as assessed by micro CT.

40 dried human cadaver vertebrae were measured on a DXA Prodigy densitometer (GE-Lunar) with a specific positioning system miming standard anterior-posterior acquisition. The block (positioning system + vertebra) was immerged in 17 cm of water in order to mimic soft tissues. A region of measurement was manually defined on each DXA acquisition and Bone Mineral Density (BMD) was evaluated in this 2D region. The DXA image was then exported on a specific workstation for TBS calculation in the same 2D region. 3D reconstructions of the 40 vertebrae were obtained by micro-Computed Tomography (eXplore Locus, GE HealthCare). The calibrated 3D grey-level images were analyzed by the use of the MicroView software (GE HealthCare) with Advanced Bone Analysis add-on. Autothreshold was applied and spine-based region of measurement was manually defined enclosing complete bone microarchitecture of the vertebral body. Standard 3D parameters {BV/TV, TbTh, TbSp, TbN, connD} were evaluated in this 3D region. Statistical analyses were performed by the use of MedCalc software.

Significant correlations were obtained between TBS and 3D bone microarchitecture (see Table 1), with higher correlation $r = 0.71$ between TBS and density of connectivity (connD). Moreover, BMD-adjusted correlations were evaluated and no significant changes were obtained.

TBS, as evaluated from AP DXA spine examination, correlated with standard characteristics of 3D bone microarchitecture, independently of BMD. TBS could constitute a good candidate to increase the evaluation of bone status in clinical routine.

Table 1

Correlation with TBS	BMD	BV/TV	TbTh	TbSp	TbN	ConnD
Direct	0.24 (ns)	0.58 (0.0001)	0.28 (ns)	−0.62 (<0.0001)	0.68 (<0.0001)	0.71 (<0.0001)
BMD matched	–	0.55 (0.0001)	0.26 (ns)	−0.59 (<0.0001)	0.66 (<0.0001)	0.69 (<0.0001)

Conflict of Interest: None declared

Tu-P312

ASSESSMENT OF VALIDITY OF IOF'S ONE-MINUTE OSTEOPOROSIS RISK TEST FOR POSTMENOPAUSAL WOMEN

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Background: This research was aimed at proving validity of IOF's One-Minute Osteoporosis Risk Test and evaluating the relation between structural-functional state of bone according to the ultrasound densitometry and results of IOF's One-Minute Osteoporosis Risk Test for postmenopausal women.

Materials and methods. We've examined 147 postmenopausal women aged 50–69 years (mean age 59,8 ± 0,7). Structural-functional state of bone was evaluated by means of an ultrasound bone densitometer ("Achilles+"). The speed of sound (SOS, m/s), broadband ultrasound attenuation (BUA, dB/MHz) and a calculated "Stiffness" index (SI, %), T and Z-range were measured. IOF's One-Minute Osteoporosis Risk Test was translated into Ukrainian.

Results: Significant correlation was found between positive answer to question 2 ("Have you broken a bone after a minor bump or fall?") and SOS ($r = -0,17$; $p = 0,042$), BUA ($r = -0,28$; $p = 0,0005$), SI ($r = -0,25$; $p = 0,002$), Z- range ($r = -0,26$; $p = 0,015$); between positive answer to question 3 ("Have you taken corticosteroid tablets for more than 3 month") and SOS ($r = -0,16$; $p = 0,047$), BUA ($r = -0,29$; $p = 0,0003$), SI ($r = -0,21$; $p < 0,000001$), and between positive answer to question 4 ("Have you lost more than 3 cm (just over 1 inch) in height?") and the following indexes of structural-functional state of bone: SOS ($r = -0,32$; $p < 0,00001$), BUA ($r = -0,27$; $p = 0,00096$), SI ($r = -0,36$; $p < 0,000001$), Z- range ($r = -0,27$; $p = 0,0015$).

Conclusion: Application of IOF's One-Minute Osteoporosis Risk Test gives an opportunity to determine structural-functional changes of bone. Among the test questions, the most reliable and informative as for postmenopausal women proved to be questions 2 ("Have you broken a bone after a minor bump or fall "), 3 ("Have you taken corticosteroid tablets for more than 3 month ") and 4 ("Have you lost more than 3 cm (just over 1 inch) in height?).

Conflict of Interest: None declared

Tu-P313

ARE HOSPITALS DANGEROUS PLACES?

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Introduction: Falls are common among hospital inpatients which often result in physical and psychological injury. This has the effect of impaired rehabilitation and increased morbidity.

The implications for the NHS are enormous not only in terms of extended inpatient stays and socio-economic cost, but also in terms of potential legal implications. An estimated 30% of falls within the hospital result in serious injury (Stevens et al 2004). Proximal femoral fractures are potentially catastrophic injuries which are relatively common following falls in the elderly.

Method: Between June 2001 and March 2003 all patients who sustained a proximal femoral fracture following a fall whilst an inpatient were identified. The hospital was a busy district general hospital. The location, type of fracture, surgical intervention performed, and 90 day survival were all recorded.

Results: 38 patients were identified between the target dates. 22 falls occurred on a geriatric ward, 3 on a surgical ward and 13 on general medical wards.

Of these 7 were male and 31 female. Average age was 80 years old (Range 54–96)

Procedures carried out included 13 dynamic hip screws, 12 hemiarthoplasties, 5 intramedullary hip screws, 2 cannulated screws and 1 total hip replacement. 4 patients did not undergo surgical intervention. Eight of the thirty eight patients died within 90 days.

Conclusion: Many patients particularly the elderly who are unwell or recovering from an illness are at significant risk from falling. Perhaps those with chronic gait instability or patients with cognitive impairment are at increased risk.

This study has illustrated the high frequency of falls and potential fatal outcomes. Fall prevention programs should be instituted and regularly assessed in all hospitals.

Conflict of Interest: None declared

Tu-P314

PRECISION OF TOTAL BODY AND REGIONAL TISSUE MEASUREMENTS BY DXA DENSITOMETRY

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Object: To determine the precision errors associated with total body and regional area measurements of bone and soft tissue masses.

Method: There were 100 patients (55 males, 45 females); mean age 60.6 years (range 22 to 83) and mean BMI 24.1 (range 13.1 to 38.8). Paired total body scans, with repositioning between scans, were obtained for each patient on a GE-Lunar Prodigy Advance densitometer. The coefficient of variation percent (%CV) and standard deviation (SD) were calculated for each pair and the short term precision error was derived from the expressions RMS-%CV = sqrt(sum CV^2/N) and RMS-SD = sqrt(sum SD^2/N)for each variable in the total body, arms, legs and trunk regions, where N is the number of patients.

Results: see table 1.

%CV and SD were also determined for the quartiles of BMD, BMC, fat mass and lean mass in the various regions. An important finding was the significantly greater %CV for fat mass in the lowest quartile(Q1) for the legs and total body, whereas the corresponding SDs were not significantly different in Q1 to Q4 by one-way ANOVA. For lean mass, %CVs and SDs in these regions did not differ significantly in Q1 to Q4. The increased %CV in the cachectic patients was probably due to the low fat mass in the denominator of the CV calculation. This implies that the SD should be used instead of %CV when utilizing the least significant change(LSD) with 95% confidence when monitoring patient progress, where LSD = 2.77(RMS-SD)gm.

Table 1 RMS-%CV gm/cm2 (RMS-SD gm) N = 100

	Arms	Legs	Arms+legs	Trunk	Total body
BMD	3.35(0.038)	1.13(0.015)		0.98(0.008)	0.84(0.010)
BMC	1.77(7.1)	0.87(8.6)	0.93(12.6)	4.02(34.2)	1.33(364)
Fat mass	4.56(66.8)	2.43(107.9)	2.15(128)	3.35(259)	1.78(276)
Lean mass	2.09(99.7)	1.42(235)	1.15(242)	1.49(341)	0.82(370)

Conflict of Interest: None declared

Tu-P315

AN OBSERVATIONAL STUDY OF NTX AND BMD IN CLINICAL PRACTICE

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Bone markers have acquired a role in clinical practice largely based on evidence from randomized controlled trials. This study examined whether observational data from clinical practice supported the trial evidence. A data base was established of new osteoporotic patients attending a bone clinic who all had a second morning fasting urine NTX measurement prior to clinic. 1027 patients are entered into the data base, 150 male and 877 female, average age 63 years. A cohort of 117 patients was examined who were treatment naïve prior to clinic, then treated and had a repeat NTX at 3–6 months and baseline and 1 year spine BMD. Most patients had postmenopausal osteoporosis or primary male osteoporosis. Of the 117 patients 63 were treated with Alendronate, 36 Risedronate and 18 other, mostly HRT, Raloxifene or Zoledronate. Mean pretreatment NTX was 59 and lumbar spine BMD 0.736 g/cm2 and post treatment NTX 33 and BMD 0.778 g/cm^2. The correlation between percentage NTX change and BMD change was r = 0.20, percentage NTX change and absolute change in BMD r = 0.18 and absolute change in NTX and BMD r = 0.08.. Thirty patients with an NTX reduction of < 37 % showed a mean rise in spine BMD of 0.030 g/cm^2, 35 with a 37–54% reduction in NTX a rise of 0.044 g/cm^2 and 36 with an NTX reduction of > 54 % arise of 0.051 g/cm^2. 23 patients with a post treatment NTX of < 20 had a mean rise in BMD of 0.048 g/cm^2, and respective results for 21 pts with NTX 21–30, 33 pts with NTX 31–40 and 34 pts with NTX > 40 were 0.045 g/cm^2, 0.049 g/cm^2 and 0.023 g/cm^2. Both the change of NTX measurement and the post treatment result provide a useful indication of the likely change in BMD and in particular those with an NTX over 40 after treatment are more likely to show a poor BMD response. Those with a post treatment NTX less than 20 always had a rise in BMD with treatment.

Conflict of Interest: None declared

Tu-P316

WHOLE BODY ASSESSMENT USING THE NORLAND XR-36 AND XR-46 SCANNER

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The evaluation of whole body bone, lean and fat is becoming an assessment of increasing interest in the clinical and research communities; however, the relationship between measurements made on different types of scanners needs to be validated by in vivo comparisons. The current study examines in vivo whole body bone, lean and fat results in a population of subjects using the Norland XR-36 and Norland XR-46 scanners.

Three whole body examinations were made on both the Norland XR-36 and XR-46 scanner on 14 adult male and female subjects. Additionally, whole body weight was obtained by scale on each subject.

Comparing whole body weight by scale to whole body weight by scanner showed strong positive regressions with both the XR-36 (y = 0.9747x + 606.83; r = 0.9997) and the XR-46 (y = 0.9515x + 959.73; r = 0.9984). When whole body bone, lean and fat measurements by the XR-36 were compared with measurements by the XR-46, strong positive regressions were seen for bone (y = 0.9629x + 93.989; r = 0.9955), lean (y = 0.9734x − 1834.5; r = 0.9988) and fat (y = 0.9614x + 2669.1; r = 0.9983). When bone, lean and fat measurements on the XR-36 were compared to measurements on the XR-46 from individual subjects the bone values were found to be almost identical while slightly more lean and less fat was seen in the XR-36 assessments.

In conclusion, this study demonstrates that whole body weight measured by the XR-36 and XR-46 are similar to whole body weight by scale supporting that measurements by these scanners are valid. Further, whole body bone, lean and fat measurements done by the XR-36 and XR-46 proved to have a close relationship.

Conflict of Interest: None declared

Tu-P317

UNIDENTIFIED VERTEBRAL FRACTURES - A COMMON PROBLEM IN GENERAL MEDICAL HOSPITAL INPATIENTS

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Background: Vertebral fragility fractures are common. They are associated with pain, functional limitation, high risk of subsequent fragility fractures and increased mortality. Identifying these fractures is important, providing an opportunity to initiate bone protective treatment and physiotherapy. However, it is estimated that only 25% of vertebral fractures come to clinical attention. This audit reviewed patients, >70 years old, with no previous diagnosis of vertebral fracture, currently occupying internal medicine beds of a UK district general hospital. The aim was to identify any patients with a vertebral fracture and initiate appropriate management.

Method: On a given day all general medicine inpatients over age 70 yrs and able and fit enough to participate, completed a questionnaire administered by a senior physiotherapist. They were also examined for the presence of kyphosis. Any patients with thoracic back pain, height loss =/> 2 inches or kyphosis were sent for a lateral thoracic spine radiograph. All X-rays and reports were viewed on PACS and the results recorded on a database.

Results: Of 73 current general medicine inpatients >70 yrs, 43 (mean age 81.6) were able to participate in the audit. 12 (27.9%) of these had previously undiagnosed vertebral fractures (mean age 87.4). 1/3 of these had multiple fractures.

Out of the 12 fracture patients, 5 were taking calcium and vitamin D and of these 2 were previously diagnosed with osteoporosis. None of the 12 fracture patients were on bone protective medication.

Thus in our "snapshot", a minimum of 16.4 % of general medical inpatients could have been identified, evaluated and treated.

As the average age of the acute medical take continues to increase, there is a clear need for programmes to bring the problem of undiagnosed fragility fracture to the attention of health care professionals caring for these patients.

Conflict of Interest: S. Tomlins, Roche Pharmaceuticals, Grant Research Support

Tu-P318

THE PREVALENCE OF HYPOCALCIURIA IN A SPECIALIST BONE CLINIC

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Introduction: Hypocalciuria is defined as calcium excretion less than 2.5 mmol/24 hour on 24 hour urinary collection. It can reflect vitamin D deficiency, malnutrition or thiazide therapy. It may be an indicator of malabsorption. It is important to identify its presence and advise appropriate dietary adjustments (once familial hypocalciuric hypercalcemia is excluded). Such patients may be at an additional increased risk of fracture. We investigated the prevalence of this condition and its causes in a specialized bone clinic.

Methods: 601 patients consecutively referred to this clinic were reviewed. Clinical history, 24 hour urinary calcium (Ur Ca), bone profile, serum intact PTH, 25 (OH) Vitamin D, bone formation markers, PINP and Osteocalcin (OC), as well as CTx, were recorded. BMD at total hip (TH) and lumbar spine (LS) and bisphosphonate and calcium therapy were documented. Results 31.6% (190) of patients had hypocalciuria. (25 male, 165 female). The mean age of patients was 77 ± 0.969 yrs. Table 1.59% were vitamin D insufficient (25–75 nmol), 20% were frankly D deficient (< 25 nmol).Only 21% of patients were vitamin D replete. 46% were on bisphosphonates and calcium, 18% were on calcium therapy alone, while 36% were on no treatment. Only 26% of patients had tissue trans-glutaminase (tTG) measured as a screening tool for coeliac disease. Of those measured, 16% were positive. 75% of patients were osteoporotic. Conclusion Hypocalciuria was very prevalent. It is a good marker for frank vitamin D deficiency. Less than a quarter of the patients were Vitamin D replete. We particularly note the high prevalence of coeliac disease presenting to our clinic with hypocalciuria as the occult indicator of underlying malabsorption. Therefore, all assessments of bone disease include a screen for hypocalciuria as it is a good indicator for underlying D deficiency, especially when this latter test may not be readily available.

Table 1

Ref Range	Mean	SEM
24hr Ur Ca (2.5–2.7mmol/24)	2.39	0.049
Serum Calcium(2.2–2.7mmol)	2.39	0.009
Phosphate (0.8–1.4mmol/L)	1.04	0.017
PTH (10–65pg/ml)	41.13	1.778
25 (OH) Vit D nmol	56.589	4.269
LS BMD g/cm2	0.852	0.017
TH BMD g/cm2	0.682	0.011
PINP (<80ug/L)	51.9	4.627
OC (11–50)	25.49	1.964
CTx (0.1–1.0)	0.356	0.025

Conflict of Interest: None declared

Tu-P319

THE RELATIONSHIP OF HYPOCALCIURIA TO BONE MINERAL DENSITY AND BIOCHEMICAL BONE MARKERS

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Introduction: Hypocalciuria is defined as calcium excretion less than 2.5 mmol/24 hour on 24 hour urinary collection. It can reflect vitamin D deficiency, hypoparathyroidism or malabsorption. It is important to identify the presence of hypocalciuria (UCa). This abnormality may be corrected by appropriate dietary or medical interventions. We clarified the relationship of hypocalciuria to BMD and biochemical bone markers in our specialized bone clinic in St James Hospital Dublin. Methods 601 patients consecutively referred to this clinic were reviewed. 24 hour urinary calcium (Ur Ca), bone

profile, serum intact PTH, 25 (OH) Vitamin D, bone formation markers, PINP and Osteocalcin (OC), as well as Ctx, were recorded. BMD at total hip (TH) and lumbar spine (LS) were noted. The correlation coefficient between urinary calcium and the above markers was calculated. Results 31.6% (190) of patients had hypocalciuria. (25 male, 165 female). Their results have been detailed in the correlation table 1 below. Significance was noticed in several biochemical variants, except with respect to vitamin D, where a positive trend was noted but this was not significant. Conclusion The adverse relationship between hypocalciuria and lower BMD is clearly demonstrated at the lumbar spine. Furthermore, hypocalciuria is associated with an increase in bone turnover, as reflected by its inverse relationship with P1NP and OC. The mechanism is likely to be a PTH-driven increase in bone turnover to compensate for this relative deficiency in calcium balance (and possibly Vitamin D). Hypocalciuric patients have two risk factors for fracture; namely low BMD and increased bone turnover. The test is easily performed. Failure to identify this deficiency is not acceptable as it is easily treated with simple dietary and medical interventions.

Table 1

	Vit D	PTH	PINP	OC	CTx	BMD LS	BMD TH
24hr Ur Ca	NS	NS	r = −0.24 p<0.01	r = 0.206 p<0.02	NS	r = 0.178 p<0.04	NS

Conflict of Interest: None declared

Tu-P320

TRABECULAR BONE REMODELLING STUDIED WITH A MARKOV MODEL

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During life trabecular bone is constantly remodelled by resorption and deposition of bone packets from its surface. This process of bone remodeling is thought to be mechanically controlled (Wolff-Roux law) to maintain the structural integrity of the trabecular network and to allow adaptation to a changing mechanical environment. A quantitative formulation in the form of remodeling rules, specifying deposition and resorption probabilities as a function of a local mechanical stimulus, is still missing. We developed a Markov model of bone remodeling to study the connection between different remodeling rules and the resulting trabecular bone architecture.

Each trabecula in a human vertebra is described by its cross-sectional area. This area can change by deposition/resorption of a bone packet. Two different remodeling rules describe the probability for bone resorption/depositon as a function of the cross-sectional area. According to the Wolff-Roux law the rules tend to protect thinner trabeculae for further resorption and eventually perforation. The outcome of an analysis of such a Markov model is a frequency distribution of the cross-sectional areas in the vertebra. The time evolution of this trabecular area distribution (TAD) has been studied for different remodeling rules proposed in literature and results have been compared with μ-CT data of vertebral bone. The model allows an insight of how much control is necessary to preserve the trabecular network at least over a life time. Further the importance of a separate description of bone resorption and deposition in two remodeling rules can be demonstrated.

Conflict of Interest: None declared

Tu-P321

HIGH PREVALENCE OF VITAMIN D INADEQUACY AMONG COMMUNITY-DWELLING POSTMENOPAUSAL WOMEN WITH OSTEOPOROSIS

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This study describes the distribution of serum 25-hydroxyvitamin D [25(OH)D] and the relationship with parathyroid hormone (PTH) levels among postmenopausal women with osteoporosis in various regions and across two seasons.

A cohort of 2589 postmenopausal women with osteoporosis from 18 countries was recruited between May 2004 and March 2005. Serum 25(OH)D and PTH were measured. Factors that could influence Vit D status were obtained by patient questionnaire. Mean serum 25(OH)D and PTH levels by season and region were used to estimate the frequency of Vit D inadequacy (25(OH)D < 30 ng/ml).

Mean age was 67.1 years, (range 41–96; 28% $>$ 70 years). 37% took a Vit D supplement $\geq$ 400 IU daily; 60% took prescription medication for osteoporosis. Overall mean 25(OH)D was 26.8 ng/ml (SD = 13.2, range 7–243). Overall prevalence of Vit D inadequacy was 64%. Among women recruited during summer, prevalence of inadequacy was 59%; among those recruited during winter, prevalence was 69%. PTH values plateau at 25(OH)D above 30 ng/ml, which is consistent with findings from other studies. Seasonal and regional distribution of Vit D inadequacy is shown below.

Results suggest that Vit D inadequacy is widespread among women with osteoporosis across all continents. In this cross-sectional sample, the prevalence of Vit D inadequacy is high, regardless of latitude or season. These results underscore a need to improve physician and patient awareness of the importance of adequate Vit D supplementation in postmenopausal women with osteoporosis.

Table 1

	25(OH)D <30 ng/ml (%)		
Region	Overall	Summer	Winter
Europe (n = 1020)	58	52	63
Middle East (n = 401)	82	81	83
Asia* (n = 549)	71	63	83
LatinAmerica* (n = 415)	53	56	51
Pacific Rim (n = 204)	61	62	59
OVERALL (n = 2589)	64	59	69

* region includes at least one equatorial country

Conflict of Interest: JC, JN, JAW, Merck and Co., Employees; SRE, DH, Merck and Co., Consulting

Tu-P322

A NEW MODEL OF OSTEOPOROTIC FRACTURE ASSESSMENT COMBINING TRABECULAR BONE SCORE (TBS) AND BONE MINERAL DENSITY (BMD) DERIVED FROM DXA SPINE IMAGING

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Osteoporosis is defined as a Bone Mineral Density (BMD) of 2.5 SD below the young adult reference mean in postmenopausal population. BMD is a measure of fracture risk but does not constitute the unique risk factor. Trabecular Bone Score (TBS) is a new grey-level texture measurement (BONE, in press). It permits to accurately differentiate between two microarchitectures that exhibit the same amount of bone, but different trabecular bone characteristics. TBS is not correlated with BMD. The aim of this study was to evaluate the fracture discrimination value of a model combining TBS and BMD against a model including BMD alone.

We present a retrospective case-control study consisting of Caucasian postmenopausal women ($n = 57$) aged between 50 and 75 years. Control (non-fractured) and fractured (non hip fracture type) groups were composed of 30 and 27 subjects respectively. BMD was measured by DXA at the lumbar spine (L1–L4) using a Prodigy densitometer (GE-Lunar). TBS was calculated at the same L1–L4 region from DXA images directly. Statistical analyses were realized in order to evaluate and compare the diagnosis values of the models (TBS + BMD) versus (BMD) alone.

Significant difference between non-fractured and fractured groups were obtained for BMD ($p = 0.0202$) and TBS ($p = 0.0022$). Odd ratio per standard deviation decreased (and their associated 95% CI) as well as the Area under the ROC Curve were 2.03 [1.08–3.84] and 0.645 for BMD and 2.92[1.41–6.02] and 0.737 for TBS. Both parameters remains significant when entered into a multivariate regression model with OR of 2.83[1.34–5.96] and 2.03[0.97–4.26] for TBS and BMD respectively. The overall OR of the model was 3.20[1.61–6.36] and the corresponding AUC = 0.779[0.649–0.878]. The overall sensitivity of the combined model was 59.26% and 76.67% for the specificity which is significantly better than the one reported for BMD alone (51.85% and 53.33%).

Combining bone microarchitecture (TBS) and density (BMD) parameters derived from the same spine DXA examination seems to significantly improve osteoporotic fracture discrimination in postmenopausal women.

Conflict of Interest: None declared

Tu-P323

HIGH MINI NUTRITIONAL ASSESSMENT (MNA) CORRELATES WITH BONE ULTRASOUND MEASUREMENTS IN ELDERLY FRACTURED WOMEN

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Background: To achieve optimum bone health, numerous factors are required such as a balanced diet, regular weight bearing physical activity and appropriate lifestyle habits. Malnutrition is a major risk factor for osteoporotic fractures. It is a very common problem in the elderly population and is often under diagnosed. The MNA test is a validated screening tool for malnutrition and is recommended for early detection of risk of malnutrition.

Aims: The aim of our study was to assess the possible relationship between MNA and bone ultrasound (QUS) measurements at the heel (bone ultrasound attenuation-BUA)

Methods: As part of on going study, we assessed MNA and QUS in 256 elderly Swiss ambulatory women who had reported a fracture in their life-time (mean age 80.6 yrs, BMI 24.6 kg/m^2, BUA 96.8). QUS was done with Achilles, Lunar Corporation as this approach was predictive of fracture risk in elderly women. The Mini Nutritional Assessment (MNA), a non invasive questionnaire which evaluates nutritional status in elderly people, was filled in by each woman. The MNA asks 18 questions to evaluate the nutritional status of the subject. The score ranges from 0–30 (< 17 indicates malnutrition, 17.5–23.5 risk of malnutrition and $>$ or $= 24$ well nourished).

Results: A higher MNA score was significantly associated with higher BUA ($r = 0.149$, $p < 0.05$). BUA increased significantly between MNA quartile 1 and MNA quartile 4 ($Q1 = 23.5$, $Q2 = 26.5$, $Q3 = 28$, $Q4 = 30$). The difference among the mean scores of BUA of the 4 groups was significant according to the multiple-range test (one-way analysis of variance with Tukey's test) ($p = 0.004$), as well as the F test linearity ($p = 0.015$).

Conclusion: High MNA (low malnutrition risk) score was correlated with higher BUA in fractured women, but the effect of the association was relatively low compared to age and BMI. The MNA appears to be a useful tool for the evaluation of osteoporotic patients.

Conflict of Interest: None declared

Tu-P324

A METHOD TO ESTIMATE FEMORAL NECK CORTICAL THICKNESS FROM CLINICAL QCT SCANS

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Age-related thinning of the femoral neck (FN) cortex may play an important role in hip fracture. Estimation of FN cortical thickness, especially at its superior-lateral aspect, from clinical QCT scans is difficult due to insufficient spatial resolution relative to cortical thickness at the FN (0.5–1.0 mm). We devised a double-width-density-weighted (DWDW) method to estimate cortical thickness and examined its performance against the full-width-half-maximum (FWHM) method.

DWDW first defines the density profile perpendicular to the periosteal surface (segmented by thresholding) and identifies the periosteal border k_p in the profile. The maximum density D_{max} and its location k_{max} in the profile are then identified. If $D_{max} < 0.2D_{FN}$ (D_{FN}: maximum density of the whole FN), which indicates a thin cortex, k_{max} is checked: if k_p-$k_{max} > 2.5$ mm, k_{max} is adjusted so that k_p-$k_{max} = 1.25$ mm. The endosteal border k_e is defined as the mirror point of k_p with respect to k_{max}, i.e., doubling the width of k_p-k_{max}. The density profile is integrated from k_e to k_p and divided by D_{FN} to arrive the estimated cortical thickness t_e.

Simulation was conducted to investigate how t_e depended on the true cortical thickness t and CT spatial resolution (described by

normalised Gaussian point spread functions, PSF). FWHM was accurate when $t >= 2$xFWHM of the PSF and over-estimated the thickness when $t < 2$–3 times of the FWHM of the PSF. DWDW always over-estimated the thickness, but performed better than FWHM when $t <$ FWHM of the PSF.

The two methods were also compared on CT scans of a pig bone with known t (0.62–3.92 mm measured by a calliper of 0.02 mm resolution). The bone was positioned at 45° to the scanner and scanned using 80 kVp and 175 mA, 1.0 mm slice thickness and 0.94 × 0.94 mm pixel size. For $t \leq 2$ mm, the root mean square error (RMSE) of DWDW was 0.09 mm, significantly smaller than FWHM (0.87 mm). When $t > 2$ mm DWDW had non-significant higher RMSE than FWHM (0.52 v. 0.34 mm).

We conclude that DWDW was superior to FWHM in estimating the thickness of thin cortices. The potential of this novel algorithm to study cortical thinning with age and its association in hip fracture in humans requires further investigation.

Conflict of Interest: None declared

Su-P325

EVALUATION OF QUALITY OF LIFE AND INCIDENCE OF VERTEBRAL FRACTURE IN POSTMENOPAUSAL WOMEN WITH OSTEOPENIA

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The study is proposed to estimate the incidence of fractures in women with osteopenia in postmenopausal age and estimating the presence of eventual factors of risk (age, familiarità for osteoporosis, reduced corporeo weight, reduced assumption of soccer with the diet, elevated concomitant caffein assumption, smoke, diseases) and the impact of the vertebral fractures on the disabilità and quality of life also in osteopeniche patients. The study has been lead on 300 women of age comprised between 44 and 83 years (medium of 72,7), consecutively recruited near the U.O.S. Reumatologia and Centro Osteoporosis (Hospital Morelli of Reggio Cal.) that, executed the study of the mineral bony density (Bone Mineral Density - BMD) to level of the femore and the rachide lumbar by means of technique DXA (Dual energy X-ray Absorptiometry) Hologic QDR 4500W, have introduced densitometrici values of T-score comprised between - 1 and - 2,5 SD (than second the WHO represents the diagnostic threshold of osteopenia). The patients who introduced at least a risk factor have executed carried out morfometrica appraisal by means of radiological technique, using the semiquantitative method of Genant. In all the anthropometric patients anamnestica card comprising given and clinicians have been carried out one; it has been moreover proposed questionnaire specific osteoporosis (miniums-OQLQ) and scales of appraisal of pain (VAS). The presence at least a documented vertebral fracture radiologically has been found in 208 patients (69%); in 92 patients (31%) the morfometrica appraisal has demonstrated the absence of vertebral deformities. The compilation of miniums OQLQ and the appraisal of the VAS have demonstrated an association between the compromissione of the quality of the general state of health, the VAS and the number of vertebral fractures radiologically documented. Between the patients who introduced vertebral fractures in 23 (11.05%) were present more of one fracture. The preliminary data demonstrate that also in the osteopeniche women a high percentage of risk of vertebral fractures is found. The open problem could be constituted from the factors that address to the demand for the morfometrico examination in these patients.

Conflict of Interest: None declared

Su-P326

INFLUENCE OF A HISTORY OF HIP FRACTURE IN FIRST-DEGREE RELATIVES ON FRACTURES IN 60+ YEAR OLD MEN

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Several factors have been linked to the risk of osteoporotic fractures in men. The aim of this study was to investigate the impact of a family history of osteoporosis or hip fracture on fracture risk in men.

Methods: A random selection of 10 000 men aged 60–74 resident in the county of Funen was obtained from the central office of civil registration. In the autumn of 2004, they received a questionnaire by mail as part of the Study of Osteoporosis and Male Ageing (**SOMA**). The questionnaire comprised topics known to or expected to be relevant to fracture risk in men i.e. genetic predisposition, calcium intake, physical activity, falls, medical history, medication, and previous fractures. If no response to the mail was registered, a single questionnaire was reissued after one month. Logistic regression analysis was used to compute the effects of a family history of hip fracture after the age of 50 and/or the diagnosis of osteoporosis in parents or siblings on the risk of experiencing fractures after the age of 50.

Results: In total, 4,953 of the questionnaires were returned. The mean age of respondents was 66.8 y (SD: 4.18), weight 82.7 kg (14.0) and height 175.3 cm (7.90). Odds ratios presented below are adjusted for weight and height. Associations with self-reported fractures were found for prior hip fracture in first-degree relatives of the same sex, but not for a family history of having been diagnosed with osteoporosis.

Conclusion: Bearing in mind the limitations inherent in self-reported information, our data suggest possible sexual dimorphism in the inheritance of fracture risk with an increase in risk in men who have a brother or father with prior hip fracture. The influence of prior fractures in female first-degree relatives was weaker and not statistically significant.

Table 1

Family history of hip fracture	Any frature (n = 484)	Hip fracture (n = 54)	Spine fracture (n = 97)
Mother	1017 (0.83–1.65)	1.43 (0.56–3.65)	1.34 (0.67–2.68)
Father	2.02 (1.11–3.67)*	No observations	1.37 (0.33–5.80)
Sister	1.38 (0.82–2.33)	No observations	1.18 (0.37–3.80)
Brother	1.41 (1.02–1.94)*	2.41 (1.11–5.19)*	1.74 (0.93–3.23)

Values represented as OR(95%CI). * p<.05

Conflict of Interest: MFN: None

The study was supported by a grant from the Novo Nordisk Foundation

Su-P327

DISTRIBUTION OF RISK FACTORS FOR FRACTURE IN WOMEN WITH AND WITHOUT A FRACTURE HISTORY: A REGIONAL COMPARISON. THE GLOBAL LONGITUDINAL REGISTRY OF OSTEOPOROSIS IN WOMEN

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Aim: To compare the real-world prevalence of fracture risk factors in 3 geographic areas in women aged 55+. Methods & results: GLOW (Global Longitudinal registry of Osteoporosis in Women) is an observational follow-up study of women aged 55+ recruited by 540 primary physician practices (17 sites, 10 countries [7 European]). Practices typical of each region were identified via primary care networks. All non-institutionalized patients visiting the practice within the prior 2 years were eligible. Self-administered questionnaires were sent (2:1 over-sampling of those aged 65+). Annual follow-up questionnaires will be sent for 5 years. Among women with a fracture history after age 45, 17% said that their mother had a hip fracture vs 12% of those without a fracture history. Overall, 29% of women with a fracture history reported weight < 125 lbs/57 kg vs 16% in those without. Canadian/Australian women with a fracture history were more likely to report having a mother with history of hip fracture (19%) vs Europeans with a fracture history (11%). Women in Australia/Canada without a fracture history reported drinking > 14 alcoholic drinks/week (4.8%) vs 3.4% of European and 2.5% of US women. Conclusions: In this international, real-world population, self-reported fracture risk factors varied by region and history (vs no history) of fracture.

Table 1

	Can/Aus* n = 33	Europe* n = 196	USA* n = 124	Can/Aus n = 2633	Europe n = 9946	USA n = 7188
Maternal hip fx	19%	11%	18%	12%	11%	13%
Weight <125lb**	28%	25%	19%	15%	18%	15%
Current smoker	25%	13%	16%	8.9%	11%	6.9%
Arms to assist	81%	59%	48%	35%	29%	32%
Parental fracture	18%	13%	22%	15%	14%	16%
Cortisone (ever)	38%	12%	30%	25%	15%	33%
Alcohol >14/week	0.7%	3.5%	1.8%	4.8%	3.4%	2.5%
Rheum arthritis	26%	21%	7.3%	10%	12%	7.8%

fx, fracture. * History of fracture; ** 57 kg

Conflict of Interest: Funding: The Alliance for Better Bone Health (Procter & Gamble Pharmaceuticals and sanofi-aventis)

Su-P328

OVEREXPRESSION OF OSTEOBLAST IGF-I BLUNTS THE DELETERIOUS EFFECTS OF LOW PROTEIN INTAKE ON BONE STRENGTH

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Isocaloric low protein intake decreases bone mass, intrinsic bone tissue quality and bone strength. These alterations are associated with decreased circulating IGF-I levels. Osteoblast IGF-I expression is also decreased by low amino-acid concentrations. Whether circulating and/or locally produced bone IGF-I are responsible for the negative effects of a low protein diet on bone damages has not yet been established. We investigated 6-month adult transgenic male mice overexpressing IGF-I in osteoblasts under the control of collagene type-1 promoter (TG-IGF) and wild type mice (WT), fed a normal or an isocaloric low protein diet, for 8 weeks. After sacrifice, tibias were tested for biomechanics, size, microstructure using microcomputerized tomography, cortical histomorphometry and bony tissue nanoindentation. Blood was also collected. In WT on a low protein diet, compression strength was significantly decreased and resistance to bending displayed a similar trend, whereas these parameters were unchanged in TG-IGF. Data in the table 1 show a cortical thinning as well as alterations of intrinsic bone tissue quality in WT but not in TG-IGF. Outer bone diameter was higher in TG-IGF, irrespective of the protein intake. Endosteal BFR was reduced in WT on a low protein diet, but not in TG-IGF. Trabecular bone mass was significantly decreased in WT only. Plasma IGF-I was similar in WT and TG-IGF, and equally decreased by the low protein diet. These results in adult male mice indicate that overexpression of locally produced bone IGF-I blunts the deleterious effects of a low protein diet, even in the presence of lower circulating IGF-I levels. These results highlight the major importance of osteoblast IGF-I production in maintaining bone integrity in the presence of altered somatotrop axis.

Table 1

	WT Control	WT Low prot	TG-IGF control	TG-IGF Low prot
Maximal load	48.5 ± 6.5	35.6 ± 3.9*	40.3 ± 3.4	41.2 ± 3.2
Cortical thickness	0.288 ± 0.009	0.250 ± 0.010*	0.285 ± 0.006	0.271 ± 0.010
Diameter	1.24 ± 0.02	1.27 ± 0.02	1.31 ± 0.02*	1.34 ± 0.02*
Hardness nano	719 ± 31	601 ± 29*	709 ± 27	704 ± 30
IGF-I	567 ± 29	344 ± 34*	614 ± 31	369 ± 29*

* $p<0.05$ vs WT Control, as evaluated by Anova

Conflict of Interest: None declared

Su-P329

PROPENSITY TO ACCUMULATE BONE MICRODAMAGES IS INCREASED IN ADULT FEMALE RATS FED AN ISOCALORIC LOW PROTEIN DIET

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Low protein intake compromise bone strength through a decrease in bone mass and alteration in microarchitecture, but also through changes in intrinsic bone tissue quality. Whether the low protein diet-induced deterioration of intrinsic bone tissue quality could favor the accumulation of bone microdamages, hence bone fragility, is not known. We investigated the effects of repeated loading on humerus bone strength in 6-month-old female rats pair-fed either a control (15% casein, n = 10) or an isocaloric low-protein (2.5%, corresponding to 50% of the minimal requisite for normal bone metabolism, n = 10) diet for 10 weeks. The humeri were cyclically loaded in three-point bending under load control for 2000 cycles. The peak load selected corresponded to 60% of the maximal load of the controlateral humerus, thus in the domain of elastic deformation. The humeri were then loaded to failure. We compared the load/displacement curve of the cyclically loaded humerus to the controlateral non-cyclically loaded humerus. Cyclic loading did not induce any deterioration in rats fed a normal protein diet, whereas the cyclic loading regimen negatively influenced the post-yield behaviour of humerus in rats fed a low protein diet, as indicated by significant decreases in post-yield load and plastic deflection. This suggests that bone microdamages could be more prominent in rats fed a low protein diet than in control bones submitted to the same loading regimen, contributing thereby to increased bone fragility.

Table 1

ProteinCyclic Loading	Normal no	Normal yes	Low no	Low yes
MaximalLoad (N)	92.3 ± 2.9	90.8 ± 2.8	87.1 ± 2.5	77.8 ± 4.8
Stiffness (N/mm)	236.6 ± 16.5	272.8 ± 17.9	237.5 ± 12.4	218.6 ± 22.5
Yield Point (N)	71.8 ± 3.6	71.4 ± 1.6	64.4 ± 2.7	67.1 ± 3.3
Post Yield Load (N)	20.5 ± 1.7	19.4 ± 2.7	22.7 ± 1.6	10.7 ± 2.0*
Plastic Defor mation (mm)	0.14 ± 0.01	0.12 ± 0.01	0.15 ± 0.01	0.08 ± 0.01*

*p<0.05 as evaluated by a Student's t-test

Conflict of Interest: None declared

Su-P330

DECREASED ACTIVITY OF LACTASE PHLORIZIN HYDROLASE AND BONE MINERAL DENSITY

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Background/Aims: The CC genotype of LCT (the gene encoding lactase phlorizin hydrolase) 13910 C/T polymorphism is perfectly matched with lactose intolerance and lower calcium intake from milk. We hypothesized that the altered calcium intake throughout life has an impact on serum calcium level and bone mineral density (BMD) in postmenopausal women. Methods: We studied 200 osteoporotic, 235 osteopenic and 160 healthy women. Genotyping, osteodensitometry and laboratory measurements (serum calcium, albumin, phosphate, crosslaps, 25-OH vitamin D3) were carried out in all subjects.

Results: Both the frequency of aversion to milk consumption (frequency for CC genotype = 19.66 % (n = 47); frequency for TT + TC genotypes = 10.43 % (n = 37); p = 0.03) and the albumin-adjusted serum calcium were changed according to polymorphism in a recessive model (albumin-adjusted calcium for CC genotype = 2.325 ± 0.09 mmol/L; albumin—adjusted calcium for TT + TC genotypes = 2.360 ± 0.16 mmol/L; p = 0.031). BMD was significantly decreased in individuals with CC compared to those with other genotypes at the total hip (Z-score for CC genotype = −0.471 ± 1.08; Z-score for TT genotype = −0.170 ± 1.09; p = 0.041), at Ward's triangle (Z-score for CC genotype = −0.334 ± 0.87; Z-score for TT + TC genotypes = −0.123 ± 0.82; p = 0.044) and at the radius (Z-score for CC genotype = 0.105 ± 1.42; Z-score for TT + TC genotypes = 0.406 ± 1.32; p = 0.038).

Conclusion: LCT 13910 C/T polymorphism appears to be associated with decreased serum calcium level and reduced BMD in postmenopausal women.

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Conflict of Interest: None declared

Su-P331

VALIDATION OF A CLINICAL DEFINITION FOR FRAGILITY FRACTURE

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Despite numerous studies examining the epidemiology of fragility fracture, there is no clinical definition available in the literature. Fractures are classically considered to be osteoporotic where the fracture is associated with decreased bone mineral density (BMD) and its incidence rises with age. This leads to the assumption that all fractures at an included site are due to osteoporosis, while fractures at an excluded site are unlikely to be osteoporosis-related. An alternative approach is to consider fragility fractures as being osteoporotic based on the mechanism of falling regardless of the site and respective BMD.

The aim of this study was to validate a clinical definition for fragility fracture: a fracture occurring spontaneously or following a minor trauma, such as a fall from standing height, a fall from the sitting position or a fall from laying down on a bed or a reclining deck chair from less than a meter high, a fall after having missed 1 to 3 steps in a staircase, after a movement outside of the typical plane of motion, or coughing.

Recognizing Osteoporosis and Its Consequences in Québec is an ongoing patient health management program aiming to improve the rate of diagnosis and treatment of osteoporosis for women 50+y that have suffered a fragility fracture. To date, 3288 women, mean age 65.2 y, have been recruited 0 to 16 weeks following a fracture and have experienced 3485 fracture events. Patients were contacted by phone to answer a short questionnaire to classify them as having

either experienced a fragility or traumatic fracture. The proportion of fragility fractures increased progressively with age [73.8% (50–59 y), 80.6% (60–69 y), 85.5% (70–79 y) and 92.8% (80+y)] and was similar between the various types of fracture [wrist (83.0%), humeral (76.1%), ankle (82.6%)], except for a higher proportion at the hip (93.0%), presumably because of its occurrence in older individuals. The proposed clinical definition of fragility fracture based on the mechanism of falling fulfills the criteria for the classical definition without the requirement of a low BMD and should be the preferred definition.

Conflict of Interest: LB, sanofi-aventis, P&G, Merck Frosst, Eli Lilly, Novartis, Amgen, Research support and/or consultant; JPB, Merck Frosst, P&G, sanofi-aventis, Novartis, Eli Lilly, Research support and/or consultant; KSD, Consultant, Servier; SJ, none declared; LGSM, Glaxo-Smith Kline, Hoffmann-Laroche, Merck Frosst, P&G, sanofi-aventis, Novartis, Eli Lilly, Servier, Research support and/or consultant.

Su-P332

THE RELATIONSHIP BETWEEN BONE MINERAL DENSITY AND ATHEROSCLEROTIC PARAMETERS IN POSTMENOPAUSAL WOMEN WITH DIABETES

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Backgound: Recent studies suggest a possible pathogenic linkage between the osteoporosis and atherosclerosis. Both atherosclerosis and osteoporosis are responsible for significant morbidity and mortality, are independent predictors of cardiovascular disease (CVD) events, and may share common regulatory mechanisms as well as histopathology. In this study we want to show the relationship between bone mineral density (BMD) and atherosclerotic parameters in postmenopausal women with diabetes.

Methods: Total 44 postmenopausal women with diabetes were enrolled in the study. We measured pulse wave velocity (PWV), carotid intima-media thickness (IMT) and carotid plaque as a atherosclerotic parameters. We also checked anthropometric and serologic including HbA1C, C-peptide, lipid profiles and bone markers. The lumbar spine, femur neck and total hip bone mineral density (BMD) was measured using dual X-ray absorptiometry (DEXA).

Results: From multiple linear regression analyses of all the study subjects, body mass index (BMI) was found to be determinants of the lumbar spine, femur neck and total hip BMD($r2 = 0.396$, $r2 = 0.405$, $r2 = 0.342$, $p < 0.05$). Serum calcium, aortic PWV, and mean IMT were significantly increased according to increase of age ($r2 = 0.386$, $r2 = 0.36$, $r2 = 0.33$, $p < 0.05$). From bivariate analyses, the lumbar spine and total hip BMD showed a negative correlation with age ($r2 = -0.26$, $r2 = -0.24$, $p < 0.05$) and duration of diabetes ($r2 = -0.35$, $r2 = -0.36$, $p < 0.05$). Lipid profile, IMT, PWV are not correlated with BMD. The prevalence of carotid plaque in our study was 32%. Only the patients with presence of plague showed significantly decreased lumbar spine BMD($p = 0.007$), increased duration of DM($p = 0.032$) and aortic PWV($p = 0.004$) compare to those with absence of plague.

Conclusion: Out of many atherosclerotic factors, carotid plague causes low BMD which may be induced by high serum calcium, increased aortic PWV and increased duration of DM.

Conflict of Interest: Non declared

Su-P333

ASSESSMENT OF RISK FACTORS FOR OSTEOPOROSIS AND PREDICTION OF 10YR FEMORAL FRACTURE RISK IN ELDERLY WOMEN FROM CANOSA OF PUGLIA

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In this study we have assessed the prevalence of osteoporosis, and of fragility fractures in 300 women who underwent medical visit in our department (Geriatric division) from Jannuary to April 2007. We have collected many information about risk factors from each woman: age, weight, sedentary or active life, smoking, early menopause (< 46 yrs), spinal deformity index, fall risk, rheumatoid arthritis (RA), prevalent non vertebral fractures, familiarity for fractures, diabetes, calcium intake from diet. We have also analyzed the relationship between clinical risk factors and bone mineral density measured with DEXA at the femoral level.

Vertebral morphometric evaluation was done on 162 women selected for the above risk factors. Mean age of patients was 70.23 (SD8.07, Min 48 Max 88), BMI 25.6 (SD 3.22, Min 20, Max 41).15% of women had an early menopause. Prevalent non vertebral fractures were present in 36% of women (femoral = 25, wrist = 37, other = 45). 40% of women have a fall risk and more than 30% have a sedentary life. The average of calcium intake from diet was less than 500 mg/die. More than 15% of patients have diabetes type 2, while RA was present in about 6%. Osteoporotic (OP) women were 26%, while the rate of osteopenia was about 47% and normal women 27%. We have done comparative analysis for each risk factor between normal and OP women and we found a statistical difference ($p < 0.05$) on the following factors: age of menopause, diagnosis of diabetes type 2, fall risk, sedentary life, familiarity for femoral fracture, non vertebral fractures and vertebral fractures. Interestingly, patients with early menopause were OP or osteopenic but none normal.

We have calculated the femoral 10 yr risk factors from SIOMMMS (Italian Society of Osteoporosis, Mineral Metabolism and Skeletal Diseases) mineral metabolism algorithm with contribution of adjunctive risk factors: 50 women out of 300 (17%) had a 10 yr femoral fracture risk score higher than 10%. We are evaluating the presence of other risk factors not included in SIOMMMS algorithm (e.g. fall risk, diabetes) in women with > 10% risk of femoral fracture in the following 10 yrs.

Conflict of Interest: None declared

Su-P334

OESTROGEN DEFICIENCY, BUT NOT THE LOW BONE MASS, INDUCES CARTILAGE DAMAGE IN HEALTHY KNEES IN AN EXPERIMENTAL MODEL IN RABBITS

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Aims: To determine the influence of ovariectomy (OVX) either alone or combined with systemic glucocorticoids administration on

healthy cartilage in an experimental model in rabbits. Methods: Twenty female NZW rabbits (10 months old; mean weight of 4.3 kg; r: 3.7–6.3) were randomly allocated in three different groups. Seven animals underwent bilateral OVX (OVX group). Low bone mineral content status was induced in 6 animals by OVX and subsequent parenteral methylprednisolone hemisuccinate (MPH;1 mg/kg/d) for 4 weeks (osteoporosis, OP group). Seven animals were used as controls (Healthy group). To evaluate the bone mass variation, bone mineral density (BMD) was measured by DXA at both baseline and 6 weeks after OVX (Hologic ® QDR-1000) in lumbar spine (L3–L4, LS), global knee (gK) and subchondral bone of the knee (sK). The histopathological cartilage damage at the end of the experimental interventions was evaluated in the medial femoral condyles following the Mankin's system.

Statistical analysis: The difference of the means of the cartilage damage and BMD between groups was calculated using the analysis of the variance (ANOVA). Correlation between DXA and histological damage was done by Spearman correlation test (SPSS, vs. 10.0).

Results: BMD (mg/cm^2)showed a significant decrease in OP rabbits when compared to both OVX and healthy rabbits at 6 wks ($p < 0.05$) (see Table 1). A significant negative correlation between BMD at LS/gK and cartilage damage was also demonstrated ($p < 0.05$), but no correlation could be established when BMD was determined at sK.

Conclusion: Since isolated OVX induced statistically significant alterations in normal cartilage in our model, oestrogen deficiency might play a direct role in the ethiopathogenesis of osteoarthritis. The fact that differences in cartilage damage using the Mankin score were not significant when OVX and OP rabbits were compared, suggests that low mineral content status and systemic glucocorticoids do not play any crucial pathogenic role in cartilage damage in healthy joints.

Table 1

Group	Mankin score	BMD-LS	BMD-gK	BMD-sK
Healthy	0.14 (0.14)	305 ± 13	473 ± 31	642 ± 47
OVX	1.43 (0.30)*	268 ± 37	426 ± 25	578 ± 70
OP (OVX+MPH)	2.42 (0.57)*&	232 ± 40*&	362 ± 86*&	490 ± 97*#

* $p < 0.05$ with respect to healthy group; & non significant differences between OVX and OP; # $p < 0.05$ between OVX and OP. Results are expressed as mean ± SD

Conflict of Interest: None declared

Su-P335

BONE REGENRATION DURING OSTEOPOROSIS

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Regulation of bone repair is provided by genetic factors, local molecular and cell influence. In addition the third system level is defined which is the regulation by hormones and hormone-like substances.

The goal of the research is to study bone repair under the conditions of experimental osteoporosis.

Materials and methods. Experiments were carried out on white rats. During 45 days animals were injected with 0.5 or 5 mg of hydrocortisone per 100 g of weight. Alimentary osteoporosis was modeled by means of keeping the rats on low calcium diet during 5 months. Traumatic defect was made in distal femur metaphysis with the help of a dental borer. Euthanasia was performed by means of ether overdose 3, 7, 14, 21 and 30 days after the operation. Material was studied with histological methods using electron microscopy and morphometry of the bone regenerate.

Results: Comparative analysis of bone regeneration in alimentary and glucocorticoid induced osteoporosis showed similar processes. At inflammation stage slowing down of haematoma reconstruction and decrease of cell 'critical' mass due to damaged chemotaxis and cell differentiation in defect zone were observed. Abnormal function of macrophages was seen. Inactive cells or phagocytes prevailed. Quantity of secreting cells decreased while the quantity of secreting macrophages increased in control animals. If compared to control group the quantity of cells of fibroblast and osteoblast differons decreased in experimental animals at the stage of cells proliferation and differentiation. The disruption of formation of cell part of regeneration (namely macrophage, fibroblast and osteoblast types) caused further slowing down of formation of tissue specific regenerate structures. During morphometric analysis it was found out that experimental groups of animals possessed similar trends of regenerate tissue formation. Connective tissue prevailed in both types of defects comparing to control group. Total area of newly formed bone tissue was decreased. Distinct slowing down of mature bone tissue formation was observed in animals injected with 5 mg/kg hydrocortisone.

Conclusion. Thus during modelling of different types of osteoporosis (alimentary and glucocorticoid induced) similar changes of regenerate formation connected to slow bone tissue formation are observed. Most probably prerequisites of disruption of reparative osteogenesis are created at the stages of inflammation, cell proliferation and differentiation.

Conflict of Interest: None declared

Su-P336

BONE REPAIR AT HYPOTHYROSIS

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Thyroid hormones (thyroxine (T4), triiodthyronine (T3) and thyrocalcitonine) influence bone metabolism. However the research works studying bone tissue regeneration under the conditions of hypothyrosis are almost unavailable.

The goal of the research is to study bone tissue regeneration under the conditions of hypothyrosis.

Materials and methods. Experiments were carried out on white rats. Animals were injected with 1 mg/100 g mercazole during 60 days. Traumatic defect was made in distal femur metaphysis with the help of a dental borer. Euthanasia was performed by means of ether overdose 3, 7, 14, 21 and 30 days after the operation. Material was studied with histological methods using electron microscopy and morphometry of the bone regenerate.

Results: In 3 days after traumatic defect quantity of neutrophils increased by 1.23 times in experimental animals in comparison with control group. The decrease of quantity of low differentiated stem cells (by 1.24 times) and fibroblasts (by 1.45 times) was seen if compared with the control group. This proves delay of cell proliferation and differentiation stages. Slowing down of formation of tissue specific regenerate structures was observed. In 14 days after trauma major area of defect was occupied by connective tissue whose area was 2.94 times bigger than the one of the control group. Bone tissue area was 1.85 times less in experimental animals than in the control ones.

In 21 days significant areas of connective tissue were seen in regenerate of experimental animals. Its relative area was 2.2 times

bigger than in control ones. The decrease of relative area of lamella bone tissue by 3.94 times (comparing to the control group) also proves slowing down or regenerate remodeling stage.

In 28 days cortex was not fully reconstructed in animals receiving mercazole. Bone regenerate was represented by thin, sparsely located trabecules with lysis sections. Enlarged vessel cavities were found in marrow.

Conclusion. Thus the carried out experimental research proved the slowing down of regeneration processes in rats femur metaphysis bone defect under the conditions of reproduced hypothyrosis.

Conflict of Interest: None declared

Su-P337

REGIONAL DIFFERENCES IN THE MANAGEMENT OF OSTEOPOROSIS: EUROPE VS OTHER GEOGRAPHIC REGIONS. THE GLOBAL LONGITUDINAL REGISTRY OF OSTEOPOROSIS IN WOMEN

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Aim: To compare use of diagnostic technology and osteoporosis medication in women aged 55+ years in 10 countries.

Methods: GLOW (Global Longitudinal registry of Osteoporosis in Women) is an observational study of women aged 55+ years recruited by 540 primary physician practices in 17 sites in 10 countries. All non-institutionalized patients visiting the practice within the prior 2 years were eligible. Self-administered questionnaires were sent (2:1 over-sampling of women aged 65+). These data refer to a preliminary group of 20,120 women. We compare the results recorded in Europe vs other regions involved in GLOW (N. America/Australia).

Results: Overall, 65% of women reported having had a bone density test; frequency of testing ranged from 51% in Europe to 80% in the other regions. Reported current use of "bone medications" (risedronate, etidronate, alendronate, ibandronate, pamidronate, raloxifene, teriparatide, tibolone, calcitonin, strontium ranelate, and zoledronate) averaged 17%, and ranged from 13% in Europe to 22% in N. America/Australia. Current use increased with age: 24% for those 75+ years vs 12% in those < 65 years. When use was assessed by self-reported diagnosis of osteoporosis or osteopenia, frequency was 50% and 26%, respectively, with 4.0% of women without either diagnosis reporting current use (age-adjusted). Use increased with clinical risk: 26% of women with Fracture Index scores $> = 5$ (indicating 5-year risk of non-vertebral fracture of 26%) reported taking bone-related medication. Frequencies ranged from 20% in Europe to 31% in N. America/Australia.

Conclusions: Management of osteoporosis in this population is not entirely consistent between Europe and the other regions involved in GLOW. Use of bone density testing and bone medications is lower in Europe than in N. America/Australia. Even in women with high Fracture Index scores, Europe appears to be more conservative than N. America/Australia, in using medications.

Table 1

	Bone mineral density and current bone medication use		
	Bone density test	Bone medication	Bone med in women Fx index score ≥ 5
Europe n/N (%)	5155/10,056 (51%)	1320/10,142 (13%)	397/1943 (20%)
N. America & Australia	7880/9886 (80%)	2161/9978 (22%)	690/2210 (31%)

Fx, fracture

Conflict of Interest: Funding: The Alliance for Better Bone Health (Procter & Gamble Pharmaceuticals and sanofi-aventis)

Su-P338

THE CHANGE IN BIOCHEMICAL BONE MARKERS AFTER 6 MONTHS OF STRONTIUM RANELATE IN ELDERLY WOMEN AND MEN WITH REDUCED BONE MINERAL DENSITY

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We have investigated the effect that 6 months of strontium ranelate therapy [StR], a bone agent that increases bone formation, has on a panel of biochemical markers of bone formation and bone resorption. We recruited 13 elderly subjects [8F, 5M] average age 66 $\pm$ 3 yr with low bone mineral density who received 2 g of StR/day Fasting serum samples were collected prior to and after 6 m of StR therapy and stored at -70°C until analysed. The biochemical markers used to study bone formation were bone alkaline phosphatase [Bone ALP], osteocalcin [OC] and the amino terminal extension peptide of type 1 collagen [P1NP] and for resorption carboxy and amino terminal telopeptides of type 1 collagen [beta CTX, NTX] and tartrate resistant acid phosphatase 5b [TRACP 5b], we also measured osteoprotegrin [OPG] and RANK Ligand [RANKL]. Analysed as a whole no significant differences [$p > 0.05$] were found between pre and 6 m of StR therapy for any of the measurements. When the group was divided by sex there were still no significant differences for any of the measurements in the female group. In the male group significant differences were found for OPG 2.3 $\pm$ 0.5 to 3.5 $\pm$ 0.6 pmol/L [$p < 0.02$], P1NP 53 $\pm$ 9.8 to 35 $\pm$ 14.7 mcg/L [$p < 0.05$], NTX 18.3 $\pm$ 3.4 to 28.8 $\pm$ 6.3 mcg/L [$p < 0.02$]. Osteoclast activity was calculated using the formula NTX/TRACP 5b; for females it decreased from 8.8 $\pm$ 2.4 to 7.3 $\pm$ 2.0 [ns] whereas in males there was an increase from 7.6 $\pm$ 1.0 to 12.2 $\pm$ 3.5 [$p < 0.05$]. Even though the number of subjects in the study was small [8F, 5M]; from our data it appears that StR has a different effect on both collagen formation and resorption, as demonstrated by the results of the various biochemical bone markers, depending on whether the subject is male or female.

Conflict of Interest: None declared

Su-P339

VALIDITY AND REPRODUCIBILITY OF QUESTIONNAIRE ABOUT PREVENTIVE BEHAVIORS AMONG FRENCH AND SPANISH POST-MENOPAUSAL WOMEN WITH OSTEOPOROSIS IN THE CALCIUM AND VITAMIN INTAKE (CAVIT) STUDY

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The purpose of this study was to validate a questionnaire used to assess amount of vitamin D from supplementation and sun exposure among post-menopausal osteoporotic women in France and Spain in the CaVit study.

One hundred women aged 50 years and older in France and Spain (50 per country) participated in two rounds of telephone interview consisting of twelve questions. Convergent and discriminant validity of the CaVit questionnaire was tested by probing its correlation with a similar question on intake of vitamin D in the National Health and Nutrition Examination Survey (NHANES) questionnaire, and another question on other prescription drug use in the NHANES questionnaire, respectively. Participants were interviewed one week after the first round interview to assess the consistency of their responses as a test for the test-retest reliability of the questionnaire. Goodman & Kruskal Tau coefficients were calculated as a measure for correlations.

Of these 100 patients, 50 women in France and 44 in Spain completed two rounds of interviews. There was a significant convergence between the multi-vitamin intake question in the CaVit study and multi-vitamin and dietary supplementation question in NHANES, with Tau = 0.16 ($p < 0.01$) for both counties together. There was no significant correlation between the CaVit questionnaire asking about multivitamins use and the NHANES question asking about prescription medication other than multi-vitamins [tau = 0.001 ($p = 0.82$) for two countries combined], indicating discriminant validity. There was a significant correlation between two rounds of interviews for multivitamin intake question (Tau = 0.82, $p < 0.01$) and for calcium supplemental intake question (0.88, $p < 0.01$).

The questionnaire used in CaVIT study showed strong convergent and discriminant validity, test-retest reliability and therefore it can be used to measure calcium and vitamin D supplementation intake among women with osteoporosis.

Conflict of Interest: SS Sen, Merck & Co., Inc., employee and Shareholders
T Fan, Merck & Co., Inc., employee and Shareholders
G Nocea, Merck & Co., Inc., employee and Shareholders

Su-P340

DIETARY AND SUPPLEMENTAL CALCIUM INTAKE AND DIETARY VITAMIN D INTAKE AMONG POST-MENOPAUSAL WOMEN WITH OSTEOPOROSIS IN FRANCE: CALCIUM AND VITAMIN INTAKE STUDY

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This study estimated the average daily intake of calcium and vitamin D from supplements and diet among women with osteoporosis 55 years and older in France.

From national physician lists, 119 general practitioners in France who treat osteoporosis were randomly contacted. These physicians referred the next 1–7 patients with osteoporosis from their clinics to participate in this study. Data were collected through telephone interviews by trained native-speaking interviewers. A French food frequency questionnaire developed in the SU.VI.MAX. study was used to collect dietary intake. A validated questionnaire was used to ask patients about their calcium supplementation and prescription drug use for osteoporosis.

Two hundred and seven osteoporotic women aged 55 years and older from France were interviewed. The average dietary calcium intake was 966 ($\pm$ 274) mg per day, which did not statistically differ from the recommended daily calcium intake of 1200 mg ($p = 0.26$). Among them, 13.0% were using calcium supplements of 1,000+ mg per day;16.4% were using supplements < 1,000 mg per day; 6.8% were unaware of their daily dosing; and 63.8% were using no calcium supplementation. The mean vitamin D intake from food was 145 ($\pm$85) IU per day and significantly lower than 400 IU ($p < 0.001$). Only 1.0% of women in France reported receiving the recommended daily intake. Only 4.8% reported taking a multivitamin on a regular basis, though 28.5% reported receiving vitamin D from their calcium product.

The mean daily intake of calcium in osteoporotic post-menopausal women in France was not statistically different from recommended daily dose, while the average dietary vitamin D intake was much lower than recommended dose. Osteoporosis patients in France need to increase vitamin D intake from other sources. Vitamin D intake from supplements needs be studied in future.

Conflict of Interest: S. Czernichow, Merck & Co., Inc., Consultant
S.S. Sen, Merck & Co., Inc., employee and Shareholders
T. Fan, Merck & Co., Inc., employee and Shareholders
G. Nocea, Merck & Co., Inc., employee and Shareholders

Su-P341

DECREASED BONE FRACTURE PREVALENCE IN POLLEN ALLERGIC MEN

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Our aim was to investigate whether pollen-allergy can affect bone mass and fractures in men. A total of 19 pollen-allergic men (mean age 56.63 yr) were compared to non-allergic subjects matched for age and body mass index (BMI). Allergic men were split into two groups according to H1 histamine receptor (H1R) antagonist treatment (treated n = 15, not treated n = 4) for at least 5 years, seasonally. Secondary causes of osteoporosis were excluded in every group. Bone mineral density, quantitative ultrasound parameters and anthropometric data were measured and bone fractures were recorded in patients and controls. Overweight and obesity (25 kg/m^2 $\leq$ BMI) were common among the allergic (57.9%) and the BMI matched

controls. Allergic patients had a slightly higher bone density at the lumbar spine, femoral neck, and at the forearm. Quantitative ultrasound parameters were slightly better among allergic, however no significant differences were found. Only one patient was found with bone fracture in the allergic group, however, 8 patients among non allergic groups suffered from bone fracture (Chi-square test, $p = 0.019$). The untreated allergic had lower bone mass (not significantly) and lower QUS parameters than the H1R antagonist treated patients (SOS 1507+/− 4.4 m/s vs 1539+/− 7.6, $p = 0.018$). In conclusion we found a lower prevalence of low-energy fractures among pollen-allergic men, than among non-allergic subjects. SOS—which is a non-mass parameter, positively correlates with bone elasticity—was higher in the H1R antagonist treated allergic group. It is possible that the H1R antagonists have positive effect on bone elasticity and protect against bone fracture in pollen allergic patients confirming our previous results in postmenopausal pollen allergic women. Further study is needed in pollen allergy to better understand the effect of H1R antagonists on bone fractures.

Conflict of Interest: None declared

Su-P342

RELATIONSHIPS BETWEEN BASELINE BONE TURNOVER, SUBSEQUENT VERTEBRAL FRACTURE AND BONE LOSS

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The use of bone turnover markers (BTM) to predict fracture and bone loss has been studied, with conflicting results. We aimed to examine associations in postmenopausal women between BTMs and subsequent vertebral fractures and rates of BMD change.

A population-based cohort of 2408 women, 55 to 80 years, was recruited at 5 European centres (the OPUS study). At baseline we obtained lateral spine radiographs and lumbar spine (LS) and femoral neck (FN) BMD by DXA. Blood and urine samples, taken between 1100 and 1500 hrs, not fasting, were measured for markers of bone formation (s-OC, s-P1NP), resorption (s-CTX, u-NTX/Cr) and parathyroid hormone (PTH). At the 6-year follow up visit 1567 subjects returned for further spine radiographs, but were only included in the analysis if at baseline they had been postmenopausal, with BTM measurements but no recent bone-affecting treatment, and had follow up BMD ($n = 924$). Annual percentage rates of BMD change were calculated. Data were analysed by multiple logistic and linear regression (SPSS).

At follow up (mean 6.03 (0.35) yrs), 44 subjects had sustained incident vertebral fractures. There was no association between any baseline BTM (log-transformed) and the incidence of fracture, either with or without adjustment for age, baseline BMD, BMI, prevalent vertebral fracture, weight change, the use of any anti-resorptive treatment after baseline, and incidence of bone-affecting conditions. However, s-CTX and s-PTH were negatively associated with the rate of FN BMD change ($p = 0.009$, $p = 0.011$), adjusted for the same variables, and PTH was also negatively associated with LS change ($p = 0.004$). The rate of FN BMD change (but not LS change) was inversely associated with the incidence of vertebral fracture (OR 0.55 (95% CI 0.38, 0.77) per SD increase in rate of change, $p = 0.001$).

We conclude that although bone turnover, measured non-fasting, does not predict vertebral fractures, higher s-CTX and s-PTH may predict a faster rate of bone loss. Greater bone loss at the femoral neck is also associated with an increased risk of vertebral fracture.

Conflict of Interest: None declared

Su-P343

EFFECTS OF AZOLE ANTIFUNGAL DRUGS ON BONE MECHANICAL PROPERTIES IN RATS

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There is a growing problem of adverse skeletal side effects of different medications. Azole antifungal drugs may potentially damage the skeletal system.

The rate of the systemic fungal infections considerably increased in recent years; azole antifungal drugs belong to the most frequently used antifungal agents. We observed that ketoconazole and fluconazole exert damaging effect on the rat skeletal system by inhibiting bone resorption and formation. The mechanism of the unfavourable effect of the drugs on the rat skeletal system involved inhibition of the formation and/or shortening of the life-span of osteoclasts as well as the tendency to decrease the osteoblast metabolic activity. Here we present the effects of fluconazole and ketoconazole on mechanical properties of the femur in rats.

The experiments were carried out on young (about 6-week-old) and adult (about 14-week-old) male and female Wistar rats, in which mechanical properties of the whole femur (load at fracture and maximal deformation) were determined after 6-week administration of fluconazole (30 mg/kg p.o. daily) or ketoconazole (50 mg/kg p.o. daily). More detailed mechanical studies were carried out after 4-week administration of fluconazole (45 mg/kg p.o. daily) or ketoconazole (75 mg/kg p.o. daily) to 9-10-week old male and female Wistar rats. Mechanical properties of the whole femur (extrinsic stiffness, ultimate and breaking load, deformation caused by the applied load) and the femoral neck (load at fracture) were examined. Bone mass, mineral content, macrometric and histomorphometric parameters were also studied. The number of rats per group was 6–8.

The effects of ketoconazole on the rat skeletal system were stronger than those of fluconazole. After 6-week administration, although both drugs impaired some bone macrometric and histomorphometric parameters, only ketoconazole impaired bone mechanical properties in young rats of both sexes, whereas fluconazole had no statistically significant effects. The 4-week administration of the azole antifungal drugs did not statistically significantly affect the examined bone mechanical properties in rats.

In conclusion, prolonged administration of azole antifungal drugs may lead to impairment of bone mechanical properties. However, the potential of various azole antifungals to damage the rat skeletal system is differential.

Conflict of Interest: None declared

Su-P344

HIP STRUCTURE IS RELATED TO MUSCLE MASS OF THE THIGH IN HEALTHY YOUNG MEN - RESULTS FROM THE ODENSE ANDROGEN STUDY

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Several studies have shown that muscle mass is at strong positive predictor of BMD. Determinants of hip structure, however, are largely unknown.

Aim: To evaluate if muscle mass of the femur is associated with BMD and hip structure in young men.

Subjects and methods: The Odense Androgen Study is a population-based, prospective, observational study on the inter-relationship between endocrine status, body composition, muscle function, and bone metabolism in young men including 783 males aged 20–30 years in Funen County, Denmark. This sub-study included 390 participants selected at random. DXA and hip structure analysis of the hip was performed using a Hologic-4500a densitometer and the APEX software (version 2.0). MRI was performed with an open, low field (0.2 Tesla) MR unit (Magnetom Open Viva, Siemens AG, Germany). The thigh muscle area was determined in one femoral slice (equidistant from the trochanter major and patella) using a T1-weighted gradient-echo sequence (repetition time: 370 msec, echo time: 15 msec, acquisition matrix: 512 × 512, field of view: 230 mm).

Results: A significant correlation was found between thigh muscle area and the parameters of hip structure as shown in table 1. The closest correlation was found with cross-sectional area of the femoral shaft ($R = 0.62$, $p < 0.001$.

Conclusions: Hip structure is closely associated with thigh muscle mass in young health males.

Acknowledgements: Kevin Wilson and Hologic Inc. are acknowledged for technical assistance.

Table 1 Data are shown as R- values

	Neck	Inter-troch	Femoral shaft
BMD standard	0.48***	0.51***	–
BMD narrow	0.47***	0.43***	0.56***
Cross-setional area	0.54***	0.53***	0.62***
Cross-sectional moment of inertia	0.49***	0.54***	0.62***
Width	0.23***	0.32***	0.19***
Section modulus	0.53***	0.59***	0.55***
Buckling ratio	−0.30***	−0.36***	−0.17**

** $p < 0.01$, *** $p < 0.001$

Conflict of Interest: None declared

Su-P345

MICROSTRUCTURE OF CORTICAL BONE AS STUDIED BY RAMAN SPECTROSCOPY (COMPOSITION VS ORIENTATION)

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The present study focuses on the basic material of bone, the collagen-mineral composite, containing nano-sized mineral platelets (essentially carbonated hydroxyapatite) and protein (especially collagen type I). These components have markedly different mechanical properties. The mineral is stiff and brittle while the protein is much softer but also much tougher than the mineral.

Mice bones of different age groups were prepared to study the mineral and organic composition as well as the sensitivity of the Raman bands to the orientation and the polarization direction of the incident light. In Raman spectroscopy the information from the mineral component and the organic matrix is obtained simultaneously, providing a complete picture of bone composition with high spatial resolution. Band integration was performed for "fingerprint" bands including phosphate u_1 at 961 cm^{-1}, collagen amide III at 1255 cm^{-1} and collagen amide I at 1671 cm^{-1}. The mineral-to-matrix ratio (phosphate-to-collagen) was calculated in different, well-defined anatomical areas. The intensity of the Raman bands showed polarization and angle dependence effects. The phosphate u_1 band was chosen as an indicator for the degree of crystal organization in addition to apatite phosphate. Raman spectra were subjected to cluster analysis and the results showed differences in the heterogeneity of the phosphate band, thus differences in the ultra structural level of mineralization.

We conclude that Raman spectroscopy provides additional insights into the standard organization of bone tissue at the ultrastructural level. Our aim is to further develop the diagnostic capability of Raman microspectroscopy so it can be implemented in a clinical setting for diagnostic purposes.

Conflict of Interest: None declared

Su-P346

LIFE-STYLE RISK FACTORS FOR OSTEOPOROSIS AND FRACTURE RATE

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The lifestyle of women in Greece and in Europe has dramatically changed during the last 30 years. The proportion of women living in the city, working in an office environment and having a smoking habit, drinking alcohol and/or coffee excessively has impressively augmented according to the statistics. Most of these women are now in menopause and an important proportion of them suffers from osteoporosis.

In order to clarify if the new lifestyle has an impact on fracture rate of postmenopausal osteoporotic women (PMOW) a population based observational retrospective study has been performed. The fracture rate of 4616 postmenopausal osteoporotic women (PMOW) (mean age = 64,1 ± 9,3 years) from 160 centers all over Greece has been compared with the five aforementioned possible risk factors. Descriptive statistics like the mean ± SD and frequencies were used to present the data. In order to assess for relationships between categorical variables the chi-square ($\chi 2$) test was performed. Statistical analysis was conducted using the software SAS, version 9.1 and statistical significance was established as 5%. The results are as follow:

1) 16,2% of these PMOW had a history of fracture and for 80,3% of them was a hip fracture.
2) 84,1% of PMOW lived in urban environment and had lower fracture rate than women living in the countryside ($p < 0,05$).
3) 47,2% of PMOW worked at home and had lower fracture rate than women working for more than 20 years in an office environment ($p < 0,0001$).
4) 60,5% of PMOW smoking more than 10 cigarettes/day for the last 3 yrs had higher fracture rate than non-smoking women ($p < 0,0043$),
5) 38,1% of PMOW drinking more than 3 coffees/day for the last 5 yrs had higher fracture rate than non drinking coffee women ($p < 0,0001$) and
6) 5,6% of PMOW drinking alcohol more than 2 portions/day had no impact on fracture rate ($p < 0,7783$).

It can be concluded that the new life-style has created more fracture-susceptible PMOW.
Conflict of Interest: None declared

Su-P347

THE IMPACT OF TYPE 2 DIABETES MELLITUS ON THE ASSOCIATION OF BONE MINERAL DENSITY WITH BODY COMPOSITION

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Diabetes mellitus type 2 has often been associated with increased bone mineral density (BMD). This finding was attributed to either mechanical (obesity) or biochemical (anabolic properties of insulin and IGFs) effects. Aim: To investigate the impact of diabetes mellitus either directly to BMD or to any correlation between BMD and body composition in postmenopausal women.

Subjects-Methods: In 23 diabetic [(DM), age 58.8 ± 4.3 years, years since menopause 9.1 ± 2.2, BMI (kg/m^2) 30.7 ± 3.7] and 38 healthy matched for BMI postmenopausal women [(PMP), 57.4 ± 4.9, 8.7 ± 2.4, 30.1 ± 3.7 respectively], BMD and body composition measurements were performed by DXA. All women aged less than 65 years and their BMI values ranged between 22–38 kg/m^2. In DM women diabetes duration was 6.7 ± 3.1 yrs and HbA1c levels were 7.1 ± 0.9%. None of the diabetic women had ever received insulin treatment. BMD measurements referred to whole body, L1–L4 vertebrae, total hip and femoral neck. We also evaluated whole body fat mass (FM) and whole body lean mass (LBM) as well as their proportions to total body mass (TBM) (FM % and LBM % respectively).

Results: No difference existed between the groups regarding age, years since menopause, TBM, FM or FM %. No significant differences existed between the 2 groups regarding either total or any regional BMD values. In either group no correlation existed between BMI and any BMD value. In contrast, BMI exerted stronger positive correlation to LBM% ($r = 0.70$–0.74, $p < 0.01$) than to FM % ($r = 0.52$–0.55, $p < 0.01$). No significant correlation existed between any lipometric and densitometric parameter in both groups. Only in PMP women a significant correlation existed between LBM % and all regional BMD values ($r = 0.37$–0.48, $p < 0.05$). In DM women no significant correlation was observed between either densitometric or somatometric parameters and the disease duration or HbA1c levels.

Conclusions: In women, BMI hardly expresses the degree of obesity since it seems to better correlate rather to body lean than the body fat mass. Nevertheless, in the absence of significant differences in BMI between diabetic and healthy postmenopausal women, diabetes mellitus type 2 does not seem to significantly benefit bone mineral density. In both diabetic and healthy women, fat mass does not seem to correlate to bone mineral density either. Larger numbers of diabetic women are required to confirm the aforementioned findings.
Conflict of Interest: None declared

Mo-P348

AVERAGE PHYSICAL ACTIVITY IS ASSOCIATED WITH HIP SIZE AND DENSITY IN BOYS BUT NOT GIRLS AT 4 YEARS OLD

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Physical inactivity is an increasing problem amongst children. In addition to concerns regarding resulting obesity, the secular decrease in load-bearing activity may be associated with reduced accrual of bone mineral to peak, and thus increased risk of osteoporotic fracture in older age. In this study we utilised an ongoing longitudinal study of mothers and their children (Southampton Women's Survey) to examine the cross sectional relationship between childhood physical activity (PA) and contemporary bone mineral.

Children were recruited at 4 years old from the Southampton Women's Survey. They attended the Osteoporosis Centre at Southampton General Hospital for measurement of bone mass at whole body, lumbar spine and hip sites (Hologic Discovery: Hologic Inc., Bedford, MA, USA), together with assessment of diet, lifestyle, health and medications. At the end of the visit the children were fitted with an Actiheart combined accelerometer and heart monitor (Cambridge Neurotechnology Ltd, Cambridge, UK), which was to be worn for 7 days continuously. At the end of this period the monitor was posted back and correlation techniques were used to compare bone mass and measures of PA.

81 children (49 boys) took part. The mean (sd) age was 4.1 (0.1) years. They were all healthy term deliveries. Mean daily PA (counts/minute) was similar in boys and girls (45.6 vs 46.1 cts/min respectively, $p = 0.836$). In the boys, greater levels of mean daily PA were statistically significantly associated with total hip bone area ($r = 0.24$, $p = 0.017$), bone mineral content ($r = 0.27$, $p = 0.007$), areal bone mineral density ($r = 0.21$, $p = 0.033$) and estimated volumetric density ($r = 0.19$, $p = 0.056$), independent of height and weight. The relationships in the girls were not statistically significant. No associations with PA were found for either gender at the whole body or lumbar spine sites.

Mean daily PA was associated positively with hip size and density at 4 years old in boys but not girls, despite similar mean levels of PA. This may reflect different patterns of activity, such that boys have shorter bursts of more intense PA, whereas girls have more prolonged moderate activity. Further analysis of the minute by minute data will help clarify this. These results may have implications for public health, and follow up at 6 years with DXA and pqCT will enable elucidation of the longitudinal relationships between PA and skeletal size and volumetric density.
Conflict of Interest: None declared

Mo-P349

LONGITUDINAL CHANGES IN BONE MINERAL DENSITY FOR HEALTHY MALE ELDERS IN SOUTHERN TAIWAN

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Background: Longitudinal data on bone decline for Chinese elderly are sparse, especially in the healthy aged male. We reported the longitudinal change in bone mineral density (BMD) at the femoral neck, the great trochanter and the Ward's triangle for healthy Taiwanese male elders.

Methods:A prospective cohort study was conducted. We screened 1500 subjects with age 65 and above. Totally 170 healthy male were eligible for hip evaluation and 167 male had the hip BMD. Two years later, 142 male had completed follow-up BMD. Linear regression was performed between aging and bone loss. Pair-t test was used for BMD changes between the intervals.

Results:In the initial study, the subjects had significant bone loss through aging by liner regression at all three sites ($P < 0.001$). Two years later, there is a significant decrease in BMD at all three sites ($P < 0.001$). For the age cohort, all the age groups showed a

significant decrease in BMD of the 3 studying sites ($P < 0.05$) except the group aged 75 and over at the Ward's triangle ($P = 0.667$) and the great trochanter ($P = 0.1$). There is a peak loss of BMD in males aged 65–69 as high as 5.57% annually at the Ward's triangle.

Conclusions: BMD is negatively related to aging in the healthy male. The loss of BMD in the age group of 65–69 years at the Ward's triangle is faster than other sites. The bone loss in Chinese male should be cautious because they have more bone loss than the Caucasians.

Table 1

Bone mineral density in male 2 years after initial exam

Age group	Number	Femoral neck	Mean Loss	Ward's triangl	Mean Loss	Great trochan	Mean Loss
65 ~ 69	27	−0.036	−2.17%	−0.070	−5.57%	−0.024	−1.56%
70–74	82	−0.027	−1.75%	−0.023	−1.57%	−0.010	−0.67%
>75	33	−0.041	−2.59%	−0.010	−0.14%	−0.012	−0.87%
total	142	−0.032	−1.87%	−0.029	−2.00%	−0.013	−0.88%

Conflict of Interest: None declared

Mo-P350

QUANTITATIVE CALCANEAL ULTRASOUND AND VERTEBRAL FRACTURES IN A POPULATION-BASED STUDY IN SOUTHERN ITALY

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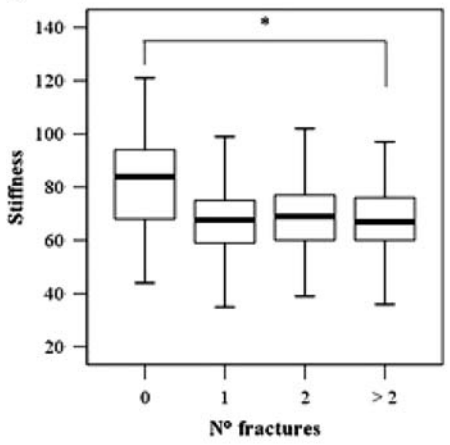
The aims of our study were to compare stiffness values to presence and number of vertebral fractures in a population of post-menopausal women which live in a Region of the Southern Italy (Campania). Methods. We examined 741 post-menopausal women, with mean age 64.4 years (range 40–86). 581 women had prevalent vertebral fractures. 160 women hadn't any vertebral fracture. We measured calcanear bone stiffness by ultrasound device (Achilles Express,GE). Results: The analysis of the data showed a significant reduction of stiffness in fractured women. Mean stiffness values in no fractured women was 82.06, while in women with at least 1 vertebral fracture it was about 68.56. The multi-variate non parametric analysis (Kruskal-Wallis test) showed that stiffness value is strongly related with the presence and number of vertebral fractures ($p < 0.001$). Also in the subgroups of patients aged under or over 65 yrs, or with a BMI higher or lower than 25, this association was confirmed. Conclusion. QUS may be an effective, acceptable, and useful tool for epidemiologic screening of osteoporotic patients. In our population-based study low calcaneal stiffness was strongly associated with the presence and number of vertebral fragility fractures.

Conflict of Interest: None declared

a



	Stiffness			
	0 Fx	1 Fx	2 Fx	>2 Fx
Mean	82.06	67.61	69.35	68.72
Median	84.00	67.50	69.00	67.00
Minimum	44.00	23.00	39.00	34.00
Maximum	121.00	112.00	102.00	118.00

A – Stiffness evaluation in 4 groups of patients, divided on the basis of the number of fractures. Stiffness decreases progressively from "0 Fx" to ">2 Fx" group (P<0.05).

B – The same observation was obtained when the population was divided on the basis of age or C – BMI (P<0.05).

b

Age < 65 years*

	Stiffness				P
	0 Fx	1 Fx	2 Fx	>2 Fx	
Mean	81.21	70.69	72.87	71.73	< 0.001
Median	81.50	70.00	71.00	72.50	
Minimum	44.00	35.00	50.00	47.00	
Maximum	121.00	112.00	99.00	97.00	

Age ≥ 65 years**

					P
	0 Fx	1 Fx	2 Fx	>2 Fx	
Mean	84.65	64.99	66.15	66.44	< 0.001
Median	88.50	64.50	64.00	66.00	
Minimum	47.00	23.00	39.00	34.00	
Maximum	117.00	99.00	102.00	118.00	

*N = 345; **N = 347

c

BMI < 25*

	Stiffness				P
	0 Fx	1 Fx	2 Fx	>2 Fx	
Mean	82.61	65.73	68.00	64.28	< 0.001
Median	83.00	66.50	68.00	64.00	
Minimum	44.00	35.00	51.00	47.00	
Maximum	120.00	95.00	91.00	82.00	

BMI ≥ 25**

					P
	0 Fx	1 Fx	2 Fx	>2 Fx	
Mean	81.75	68.26	69.67	69.77	< 0.001
Median	84.50	68.00	69.00	67.50	
Minimum	47.00	23.00	39.00	34.00	
Maximum	121.00	112.00	102.00	118.00	

*N = 180; **N = 514

Mo-P351

CALCIUM INTAKE AND VERTEBRAL FRACTURES IN A SOUTHERN ITALIAN POPULATION

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The aims of our study were to quantify nutritional calcium intake in a population of post-menopausal women which live in a Southern Italian region (Campania) and to verify the relationship between a lower nutritional calcium intake and vertebral fractures.

Methods. We examined 741 post-menopausal women, with mean age 64.4 years (range 40–86). 581 women had prevalent vertebral fractures. 160 women hadn't any vertebral fracture.

Results: The mean daily calcium dietary intake was 480,63 mg/die. Calcium intake was slightly higher in no fractured women (mean 502.88 mg/die), than in women with more than 1 vertebral fracture (mean 455.40 mg/die). The multi-variate non parametric analysis (Kruskal-Wallis test) showed that the daily calcium nutritional intake is related with the presence and number of vertebral fractures ($p < 0.05$). Dividing our sample population in two groups depending on their age (below and over 65 years old), the statistical significance remained only for the younger population group ($p = 0.03$).

Conclusion. In our post-menopausal women sample the daily calcium intake is much lower than 1000–1500 mg/day. Despite the typical cheese-based alimentary habits, calcium intake appears to be a risk for osteoporotic fragility fractures, suggesting that a calcium supplementation is always necessary.

Conflict of Interest: None declared

Mo-P352

BMD AND SERUM LEPTIN LEVEL IN TYPE II DM PATIENTS COMPARED WITH NORMAL GROUP IN POETMENOPAUSAL KOREAN WOMEN

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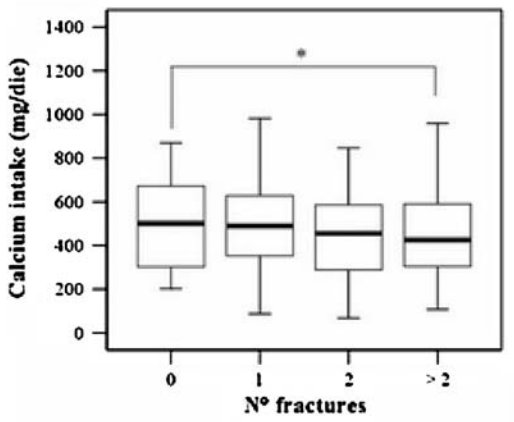
Objectives: Type II DM is associated with obesity, which is one of the factors that protect against the loss of bone mass. Concerning that Leptin is encoded by obesity gene, and produced and secreted by adipocytes, we designed the cross-sectional comparative study to evaluate BMD and serum leptin level between type II DM group and normal group.

Subjects and methods: 562 patients who visited one of the health care center in Seoul were classified to type II DM group($N = 206$) and normal group ($N = 256$). BMD and serum leptin level in type II DM group were evaluated compared to that of the normal group in poetmenopausal Korean women.

Results: Mean serum leptin level in type II DM group was 9.3 ± 2.1, while 8.0 ± 1.4 in normal group. Each of mean lumbar and femoral neck BMD was 0.917 ± 0.119, 0.493 ± 0.132 in type II DM group, while 0.951 ± 0.135, 0.527 ± 0.031 in normal group. However, there was no correlation between serum leptin level and BMD with statistical significance.

Conclusions: Our results suggested lower BMD and higher serum leptin were observed in type II DM patients. Comprared with Caucasian, Korean has lower body mass index in type II DM patients. However, further well-designed study evaluating leptin's effect on bone which might inhibit bone formation in Korean women will be needed.



	Calcium intake (mg/die)			
	0 Fx	1 Fx	2 Fx	>2 Fx
Mean	502.88	498.76	455.40	454.67
Median	500.71	490.00	455.00	425.71
Minimum	202.86	87.14	68.57	107.14
Maximum	868.57	1174.29	847.14	960.00

b Age < 65 years*

	Calcium intake (mg/die)				P
	0 Fx	1 Fx	2 Fx	>2 Fx	
Mean	502.50	518.52	457.12	435.56	0.03
Median	471.43	520.00	462.86	425.00	
Minimum	218.57	171.43	68.57	107.14	
Maximum	868.57	1044.29	847.14	808.57	

Age ≥ 65 years**

	0 Fx	1 Fx	2 Fx	>2 Fx	P
Mean	515.60	482.47	453.90	471.28	N.S.
Median	523.57	485.71	447.14	431.43	
Minimum	227.14	87.14	105.71	142.86	
Maximum	861.43	1174.29	795.71	960.00	

*N = 345; **N = 347

A – Calcium intake evaluation in 4 groups of patients, divided on the basis of the number of fractures. Calcium intake decreases progressively from "0 Fx" to ">2 Fx" group ($P<0.05$).
B- When the population was divided on the basis of age, this observation was conserved only for younger (<65 yrs) people

Table 1

	Lumbar , femoral BMD and leptins in each groups		
	Normal (N = 256)	Type II DM(N = 206)	P value
Age(years)	50.2 ± 1.3	51.3 ± 2.9	0.760
Weight(kg)	59.5 ± 7.2	63.2 ± 7.51	0.049
L-BMD(mg/cm2)	0.951 ± 0.135	0.917 ± 0.119	0.032
L-BMD(mg/cm2)	0.527 ± 0.031	0.493 ± 0.031	0.049
Leptin(nmol/dl)	8.0 ± 1.4	9.3 ± 2.1	0.043

Conflict of Interest: None declared

Mo-P353

POSSIBLE MECHANISM OF OUCH-OUCH DISEASE CAUSED BY CADMIUM EXPOSURE

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It is well known that exposure to environmental cadmium causes bone disease so-called itai-itai (ouch-ouch) disease. However, the exact mechanism of itai-itai disease caused by cadmium exposure remains unclear. From the viewpoint of the calcification mechanism, we conducted this study to clarify the biological effects of cadmium ions on the crystal formation with the possible mechanism of osteoporosis in mind. In the belief that the structural defect of crystals might take place if cadmium ions take part in the process of crystal formation, we examined the developing tooth enamel of rats that were fed water containing cadmium ions. Electron microscopy demonstrated the presence of crystal perforation in the developing tooth enamel of rats, indicating that the crystal nucleation process might be interrupted by cadmium ions. Furthermore, the enzymatic analyses revealed that the catalytic activity of carbonic anhydrase declined remarkably despite showing little quantitative reduction of this enzyme, suggesting that the reduction of catalytic activity might be due to the replacement of zinc with cadmium ions. From the present study, we conclude that cadmium-binding carbonic anhydrase could directly interrupt the nucleation process.

We greatly appreciate the valuable assistance provided by the members of LEBRA (Laboratory for Electron Beam Research and Application) at Nihon University. This study was supported in part by the Frontier Science Projects to LEBRA at Nihon University and subsidized by the Japanese Ministry of Education, Science, Sports and Culture (2000–2004, 2005–2007).

Conflict of Interest: None declared

Mo-P354

SERUM BETA-CTX LEVELS ARE RELATED TO LOW BONE MASS, SKELETAL FRACTURES AND OSTEOPOROSIS THERAPY IN POSTMENOPAUSAL WOMEN. PRELIMINARY DATA FROM THE FRODOS STUDY

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The FRODOS Study is a large population-based prospective study aimed at building a representative cohort of 2900 postmenopausal women from the Osona district (Catalonia, Spain) to analyse the prevalence, incidence and risk factors for fractures and bone loss. We present preliminary data on the relationship between bone turnover, antiosteoporotic therapy, bone mineral density (BMD) and prevalent fractures.

Patients and Methods: At present 1144 postmenopausal women aged 59–70 yrs (65.0 ± 3.6), years since menopause 15.1 ± 2.3, have been included. In all participants we measured at baseline BMD by DXA at the spine and hip, bone turnover markers (serum β-CTX) and clinical risk factors for osteoporosis. In addition, prevalent vertebral deformities compatible with vertebral fractures have been identified by X-ray lateral absorptiometry (MXA), with a fracture definition criterion of -3 SD at wedge and mid-wedge ratios. Reference range for β-CTX (0.305 ± 0,150 ng/ml) was obtained from a sample of 78 healthy pre-menopausal women aged 30–40 yrs (mean 34.5 ± 1.2).

Results: 17.6 % (n = 201) of women had prevalent skeletal fractures. 9.5% of them had vertebral fractures and 33.3 % had densitometric osteoporosis.

Two-hundred women (17.5%) were taking bone active drugs (BAD). Mean β-CTX values from the whole population was 0.408 ± 0.220. When β-CTX was analysed as a function of age (> or < 65 yrs), non-significant differences were found. Women taking BAD showed lower β-CTX values than those who were not (0.322 ± 0.217 vs 0.425 ± 0.216, $p < 0.001$). When participants taking BAD were excluded from the analysis, we observed that women with densitometric osteoporosis showed higher CTX values(0.507 ± 0.263*) than those with osteopenia (0.419 ± 0.196*) and with normal BMD (0.366 ± 0.189*) (*$p < 0.001$). Moreover, women with prevalent fractures showed higher β-CTX values than those without (0.446 ± 0.214 vs 0.414 ± 0.217, $p = 0.031$).

Conclusions: In this population-based cohort of unselected postmenopausal women, high β-CTX serum values are associated with low bone mass and prevalent fractures. In women taking bone active drugs lower β-CTX levels could help to assess therapy compliance.

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Conflict of Interest: None declared

Mo-P355

LIFESTYLE DETERMINANTS OF BONE MINERAL DENSITY IN HEALTHY INDIAN MEN

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Background: Alcohol intake and smoking have been implicated as risk factors for osteoporosis and related fracture. In India, the hip fracture incidence among men is higher. The pathophysiology of low mass is complex involving a broad spectrum of endogenous as well as environmental factors. Studies on the effect of environmental factors such as alcohol intake, smoking and exercise on BMD in men are inconsistent and the combined effect of these variables on BMD is yet to be explored. Inconsistent results may be due to differences with regards to source of subjects, control of confounding variables including the health status of individuals. The aim of the study was to investigate the influence of alcohol intake, smoking and exercise on BMD in a cohort of males with well-defined lifestyle conditions that minimizes the effect of confounding variables.

Materials and Methods: Men from armed forces (n = 330) having uniform and defined routines and diet were enrolled. BMD at spine and hip was measured by DXA. The subjects were classified according to their lifestyle variables. The association between the lifestyle variables and BMD were assessed by ANCOVA. Bonferroni multiple comparison test was applied to determine the post hoc significance between the various categories of alcohol intake compared with non-consumers. Multiple regression models were used to assess the independent influence of lifestyle variables on BMD.

Results: The subjects with 15000 or more of g-alcohol-years had significantly higher BMD at femur compared with non-alcohol consumers ($p < 0.05$) and a linear increase in mean femoral BMD over increasing categories of alcohol intake ($p < 0.05$) was observed. The relationship between measures of smoking and BMD was not significant ($r = 0.05$, $p = 0.43$). Exercise showed a positive correlation with femoral BMD ($r = 0.147$, $p = 0.02$). Furthermore, in the combined effect of alcohol consumption and exercise, routine exercisers in alcohol consumers group had 5% higher BMD after adjusting for age and BMI. In stepwise multiple regression analysis, age ($\beta \pm SE = -0.31 \pm 0.07$, $p < 0.0001$) and alcohol consumption ($\beta \pm SE = 0.235 \pm 0.06$, $p = 0.0002$) were independent predictors of femoral BMD. At the lumbar spine, age ($\beta \pm SE = -0.33 \pm 0.07$, $p < 0.0001$) and BMI ($\beta \pm SE = 0.16 \pm 0.07$, $p = 0.02$) were independent predictors.

Conclusions: In physically active men with well-defined lifestyle conditions, alcohol consumption was associated with higher BMD and exercise further improved the beneficial effect of alcohol.

Conflict of Interest: None declared

Mo-P356

INSULIN SECRETORY CAPACITY AND BONE MINERAL DENSITY IN TYPE 2 DIABETES MELLITUS

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Background: Hyperinsulinemia is associated with bone formation in type 2 diabetes. In type 2 diabetic patients, reduced insulin secretion and impaired insulin action may coexist in the same patients. Type 2 diabetic patients have lower endogeneous insulin levels as diabetes progress. We studied the relationships between ß-cell secretory capacity, bone turnover markers and bone mineral density (BMD) in type 2 diabetic patients.

Method: A total of 50 type 2 diabetes patients were consecutively enrolled. Dual-energy X-ray absorptiometry was performed to measure BMD in the lumbar spine, femoral neck, and total femur. ß-cell secretory capacity was assessed in all patients in two ways: fasting plasma C-peptide concentration, plasma C-peptide response to glucagons (glucagons stimulation test; GST).

Results: The mean age was 64.9 ± 9.8 years and the mean duration of diabetes was 15.5 ± 8.4 years. The mean $\pm$ SD concentrations of fasting C-peptide and glucagon stimulated C-peptide were 1.79 ± 1.12 and 3.14 ± 1.70 ng/ml, respectively. C-peptide concentrations were negative correlated with duration of diabetes (fasting C-peptide; $r = -0.21$, $p = 0.042$, glucagon stimulated C-peptide; $r = -0.415$, $p = 0.03$). But C-peptide concentrations were not associated with bone mineral density at any site or bone turnover markers, such as osteocalcin and ICTP.

Conclusions: Although type 2 diabetic patients have a diminished insulin secretion as diabetes progress, insulin secretory capacity in type 2 diabetic patients is not associated with bone mineral density and bone turnover markers.

Conflict of Interest: None declared

Mo-P357

LIFELONG DISTURBANCE OF CALCIUM HOMEOSTASIS IN TRPV5 KNOCK-OUT MICE HAVE PROFOUND EFFECTS ON BONE WITH AGEING

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Objective: Previous studies showed the transient receptor potential channel V5(TRPV5) to play a role in transcellular calcium transport in kidney and bone and maintenance of calcium homeostasis. Moreover young TRPV5 knockout(KO) mice were shown to have reduced bone thickness compared to wildtype(WT) littermates. *Ex vivo* bone marrow cultures showed increased osteoclast formation but with strongly reduced resorption activity.

Aim: to assess the effect of lifelong disturbance in calcium homeostasis on bone.

Methods: 10, 52 and 80 weeks old male WT and KO mice were studied for their bone phenotype by μCT, real-time PCR, and ability to form osteoclasts *ex vivo*.

Results: μCT analyses show that in femoral head trabecular thickness is significantly reduced compared to WT mice in 52 and 80 weeks old mice($p = 0.04$ and $p = 0.01$) but not in 10 weeks old mice. Other parameters were not significantly affected, though, endocortical volume was slightly higher in 80 but not in 10 and 52 weeks old KO compared to WT mice($p = 0.06$). In the diaphysis, cortical thickness and cortical volume were reduced in KO at all 3 ages. The difference in cortical volume between KO and WT was largest at 80 weeks of age. In 80 weeks old mice but not in 52 and 10 weeks old mice the endocortical volume was higher in the KO compared to WT($p = 0.006$). RNA expression of calcium transport related markers in femurs of these mice showed higher expression of the NCX1 gene in KO versus WT($p = 0.02$). The expression of the bone resorption osteoclast markers cathepsin K and tartrate-resistant acid phosphatase(TRAP) but not that of CLC7 and H^+-ATPase was higher in KO than in WT femurs($p = 0.006$ and $p = 0.03$). VDR expression in femurs and serum $1,25\alpha\text{-}(OH)_2D_3$ levels were higher in KO compared to WT($p = 0.02$ and $p = 4{,}0 \times 10^{-4}$). Serum $1,25\alpha\text{-}(OH)_2D_3$ levels in KO increased from 686 pmol/l at 10 weeks to 1306 pmol/l at 80 weeks whereas in WT it increased from 121 pmol/l at 10 weeks to 462 pmol/l at 80 weeks. Serum PTH and serum calcium levels did not differ between WT and KO. *Ex vivo* bone marrow cultures showed a higher number of TRAP positive osteoclasts in WT mice compared to KO($p = 0.02$).

Conclusion: The aging studies on TPRV5 knockout mice demonstrate that despite maintaining calcium homeostasis by increased $1,25\alpha\text{-}(OH)_2D_3$ levels a lifelong challenge of the calcium homeostasis affects bone mass and bone quality and bone cell function. These data stress the importance of optimal calcium balance for healthy bones with ageing.

Conflict of Interest: None declared

Mo-P358

RELATIONSHIPS BETWEEN LIPID METABOLISM AND BONE MINERAL DENSITY IN COMMUNITY-DWELLING ELDERLY

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Purpose: A glucose metabolism disorder such as diabetes can cause decreased bone mass and osteoporosis. Furthermore, decreased QOL and ADL, an extended time period for healing of bone fracture, and increased medical costs can also result. It is also considered that lipid metabolism may have a relationship with bone metabolism. However, the mechanism is not yet clear and further research is necessary.

The purpose of this study was to clarify bone strength and its relationship to lipid metabolism, which would contribute to basic guidelines to prevent bone fracture from osteoporosis. Subjects of this study were falls prevention program participants.

Subjects and method: From December 1997 until October 1997, 510 community dwelling elderly participated in fall preventions programs. Average age upon entering the program was 70 years, average weight 51.1 ± 8.4 kg, and average BMI 22.2 ± 3.4. Bone mineral density [BMD] was measured by using dual-energy X-ray absorptiometry [DXA] (QDR 2000, Hologic Co., Bedford, MA, USA). Based on results, subjects were divided into 3 groups: normal group, osteopenia group and osteoporosis group(according to guidelines of the Japanese Bone and Mineral Metabolism Society) by the YAM. Additionally, although the program mainly focused on exercise guidance, an electrocardiogram and joint function assessment by x-p were performed. Also, lipid metabolism was assessed by measurements of HDL cholesterol, HDL/LDL cholesterol ratio, and triglycerides.

Analysis of variance was performed for each of the 3 groups according to lipid metabolism. Multiple comparisons after the analysis corrected for the influence of gender and age. The statistical package SPSS (Ver.15.0) was used for statistical analysis with 5% as the level of significance.

Results: HDL cholesterol and the LDL/HDL cholesterol ratio were significantly associated with BMD in the osteoporosis group in comparison with each of the other 2 groups ($p < 0.05$). LDL cholesterol, which was higher than in the osteopenia group, did not differ significantly. In addition, HDL cholesterol had an equilateral correlation with BMD ($p < 0.05$), but LDL cholesterol had a negative correlation ($p < 0.05$).

Conclusion: There was a negative correlation between HDL cholesterol and BMD about lipid metabolism and the relationship was high for LDL/HDL cholesterol (Adami, 2004). It is necessary to establish that more specific lifestyle guidance for prevention of osteoporosis is needed.

Conflict of Interest: None declared

Mo-P359

THE ROLE OF FAS/FAS LIGAND SYSTEM IN ESTROGEN DEFICIENCY-INDUCED OSTEOPOROSIS

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Background/aims: Fas is a death receptor whose major function is mediation of T cell cytotoxicity and regulation of immune response. It is also ubiquitously expressed on various cell types where it is involved in regulation of differentiation and survival. Fas ligation induces apoptosis, but there is increasing evidence of non-apoptotic functions of Fas mediated by distinct signaling mechanisms dependent on the tissue type and other regulatory factors including cytokines, chemokines and growth factors. Fas is expressed on osteoblastic and osteoclastic cells, where it may, under specific circumstances, induce apoptosis. Fas may also inhibit osteoblast differentiation via caspase 8 dependent mechanism. It is hypothesized that estrogen deficiency upregulates Fas on osteoblasts, which may lead to increased osteoblast apoptosis and/or their decreased differentiation and contribute to bone loss.

The aim of this study was to estimate importance of the proposed pathogenic mechanism in vivo.

Methods: We first analyzed the expression of Fas gene four weeks after the ovariectomy in bones and bone cell cultures from wild-type mice, and confirmed increased Fas expression in total bone tissue and osteoblasts from ovariectomized compared to sham operated animals.

Results: Four weeks after ovariectomy in mice deficient for Fas gene (Fas −/−) we analyzed bone histomorphometric parameters and osteoblast and osteoclast differentiation in vitro. Bone volume was generally higher in Fas −/− mice than in wild-type controls, and significantly decreased after ovariectomy in wild-type mice, whereas it was unaltered in Fas −/− mice. Number of osteoclasts in vivo and osteoclastogenesis in vitro were increased after ovariectomy in wild-type mice, but unchanged in Fas −/− mice. Osteoblastogenesis in vitro was stimulated by ovariectomy in both mouse strains, and this effect was more pronounced in Fas −/− mice. Fas −/− osteoblasts expressed higher levels of osteoblast specific genes than the control osteoblasts. Osteoblast differentiation genes had similar expression patterns in sham operated and ovariectomized mice.

Conclusion: Our findings show that Fas/Fas ligand system may have an important role in the pathogenesis of postmenopausal osteoporosis. Modulation of its effects on bone cells may contribute to the development of new strategies for osteoporosis treatment.

Conflict of Interest: None declared

Mo-P360

EPIDEMIOLOGICAL SURVEY OF OSTEOPOROSIS IN PEOPLE FROM NAPLES: RISK FACTORS AND MORPHOMETRIC EVALUATION OF VERTEBRAL DEFORMITIES

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Goal of this study was to evaluate the impact of risk factors with the status of bone fragility and fragility fractures on 1300 women that underwent medical visit in our division ambulatory from January to December 2005. Furthermore, we have enrolled 348 patients for morphometric evaluation of vertebral deformities because of the presence of risk factors for fractures. A large array of risk factors was investigated: age, familiarity for fractures, calcium intake, sedentary life, early o surgical menopause, smoke addition, low BMI, concomitant pathologies and self-reported history of fractures. After the patient had undergone interview and a brief physical examination, QUS of the heel was performed, using the Achilles Express apparatus (GE-Lunar, Madison, USA).

The prevalence rate of osteoporosis (OP) was approximately 32%, while the rate of osteopenia was about 49% (women age 60.9 yrs SD 9.6). An association with fractures, sedentary life and low calcium intake was found for OP and osteopenia. Our results show an association between the duration of the fertility period and risk for OP in women who underwent surgical menopause (fertility period mean 30.2 yrs in OP women vs 35.5 yrs in normal women $p < 0.05$). A total of 348 women were studied with MorphoXpress based on previously scanned spine X ray; mean age in women was 66.6 years (SD 8.8). We detected at least one vertebral deformity in 67% of patients. The majority of patients have a thoracic deformity (90%), while 8% have a lumbar deformity and only 2% have both thoracic and lumbar deformities. Wedge deformities were the most frequent deformity and tend to cluster at the mid-thoracic (T7–T9) regions of the spine, and decline in frequency at thoracic-lumbar and lumbar vertebral levels. There was some evidence that the thoracic-lumbar (T12–L3) deformities occur less likely amongst individuals with more than two deformities ($p < 0.05$) vs. individual with one deformity. We are evaluating the impact of risk factors with the incidence of vertebral deformities, their spine localization, and their degree of deformities and presence of multiple deformities.

Conflict of Interest: None declared

Mo-P361

COMPARISON OF DXA AND QUS PARAMETERS OF UPPER EXTREMITIES IN HEMODIALYSIS PATIENTS

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Surgically created arteriovenosus fistulas are widely used for the access of hemodialysis in patients suffering from end-stage renal failure. An arteriovenosus fistula is an abnormal connection between an artery and vein. The fistula always provides a danger to the blood supply of the peripheral (bone) tissue.

Our study was initiated to evaluate whether there are differences between the two sides, depending on fistula, in densitometry values and quantitative ultrasound parameters (QUS) of the upper limb in hemodialysis patients.

Twelve women and 8 men were involved (range: 45–72 ys.). The bone mineral density (BMD) of the left and the right radius were measured by dual-energy X-ray absorpiometry (DXA, pDEXA, Norland) and peripheral quantitative computed tomography (pQCT, Stratec). We also performed quantitative ultrasound parameter at the phalanxes: amplitude dependent speed of sound (AdSOS), by Bone Profiler (IGEA).

There was no significant difference in the measurements of extremities with or without a fistula. The BMD levels were similar in both arms [pDEXA BMD(g/cm2): 0.674 vs 0.666, pQCT BMD (g/cm^3): 248.1 vs 246.2]. The AdSOS of the phalanxes of the right side did not differ from that of the left side, too [AdSOS(m/sec) 1811.7 vs 1801.0].

This result suggests that the changes of local circulation caused by the arteriovenosus fistula on the upper limb do not influence the bone mineral content of the radius and AdSOS result of phalanxes. Further data are currently being collected to increase the statistical power of the study.

Conflict of Interest: None declared

Mo-P362

HIP FRACTURE INCIDENCE AND MORTALITY IN QUEBEC- 1992 TO 2002

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Background: Osteoporotic hip fractures have a substantial impact on health care systems around the world. As populations age, the incidence of hip fractures is predicted to increase exponentially. However, recent reports have documented stabilization and even decreases in the rates of hip fractures in certain regions. Geographical variations in the incidence of fractures mandate the confirmation that trends observed in other populations are comparable in Quebec. The goal of this study was to ascertain the evolution of the incidence of hip fractures and mortality rates in men and women from 1996 to 2002.

Methods: We designed a population based, retrospective cohort study, using administrative databases and identified patients 65 years and older who have been discharged from hospital with a primary diagnosis of hip fracture between 1996 and 2002. We calculated age- and sex-specific hip fracture incidence rates and related-mortality rates for the province of Québec during the years 1996 to 2002.

Results: We identified 33,243 patients with hospital discharges for hip fractures. The age-adjusted annual rates of hip fractures decreased in women from 711 per 100,000 person-years in 1996 to 631 per 100,000 person-years ($p = 0.008$) in 2002; there was a similar trend in men from 302 per 100,000 person-years to 271 per 100,000 person-years ($p = 0.169$). There were 8,248 deaths within one year after the initial hip fracture amongst men and women for an overall age-adjusted mortality rate of 24 per 100 person-years in women and 37 per 100 person-years in men. The standardized mortality ratios were approximately 6 fold higher in women and 7 fold higher in men compared to age-and sex-matched Québec population for each calendar year.

Conclusions: We have documented hip fracture rate reduction between 1996 and 2002 in women. The cause of this decline is yet undefined. Mortality rates remain elevated and are higher in men compared to women. Nevertheless, inasmuch as hip fracture is a prevalent and serious disease, the projected significant increase in the aging of the population may counter the effect of this reduction.

Conflict of Interest: None declared

Mo-P363

SEXUAL DIMORPHISM FOR BONE MINERAL DENSITY AS A PROSPECTIVE RISK FACTOR FOR STROKE

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Background: A few recent studies suggest a relationship between risk factors for stroke and osteoporosis. In the present study we investigated whether bone mineral density was prospectively related to strokes.

Method: The material consisted of 6201 women (mean 56 yr, range 20–95) and 1812 men (mean 46 yr, range 20–92). Bone mineral density (BMD, g/cm^2) was measured by Dual Energy X-ray absorptiometry at the femoral neck and lumbar spine. Strokes incidents were registered and validated. Cholesterol, systolic and diastolic blood pressure, physical activity, and smoking were analyzed in a subgroup of the cohort consisting of 2186 women and 440 men.

Results: During a mean follow up time of 2128 days, 242 strokes occurred in 170 women and 72 men. In women, BMD of the femoral neck was lower at baseline in those 72 women (mean age 64 yr) who later sustained a stroke before 75 years of age compared to those 6031 women (mean 56 yr) who did not (0.85 g/cm^2 vs. 0.78 g/cm^2, $p < 0.001$). Using a survival analysis, these differences remained after adjustment for the influence of age, follow up time, and body mass index (Hazard Ratio = 1.49, $p = 0.008$). No significant associations between BMD and stroke were found in men. In the subgroup of 2186 women, BMD of the femoral neck was found to be related to levels of cholesterol, systolic and diastolic blood pressure, physical activity, and amount of walking each week ($p < 0.05$ for all). However all these associations disappeared when adjusting for the influence of age.

Conclusions: In summary we have found an independent prospective relationship between BMD of the femoral neck and later strokes in women. This relationship seems to be independent of traditional risk factors for strokes such as serum lipid levels, blood pressure, blood glucose levels, and physical activity.

Conflict of Interest: None declared

Mo-P364

FRACTURES AND OTHER RISK FACTORS FOR DEATH

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Background: The independent risk of death from different types of fractures, cardiovascular risk markers, diabetes, and life style factors has not been investigated.

Material: In this cohort study, a total of 16612 women (mean age 46.3 ± 9.3 yr) and 16013 men (mean age 46.5 ± 9.5 yr), were included. All subjects were included in the Västerbotten Intervention Programme (VIP), which is a health investigation that started in Västerbotten County, Northern Sweden, in 1985. At baseline, information about life style factors, medications, and disease, were collected using questionnaires. Blood pressure was measured, blood lipids were analyzed in a fasting state, and a 75 gram oral glucose tolerance test was performed.

Results: During a mean follow up time of 3556 days (range 1–6535 days) a total of 2698 validated fractures were recorded in the total cohort. During the follow up time 437 women and 656 men died. In survival analyses, prospective fractures were included together with obesity (body mass index ≥ 30), previous heart infarction, hypertension, hypercholesterolemia, diabetes, smoking habits, and physical activity at baseline. In women, independent risk factors of death were age (hazard ratio (HR) = 1.06, $p < 0.001$), diabetes (HR = 1.92, $p < 0.001$), previous heart infarction (HR = 2.64, $p = 0.03$), obesity (HR = 1.35, $p = 0.03$), and smoking (HR = 1.64, $p < 0.001$), while physical activity were related to an decreased risk of death (HR = 0.83, $p = 0.03$). In men, independent risk factors of death were age (HR = 1.08, $p < 0.001$), diabetes (HR = 1.58, $p = 0.002$), previous heart infarction (HR = 2.51, $p < 001$), hypertension (HR = 1.19, $p = 0.049$), and smoking (HR = 1.89, $p < 0.001$). Overall fractures were not related to an increased risk of death. However, in separate analysis, sustaining a hip fracture was related to an increased independent risk of death in women (HR = 2.60, $p = 0.008$), while sustaining a wrist fracture (HR = 0.57, $p = 0.04$), was related to a decreased risk of death.

Conclusion: In both men and women we show that smoking, diabetes, and a previous heart infarction increase the risk of death independently. However, after adjusting for these and other factors known to influence the risk of death, sustaining a hip fracture is related to an increased risk of death in women. In contrast, to sustain a wrist fracture decreased the risk of death.

Conflict of Interest: None declared

Mo-P365

ELECTRON MICROSCOPY ON THE CRYSTAL STRUCTURE IN VASCULAR CALCIFICATION

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In general, vascular calcification occurs under pathological conditions and consists of calcium phosphate mineral, known as hydroxyapatite. Recently, it has been proposed that the vascular calcification might be regulated by the manner similar to bone mineralization. Although the crystal type in vascular calcification is reported to be similar to that in bone, little information regarding the detailed structure of crystal is available. From the viewpoint of crystal structure, we conducted this study to clarify the type of crystal structure in vascular calcification and compare to that in bone. In this study, we investigated calcification in the femoral artery, using transmission electron microscope. Electron micrographs demonstrated that vascular calcification consisted of two types of apatite crystal as judged from the lattice image. One type has the central dark lines (CDLs), which represent the platy nuclei of apatite crystals in the ordinary hard tissues of vertebrates, while the other does not have lines. We also noted that the cell debris originated from degenerated cells seemed to play an active role in the crystal development. From these findings, we conclude that the mechanism of vascular calcification might employ two pathways for crystal formation. Furthermore, it is plausible to consider that degenerative process may give rise to the vascular calcification to prevent the necrotic rupture of the vessel. This study was supported in part by Grant-in-Aid from Saitama Pref. University Encouragement Fund.

Conflict of Interest: None declared

Mo-P366

INCIDENCE OF NONSURGICAL FRAGILITY FRACTURES. NATIONAL STUDY OF OSTEOPOROTIC FRACTURES IN OUTPATIENT TRAUMA CARE

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Evaluate the number of patients with fragility factures after low-energy trauma, their epidemiological characteristics and the care burden generated by these patients in outpatient trauma departments.

This prospective, observational, multicenter national study collected data for five months in 358 specialist centers on all osteoporotic fractures (wrist, proximal humerus, vertebra or rib) treated for a period of 30 consecutive days in postmenopausal women aged $\geq$50 years. Fractures were classified as osteoporotic if they were due to low-energy trauma, such as a fall from a standing height. Pathological fractures and those requiring surgery were excluded. The incidence of the different fragility fractures was calculated

5752 women with osteoporotic fractures were recruited and 5147 (90%) met all inclusion criteria and were included in the study. The mean age was 72.6 years. A total of 5317 fractures was recorded. Distal radius fractures were the most common fracture type (2364 fractures, 46%) followed by vertebral fractures (1812 fractures, 35%) and 919 proximal humerus fractures, which accounted for 18% of all fragility fractures. The highest number of fragility fractures occurred between 61 and 74 years of age, with a peak at age 70, except for proximal humerus fractures, which were the only fractures that increased in patients over 79 years of age (249 fractures, 25.5%). Distal radius fracture was the most common fracture type in all age ranges. Osteoporotic fractures have a high prevalence in trauma outpatient clinics; 34.2% of patients having a fracture were diagnosed with an osteoporotic fracture, and the mean number of patients with osteoporotic fracture treated per month on an outpatient basis was 26.5

Fragility fractures are a major health problem due to their high incidence and impact on quality of life in the population aged 50 years and older. Nonsurgical osteoporotic fractures have a high prevalence in trauma outpatient clinics and create a significant health care burden. Distal radius fracture was the most common fracture type in all age ranges

Conflict of Interest: F.E.Osorio-Picone is MSD full-time employee

Mo-P367

THE PREVALENCE OF SECONDARY HYPERPARATHYROIDISM AMONG POSTMENOPAUSAL WOMEN SUFFERING FROM END-STAGE KNEE OSTEOARTHRITIS SCHEDULED TO UNDERGO TOTAL KNEE ARTHROPLASTY. SHOULD WE CARE ABOUT IT?

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Introduction: Parathyroid hormone (PTH) seems to play a crucial role in the orthopaedic implants' incorporation/fixation procedures. Aim of this prospective study was the evaluation of the prevalence of Secondary Hyperparathyroidism (SH) among postmenopausal women suffering from end-stage Knee Osteoarthritis scheduled to undergo Total Knee Replacement (TKA), as continuously elevated levels of PTH may potentially play a negative role in the implant's incorporation process.

Methods: During a period of 29 months, 281 women were enrolled. The serum levels of Intact-PTH (I-PTH), Calcium, Phosphorus and Creatinine were evaluated and the clearance of creatinine was calculated. Patients suffering from any endocrine disorder, rheumatoid or other secondary arthritis, any disease interfering with bone homeostasis or receiving medication affecting bone metabolism, were excluded from the study. None had suffered a fracture or underwent any orthopaedic surgical operation during the 36 months prior to enrollment.

Results: The patients' mean age was 70.02 years (range:49–81). The years that had passed since their menopause ranged from 7 to 31 (mean of 18.8 years). Ninety-eight patients (34.9%) were suffering from SH; two from Primary Hyperparathyroidism. Statistically significant positive correlation was found between I-PTH and age ($r = 0.158$, $p = 0.008$) and between I-PTH and creatinine ($r = 0.138$ $p = 0.021$). A statistically significant negative correlation was found between I-PTH and creatinine clearance ($r = -0.169$, $p = 0.004$). Multiple regression analysis estimated how well a set of variables (age, weight, creatinine, creatinine clearance) could predict the value of I-PTH. This model explained 7.3% of the variance in I-PTH values (R square $= 0.073$, $p = 0.000$) reaching statistical significance. Creatinine was the strongest unique significant contributor (Beta value $= 0.275$, $p = 0.008$) to the this model.

Discussion/Conclusion: SH appears to be a 'silent' epidemic among elderly postmenopausal women. Regardless of its actual cause, the negative impact of continuously elevated PTH on bone formation, may well interfere with an implant's incorporation procedure. Given the fact that the risk of prosthetic loosening seems to be determined during the first post-operative months it is not certain how well an implant's incorporation process may advance in patients with elevated PTH levels, hence the pre-operative evaluation of I-PTH in patients undergoing TKA is strongly recommended.

Conflict of Interest: None declared

Mo-P368

ASSOCIATION BETWEEN BONE MINERAL DENSITY AND LOWER-LIMB FUNCTION IN OLDER ADULTS: CROSS-SECTIONAL DATA FROM THE JAPANESE FALL PREVENTION PROGRAM FOR SENIORS

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In 1997, Japanese Fall Prevention Programs for Seniors was established to prevent osteoporosis, and fall risks and related fractures in community-dwelling older adults. We examined the association of osteoporosis and the different aspects of lower-limb physical function (Komatsu et. al. 2006, Park et. al. 2008). Five hundred and twenty-three older adults (average age 71.2 ± 4.35 y, body mass 52.3 ± 8.1 kg, body mass index 22.2 ± 3.1 kg/m^2, and serum albumin level 4.2 ± 0.3) participated in the Fall Prevention Program between December, 1997 and September, 2007. The bone mineral density (BMD) of the femoral neck was measured by dual-energy X-ray absorptiometry using the Hologic Inc. QDR2000. The World Health Organization criteria for normal (n = 140, 26.8%), osteopenia (n = 220, 46.1%), osteoporosis (n = 128, 24.5) severe osteoporosis (n = 35, 6.7%) were used. For the linear relationship, we calculated the partial correlation coefficients. Using analyses of covariance, we compared BMD measurements and lower limb physical function such as maximal walking speed, maximal step length and one-legged stand time while controlling for sex, age, BMI and menstrual status, and we performed an multivariable-adjusted logistic regression analysis to determine odds ratios and their 95% confidence intervals in order to assess association between the risk of osteoporosis and the lower-limb physical function. A significant correlation was observed between the BMD at the site of femoral neck and the maximal walking speed ($r = 0.32$, $P = 0.03$), and maximal step length ($r = 0.26$, $P = 0.04$). Subjects who are in the lowest quartile of the maximal walking speed were 1.99 folds more at risk of osteoporosis than the highest quartile (Table 1). Older adults with osteoporosis had slower maximal walking speed, even after multivariate adjustment including age, gender, nutritional status and other potential confounding variables. Therefore, we concluded that walking speed is an important risk factor in determining fall and osteoporosis-related fracture risk (Kanis 2005 and Ensrud KE.2007).

Table 1 Multi-adjusted odds ratio for risk of osteoporosis

Gait speed (m/s)	Odds ratio	95% confidence interval
<1.56	1.99	1.32–2.58
1.59–1.76	1.47	1.03–1.96
1.80–1.96	1.04	0.33–1.89
>1.97	1 (reference)	

Conflict of Interest: None declared

Mo-P369

EFFECT OF BISPHOSPHONATE TREATMENT ON COLLAGEN CROSS-LINKS IN TRANSGENIC MICE OVEREXPRESSING RUNX2

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Transgenic mice overexpressing Runx2 in osteoblasts exhibit increased bone resorption and remodeling that leads to osteopenia and spontaneous vertebral fractures after 1 mo of age. The primary objective of this study was to determine whether the bisphosphonates risedronate (RIS) and alendronate (ALN) had an anti-fracture effect in this mouse model of bone loss and fracture. In addition, we examined bisphosphonate effects on several components of bone material quality. In this analysis we determined the effect of alendronate and risedronate on the organic matrix aspect of bone material quality. Specifically, changes in two of the major bone type I collagen cross-links, pyridinoline (Pyr) and dehydro-dihydroxylysinonorleucine (deH-DHLNL), were determined by Fourier Transform Infrared Imaging analysis as a function of trabecular surface metabolic activity. Five-wk old female Runx2 mice were randomized to receive ALN (10 ug/kg 2 × week subcutaneously; n = 11) or RIS (10 ug/kg 2 × week subcutaneously; n = 15) for 12 weeks. Runx2 (TR, n = 7) and matched wild type (WT, n = 8) mice received vehicle as controls. Fourier transform infrared imaging was used to determine the ratio of non reducible Pyr to reducible deH-DHLNL (Pyr/deH-DHLNL) collagen cross-links in 2-to-4-μm-thick sections from vertebrae. Samples were measured for the ratio of the relative intensities of the peaks at 1660 and 1690 cm^{-1} for Pyr/deH-DHLNL in forming and resorbing trabecular bone areas. In bone formation areas, TR mice had a decrease in Pyr/deH-DHLNL compared to the WT controls. This observation is consistent with the accelerated bone turnover in these transgenic mice, with the tissue having less time to mature. Treatment with ALN and RIS restored the Pyr/deH-DHLNL ratio to WT control values. There was no significant difference observed between the two bisphosphonates. In bone resorption areas, there were no significant differences in Pyr/deH-DHLNL between WT, TR and ALN-treated mice. RIS-treated mice however had a significantly higher Pyr/deH-DHLNL ratio compared to TR controls and ALN-treated mice. In mice overexpressing Runx2, there was delayed maturation of the collagen (due to a high remodeling), which was prevented by the bisphosphonates ALN and RIS. At the dose levels used, RIS had a slightly larger effect than ALN at bone resorbing areas. Whether this difference contributes to the greater anti-fracture effect seen with RIS in this animal model remains to be determined.

Conflict of Interest: Dr Paschalis has received research grants from Procter & Gamble Pharmaceuticals

Mo-P370

DIET-INDUCED HYPERINSULINAEMIA NEGATIVELY AFFECTS BONE IN MALE C57/BL MICE

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Background/Aims: Although diabetes mellitus type 2 and osteoporosis often coincide, it is unclear how and at which time point and pace the diseases mutually influence each other. The aim of this study was to assess bone metabolisms and bone quality in a mouse model of diet-induced obesity and hyperinsulinaemia. Methods: At 7 weeks of age, 21 male C57/BL mice were randomized into 3 diet-groups: low fat LF (normal chow for 24 weeks), high fat HF (high fat chow for 24 weeks) and short term high fat ST (LF for 20 weeks then HF for 3 weeks). In the HF diet, 60% of total calorie intake was based on lipids. At week 31, mice were sacrificed and fasting glucose, insulin, CRP, IL-6 and CTX were measured. Lumbar bone density was assessed by conventional DXA. Results: Body weight was significantly different between all groups with HF being the most obese (LF 33,2 g +/−1,0; HF 50,7 g +/−0,7; ST 41,2 g +/− 1,5). Significant hyperinsulinaemia was detectable in HF and ST (LF 5,0 μU/ml +/−1,4; HF 39,6 μU/ml +/−14,1; ST 15,2 μU/ml +/−4,0), whereas blood glucose, CRP and IL-6 levels did not differ between the groups. DXA scans revealed significantly lower bone density in HF and ST animals (LF 0,103 g/cm^2 +/−0,006; HF 0,075 g/cm^2 +/− 0,002; ST 0,076 g/cm^2 +/− 0,002). CTX were consistent with spinal bone loss and displayed significantly lower values in HF and ST (LF 26,1 ng/ml +/−2,1; HF 31,1 ng/ml +/− 2,7; ST 31,8 ng/ml +/−1,9). Expressing the negative effect of obesity and hyperinsulinaemia/type 2 diabetes on murine bone health, lumbar bone density was negatively correlated with insulin levels, body weight and the resorption parameter CTX ($r = -0{,}494$; $r = -0{,}603$; $r = -0{,}583$). Reaffirming the study's coherence, insulin levels were positively associated with CTX ($r = 0{,}635$). Conclusion: High fat diet-induced adiposity and hyperinsulinaemia negatively affect bone density in male C57/BL mice. Moreover—in our study—poor metabolic control increases bone resorption as measured by plasma CTX. Interestingly, short term high fat chow leads to similar bone loss as long term HF. In contrast to reports on normal or even high bone mass in humans with type 2 diabetes, our experiment reveals a catabolic bone state in male obese, hyperinsulinaemic C57/BL mice.

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Conflict of Interest: None declared

Tu-P371

RISK FACTORS FOR COMPLEX REGIONAL PAIN SYNDROME TYPE I AFTER WRIST FRACTURES

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Aims: Assessment of the occurrence of Complex Regional Pain Syndrome (CRPS) type I in patients with wrist fracture and identification of possible risk factors for this nosological entity.

Methods: A retrospective analysis of the 52 cases addressed to the Rehabilitation Department after wrist fracture during one year. For all patients the subsequent data were analyzed: demographic factors (age, gender, profession, socio-economic and educational backgrounds), fracture type, initial treatment (surgical / orthopedic), immobilization period, rehabilitation treatment, associated diseases (especially osteoporosis and osteoporotic fractures), occurrence of CRPS or other complications. The eventual emotional disturbance was estimated by Depression Anxiety Stress Scale (DASS 42). The SPSS programme was used for statistical analysis.

Results: CRPS type I occurred in 38 patients (73%). An immobilization period longer than 42 days (recommended especially in comminuted, unstable, non-operable fractures) was always associated with CRPS type I ($p < 0.01$). The presence of anxiety or depression was significantly associated with CRPS occurrence ($p < 0.05$). A lower socio-economic level was associated with a higher rate of CRPS development, although no statistical significance was met ($p > 0.05$). Osteoporosis, osteoporotic fractures and precocious menopause were significantly predisposing to CRPS ($p < 0.05$).

Conclusions: Special attention must be paid to patients with unstable, respectively prolonged immobilization requiring fractures, with osteoporosis or associated conditions, with emotional disturbance or poor socio-economic level. In these categories a closer, careful monitorisation is necessary, for early detection of CRPS and its adequate treatment.

Conflict of Interest: None declared

Tu-P372

INFLUENCE OF ORCHECTOMY ON BONE MINERAL DENSITY IN MALE RATS OF REPRODUCTIVE AGE

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Aim: The aim of the present study is to evaluate the influence of orchectomy on bone mineral density and bone mineral content in male rats of reproductive age.

Research Object: There were inspected 16 male rats of reproductive age, "Vistar" line, under vivarium conditions of Institute of Gerontology. 10 rats (mass−0,18 ± 0,005 kg) made up a control group (CG); 8 animals of experimental group (mass−0,20 ± 0,006 kg) have undergone orchectomy (ORC).

Research Methods: Bone mineral density (BMD) and bone mineral content (BMC) were measured using dual energy X-ray densitometry (DEXA) and «Experimental animals» software. Examination was made before orchectomy and over 30 days after operation. Increase was determined in % of bone mineral density and bone mineral content of the entire body. The index was calculated according to the formula: Delta BMD(%) = (DeltaBMD/BMD ref.) × 100

Research Results: Comparative dynamics indexes of bone mineral density and bone mineral content in male rats of control group and group after orchectomy is presented in Table 1.

Conclusions: The orchectomy leads to a substantial decrease of bone mineral density and bone mineral content in male rats of reproductive age, allowing this method to be used for creation of experimental model of osteoporosis.

Annotation: M ± m; CG - animals of control group; ORC-animals with orchectomy; BMD ref. - initial indexes of bone mineral density of the entire body; BMC - initial indexes of bone mineral content of the entire body; F - Fisher index.

Table 1 Dynamic of BMD and BMC in male rats of reproductive age

Group	BMD ref.	Δ BMD	BMD (%)	BMD ref.	Δ BMC	Δ BMC(%)
CG	0,10 ± 0,00	0,02 ± 0,01	19,33 ± 9.8	9,65 ± 0,3	2,44 ± 0,3	25,88 ± 3.5
ORC	0,11 ± 0,00	−0,003 ± ,0	−2,87 ± 2,4	11,62 ± 0,3	−0,30 ± 0,3	−2,41 ± 2,6
F	5,84	3,89	4,01	7,12	32,52	26,7
P	0,015	0,047	0,041	0,009	<0,00001	<0,00001

M ± m;

Conflict of Interest: None declared

Tu-P373

A COHORT STUDY OF THE EFFECTS OF PROTEIN INTAKE ON BODY COMPOSITION

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In a previous study in elderly women we showed a beneficial effects of protein above current recommended levels on bone structure one year later. This prospective cohort study examined the effect of dietary protein intake at baseline on lean body mass and bone density 5 yrs later.

862 ambulant female patients aged 75 ± 3 y were recruited from the population for a longitudinal study health in the elderly. Protein intake was assessed at baseline by a validated food frequency questionnaire and body composition measured by whole body DXA (Hologic QDR 4500) at 5 yrs.

The baseline protein intake was 81 ± 28 g per day (1.22 ± 0.45 g/kg body weight per day) contributing 19 ± 3% of total energy intake, the baseline BMI was 26.8 ± 4.4 kg/m^2. At 5 yrs the bone-free lean body mass was 36.4 ± 4.7 kg, and whole body BMD (minus head) was 844 ± 87 mg/cm^2.

When divided into tertiles of protein intake baseline intake was positively associated with bone-free lean body mass and BMD before (Table 1) and after adjustment for age and body size.

Protein intake over 86 g/day compared to protein intake under 66 g/day had a long term beneficial effect on muscle mass of 5–6% and bone structure of about 2–3 %.

These epidemiological data suggest substantial benefits on muscle and bone mass from a high protein intake which may translate into increased health benefits in terms of fracture and falls reduction.

Table 1

	Effect of protein intake on muscle and bone at 5 years		
	1st tertile Protein < 66 g/d	2nd tertile Protein 66–87 g/d	3rd tertile Protein >87 g/d
DXA lean mass (kg)			
Whole body	35.55 ± 4.47	36.25 ± 4.48	37.42 ± 4.85*‡
Legs	11.28 ± 1.82	11.56 ± 1.81	11.98 ± 1.95*‡
DXA BMD (mg/cm2)			
Whole body	833 ± 82	842 ± 86	856 ± 90*
Legs	987 ± 107	1001 ± 114	1014 ± 117*

* p< 0 .05 cf 1st tertile ‡ < 0 .05 cf 2nd tertile

Conflict of Interest: None declared

Tu-P374

THERE IS A SECULAR DECREASE IN BMD BUT NO CORRESPONDING INCREASE IN AGE-ADJUSTED HIP FRACTURE INCIDENCE IN SWEDISH MEN DURING THE LAST DECADE

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Introduction: Osteoporosis, with hip fractures, represents a major public health problem. Although the incidence of hip fractures during the last decades has been inferred to increase, this view has recently been opposed. There exist to our knowledge no reports that evaluate if this decrease depends on secular changes in bone mineral density (BMD) or other factors.

Material and Methods: This prospective study evaluated secular changes in BMD (mg/cm^2) in one population based sample of urban and one sample of rural men aged 60 to 80 years in 1988/1989 (n = 202 vs. 121) and in 1998/1999 (n = 79 vs. 69). BMD was measured at the distal radius of the dominant side by single-photon absorptiometry (SPA). All hip fractures between 1987 and 2002 were

registered in the same urban (n = 25491) and rural populations (n = 16432) of men aged 60 years or above. The age adjusted hip fracture incidence was calculated by use of national demographic data by direct standardisation with the population 1987/1988 as the standard population. Time-trend analysis was done by linear regression. Age adjusted BMD was calculated by analysis of covariance (ANCOVA) when comparing men measured 1988/1989 with the men measured 1998/1999.

Result: There was a secular decrease in BMD during the study period when evaluating all men ($p < 0.05$), a non significant decrease in the urban men ($p = 0.08$) and the rural men ($p = 0.11$). The age-adjusted hip fracture incidence was unchanged when including all men ($p = 0.99$), urban men ($p = 0.65$) and rural men (0.57).

Conclusion: In spite of finding a secular decrease in BMD over the last decade we did not find any changes in the age-adjusted hip fracture incidence. This implies that the sum effect of other risk factors apart from BMD has undergone secular changes counteracting the decrease in BMD. We could not in our setting verify the by others reported decrease in age-adjusted hip fracture incidence.

Conflict of Interest: None declared

Tu-P375

THE EARLY DEVELOPMENT OF OSTEOPATHIES IN OFFSPRING FROM MOTHERS STRESSED IN PREGNANCY

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Objective. The impact of fetal exposure to increased levels of maternal or exogenic glucocorticoids on the offspring birth weight and the development of numerous diseases in adulthood have been demonstrated. The bone pathology manifestations throughout the life of the experimental animals at the embryonal stage are worth being studied.

The aim of this study was to determine the bone formation peculiarities in utero stressed offspring (Gr-OS) and the influence of stress-developed osteopathies on their future life.

Material and Methods. The object of investigation—the offspring bone tissue and hormonal status after mothers with social stress in early pregnancy. We have studied the 3–12 month animals (n = 72) in pre- and post-immobilization states. Hormonal concentrations were determined by IFA methods. The histological indications of the hip bone tissue were performed using a microscope "Olimpus" with a computer analysis of microstructure and statistical values.

Results. It has been established that HPA axis activation in Gr-OS was higher than that in a control group ($P < 0.05$) during the whole examined periods. Levels of sexual hormones, calcitonine, alkaline, phosphates, osteocalcine decreased; levels of T3, insulin increased in stressed offspring. After immobilization the Gr-OS corticosterone level showed an increase of 20%, but the sex hormone degraded of 25–40 %. The histological indications revealed that the process of bone formation from cartilage in Gr-OS of the young animals has been reduced; the age dynamics of the bone mass growth degraded; starting from the age of 8–10 months the processes of bone resorption (particularly, in spongy substance) prevailed over the bone formation in Gr-OS as well as symptomised by osteoporotic loci in hip diaphysis after immobilization.

Conclusion. The findings confirmed that the stress in utero resulted in the disturbances in hormonal regulation of bone formation and early osteopathies in the postnatal life.

Conflict of Interest: None declared

Tu-P376

BONE MINERAL DENSITY, BONE TURNOVER MARKERS IN TYPE 1 DIABETIC MEN

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Background/Aims: Although osteopenia is reported as a complication of type 1 diabetes mellitus (DM), its frequency and severity remain unclear. Studies of bone mineral density (BMD), bone turnover markers in type 1 diabetic men have yielded conflicting results.

Materials and Methods: In the study were included 78 men with type 1 DM (mean age 36,32 + 3,52 yrs, duration of disease 11,25 + 3,42 yrs, HbA1c 8,6 + 1,12 %). BMD was measured by DXA (L2–L4, femoural neck), markers of bone formation (serum alkaline phosphatase (ALP), serum N-MID osteocalcin (OC) and bone resorption (cross-linked C-telopeptide (CTX) were measured in the diabetics and in 28 healthy matched controls.

Results: Osteopenia was revealed in 28 % of the diabetic men (Z-score: −1,6 + 0,42 vs −1,22 + 0,62 in controls), predominantly in the femour neck (22%). The levels of serum ALP (92,22 + 39,45 IU/l) and osteocalcin (17 + 6,65 ng/ml) were statisticly ($p < 0,05$) lower in the patients with osteopenia than in those without osteopenia (115,32 + 25,16 U/l and 28 + 5,9 ng/ml accordingly), that suggested lowering bone formation. In the patients with osteopenia mean CTX (521,76 + 42,56 pg/ml) was statistically higher ($p < 0,01$) than in those without osteopenia (420,76 + 36,15 pg/ml). There were no differences in the mean levels of HbA1c between the diabetic patients with and without osteopenia (8,25 + 0,97% vs 7,99 + 1,15%).

Conclusions: The data confirm the high prevalence of osteopenia in type 1 diabetic men and demonstrate that in type 1 DM men, osteopenia is the consequence of a lowered bone formation with a predominance of bone resorption over formation.

Conflict of Interest: None declared

Tu-P377

ON THE THRESHOLD OF OSTEOPOROSIS EPIDEMY: FOOD CALCIUM CONSUMPTION AMONG MOSCOW STUDENTS

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Background/Aim: By the beginning of XXI century the problem of osteoporosis became one of leading and now it ranks 4th place in the rating of diseases with maximum medico-social importance—right after cardiovascular, oncological diseases and diabetes mellitus. Among the abundance of risk factors alimentary calcium consumption (ACC) is one of the most critical because it refers to potentially modifiable. Considering changes in nutritional behavior of Russians in recent years the major aim of this research became examination of ACC among Moscow youth. Materials and methods: in random cohort of students of Sechenov Moscow Medical Academy (n = 220, 63 boys and 157 girls at age of 18–24 years old) there was made a cross-sectional research aimed to calculate ACC. Data acquisition was made in October 2007 using method of formalized interview (ACC questionnaire). Statistical analysis was performed using special electronic software provided by "Nycomed" (Norway). Results: according to the optimal ACC for this

age group recommended by the international experts (1200–1500 mg/day; National Institute of Health, 1994) there was determined substantial fall of ACC in young Moscow population (median 398 mg/day with limits from near 0 to 2177) and mean ACC was 29% of normal. Moreover we reported food calcium deficit with ACC lower than physiological requirement in absolute majority of respondents (90%). Most respondents had a severe deficiency (ACC within 0–400 mg/day in 50%, 400–800 in 28%, 800–1200 in 12%). It follows that total calcium debt of young organism at the age of peak bone mass accumulation attains 88%, 55% and 31% respectively against the normal intake (median 1350 mg/day). In whole, girls had worst ACC that is 31% lower that in boys (median 366 mg/day versus 527, $p = 0.0014$, test based on signs). Conclusion: preliminary data about current insufficiency of dietary consumption of calcium (the major mineral component of calcified tissues) was obtained in young Moscowites at first. This information gives a reason to predict various bone pathology, including rise of postmenopausal osteoporosis, within Russian population in future decades.

Conflict of Interest: None declared

Tu-P378

VITAMIN D STATUS AND BONE DESITY IN VEGETARIANS AND NONVEGETARIANS

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Background: Nutrition is an important component of bone health. The value of nutrients and vitamins such as calcium and vitamin D is well documented. Intake of other nutrients, such as proteins, remains controversial. Different nutrition with different sources of calcium, vitamins (especially vitamin D) and protein may have different effects on bone metabolism and bone density. Low concentrations of serum 25(OH) vitamin D are associated with high parathormone (PTH) concentration and low bone mass density (BMD).

The aim of the study was to investigate the relationship between nutrition, vitamin D status and bone density in healthy, pre- and postmenopausal vegetarians and nonvegetarians with similar background characteristics.

Methods: 273 women (old (O):113 in age 60–70 y; young (Y):160 in age 20–30 y) were enrolled into the study. Both groups were divided to the next 2 subgroups according to the food intake (vegetarian and nonvegetarian). Serum 25(OH) vitamin D, PTH, Ca, iCa, P, albumin, total proteins, cholesterol, LDL-C, HDL-C and TAG were evaluated in all groups. In all probands BMD was examined.

Results: There were no significant differences in any serum parameters between vegetarians and nonvegetarians in group O as well as in group Y. Only 14 % of probands had 25(OH) vitamin D levels in normal range (>30 ng/ml), 52% in mild insufficiency (15–30 ng/ml) and 34% were in 25(OH) vitamin D deficiency (< 15 ng/ml). Nonvegetarians had significantly higher concentration of 25(OH) vitamin D than vegetarians, but the concentration was still in deficiency range.

BMD was significantly decreased in old group independently on food intake if compared with young group. The old vegetarians had significantly lower total proximal femur BMD ($p < 0,01$) and trochanter BMD ($p < 0,01$) in comparison with old nonvegetarians. Significant differences in BMI, % of body fat and lipid parameters were detected between old and young groups.

Conclusion: There was high prevalence of 25(OH) vitamin D deficiency in studied cohort of women. Bone density was significantly decreased in old group comparing with young group. The differences between old and young probands are not affected by different nutrition intake, however vegetarian nutrition could have negative impact in older age.

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Conflict of Interest: None declared

Tu-P379

INCIDENCE OF KNEE INJURIES AND CORRELATION IN CLINICAL PATHOLOGY

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Incidence of knee injuries and correlation in clinical pathology Purpose: The aim of the present study was to investigate knee joint injury in young adult with age range from 20 to 30 years old.

Methods: A retrospective analysis to investigate knee joint injury in young soldiers with age range from 20 to 30 years old. During a period of 32 months, 3726 orthopedic hospitalized patients. A total of 973 patients with knee joint was conducted.

Results: Here we addressed that the cruciate ligament disruption, associated with or without other joint structure injuries, was the most common type of the knee joint disease and high prevalence to induce chondromalacia patellae as well. All patients with the anterior cruciate ligament tear showed different types of physical insult to the knee joints. Other important issues such as the amount of exercise, injured physical factors, or even the long term follow up were then evoked and may be identified in the near future in the young adulthood.

Conclusions: In conclusion, the general prevalence of the knee joint diseases is established. Further biochemical investigation by the animal tissues and also the human specimens would be followed. It is suggested that knee injury prevention such as knee brace utilization and warm-up before training, rehabilitation after knee injuries, regular exercise, maintaining body weight, and a changed locomotion pattern all may prevent osteoarthritis initiation and progression in young adults.

Conflict of Interest: None declared

Tu-P380

THE CORRELATION OF SERUM HOMOCYSTEINE CONCENTRATION AND BONE MINERAL DENSITY IN THE PERIMENOPAUSAL AND POSTMENOPAUSAL KOREAN WOMEN

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Background: Interests in serum homocysteine concentration and BMD are currently on the increase. Hence the objective of this research was to find out the relevance of serum homocysteine concentration and BMD using perimenopausal and postmenopausal Korean women.

Method: In total of 109 perimenopausal and postmenopausal female participants were recruited who visited the department of

family medicine at certain University Hospital from August 2006 to January 2007. The blood sampling, lumbar and femur neck BMD, smoking, drinking, caffeine and milk consumption of the participants were investigated. The participants were divided into three groups according to their state of BMD(osteoporosis, osteopenia, normal), and their differences between three groups through ANOVA or Fisher's exact test were analyzed. The multiple regression analysis was used to analyze factors affecting to serum homocysteine. And the correlation analysis was performed to find out the relationship between the serum homocysteine concentration and BMD adjusted by factors affecting to homocysteine in the multiple regression analysis result.

Result: In BMD, the three groups exhibited some differences ($P < 0.05$) in terms of age and milk consumption but serum homocysteine was no difference between three groups. Serum homocysteine is associated ($P < 0.05$) with serum glucose, serum creatinine, HDL cholesterol, femur neck BMD each independently. Although a correlation relationship was established ($\beta = -0.25$, $P = 0.01$) between the femur neck BMD and homocysteine adjusted by serum glucose, serum creatinine and HDL cholesterol, there was no such relationship present ($\beta = 0.027$, $P = 0.786$) between the lumber BMD and homocysteine.

Conclusion: Increasing serum homocysteine in perimenopausal and postmenopausal women is associated with the reduction of the femur neck BMD. Hence the increase in the serum homocysteine can be considered to be one of the risk factors of osteoporosis.

Conflict of Interest: None declared

Tu-P381

KNEE INJURY AND OSTEOARTHRITIS OUTCOME SCORE (KOOS) AND CARTILAGE BIOMARKERS IN MIDDLE-AGED WOMEN WITH EARLY OSTEOARTHRITIS

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There is evidence that intensive degradation and synthesis in joint tissues take place in early knee OA (KOA). How-ever, it is not clear whether functional limitations in early radiographic KOA are linked to changes of serum and/or urinary cartilage biomarkers. AIM: To investigate associations between changes in some of the KOOS subscales and serum and/or urinary cartilage biomarkers in middle-aged women with early radiographic KOA. Methods: We studied 99 women from the Elva population based cohort, aged 34–54 (mean 45)years.Weight-bearing radiographs of the tibio-femoral(TF) compartment and axial radiographs of the patello-femoral(PF) compartment of the knee joint were used. Grade 1 OA was found in 53% the subjects, and grade 2 or 3 OA was found in 9%. The KOOS questionnaire includes the subscales: Symptoms(S), Pain(P), Activities of daily life(ADL), Sport/recreation(SP/Rec)and Quality of life(QL). An index value was calculated for every subscale (0–100). The degradation of cartilage was assessed by urinary excre-tion of the C-telopeptides of type II collagen, U-CTX-II (Nordic Bioscience Diagnostics) and by the serum cartilage oligomeric matrix protein, S-COMP (AnaMar Medical), both measured by ELISA. S-COMP is associated with several joint structures (Kumm, 2006). S-high sensitive CRP (S-hs-CRP) was measured by particle-enchanced immunoturbidimetric assay. Results: Correlations were observed between U-CTX-II and SP/Rec (rho $= -0.274$, $p = 0.007$) as well as QL (rho $= -0.213$, $p = 0.037$). The borderline association between U-CTX-II and pain(P)was found ($p = 0.055$). S-hs-CRP was associated with SP/Rec (rho $= -0.261$, $p = 0.01$) and ADL($p = -0.226$, $p = 0.027$). Correlations between S-COMP and all KOOS subscales were statistically non-significant. S-CRP associated with U-CTX-II(rho $= 0.318$, $p = 0.002$) and also with S-COMP (0.210, $p = 0.04$). On the KOOS subscale SP/Rec, out of 5 different demanding activities 4 (squatting, running, jumping and kneeling) were statistically significantly correlated with S-CRP ($p = 0.025$–0.009)and U-CTX-II ($p = 0.04$–0.003). Conclusions: 1. In some subjects limitations in demanding activities for the knee joint, according to the KOOS SP/Rec subscale, are associated with increased degradation of the type II cartilage. 2. Functional limitations in using the knees, expressed by subscales of ADL and SP/Rec, are associ-ated with low grade inflammation (based on S-hs-CRP) 3. Li-mitations in squatting, running, jumping and kneeling are associated with an increase in U-CTX-II and S-CRP levels.

Conflict of Interest: None declared

Tu-P382

CHARACTERISTICS OF OSTEOPOROTIC HIP FRACTURE PATIENTS IN HEALTH CARE AREA 2 OF THE COMMUNITY OF MADRID (SPAIN)

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Objective: To describe the sociodemographic and clinical characteristics of the patients who suffered osteoporotic hip fracture during 2006 in Health Care Area 2 of the Community of Madrid (Spain).

Material and Methods: A prospective, observational incident case study was made. The study included all patients admitted with osteoporotic hip fracture between January 1 to December 31, 2006, in the only public hospital serving the adult population of Health Care Area 2. All patients, with the help of a relative, were administered a previously designed questionnaire comprising sociodemographic data, prior diagnosis of osteoporosis, treatment with calcium / vitamin D supplements, antiresorptive drugs or bone forming agents in the case of a positive diagnosis, and the number and location of previous osteoporotic fractures. This information was completed with the data obtained from the Hygea registry - an integrated clinical information system documenting the events seen in Primary and Specialized Care. The mean, standard deviation, statistical proportions and corresponding confidence intervals were determined.

Results: During 2006, a total of 397 patients with osteoporotic hip fractures were admitted. The mean age was 82.9 ± 9.4 years, and 318 (80.1%) were women. The principal characteristics in terms of the diagnosis and treatment of osteoporosis before hip fracture are shown in the following Table 1.

Of the 100 patients with a prior diagnosis of osteoporosis, 62 (62%) received calcium and vitamin D supplements, 32 (32%) antiresorptive therapy, and 2 (2%) bone forming treatment. A total of 140 patients (35.3%) had previous osteoporotic fractures, though only 61 (43.5%) were diagnosed with osteoporosis, and only 33 (23.6%) received calcium and vitamin D, and 21 (15.5%) antiresorptive treatment.

Conclusions: In our area, the patients with hip fractures are close to 83 years old. Although 35.3% of the patients had previous

Table 1

Diagnosis osteoporosis	Osteoporotic fractures	Hip fracture	Vertebral fracture	Calcium and vitamin D supplements	Antiresoptive drugs	Bone forming agents
100 (25.2%)	140 (35.3%)	58		(14.7%)	30 (7.6%)	66 (16.6%)
32 (8%)	2 (0.6%)					

osteoporotic fractures, only 25.2% were diagnosed with osteoporosis, and only 8.6% received antiresorptive and/or bone forming treatment. Physicians must be aware of the need to diagnose and treat osteoporosis in the elderly population, and particularly among patients with a prior history of fractures, where the risk of hip fracture is high.
Conflict of Interest: This research study has been carried out with a grant from Merck Sharp & Dohme (MSD) Spain.

Tu-P383

ANNUAL INCIDENCE OF OSTEOPOROTIC HIP FRACTURE IN HEALTH CARE AREA 2 OF THE COMMUNITY OF MADRID (SPAIN)

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Objective: To estimate the annual incidence of osteoporotic hip fracture in Health Care Area 2 of the Community of Madrid (Spain) during the year 2006.

Material and Methods: The recruitment population of Health Care Area 2 of the Community of Madrid totals 554,274 urban inhabitants. Based on the corresponding population pyramid, the elderly population (over 65 years) is estimated to total 16.18% (89,678 inhabitants). The male/female ratio in this age segment is 1/4. A prospective, observational incident case study was made to estimate the annual incidence of osteoporotic hip fractures in Health Care Area 2. The study included all patients admitted with low-impact hip trauma between January 1 to December 31, 2006, in the only public hospital serving the adult population of Health Care Area 2. All patients, with the help of a relative, were administered a previously designed questionnaire comprising sociodemographic data, prior diagnosis of osteoporosis, treatment with calcium / vitamin D supplements, antiresorptive drugs or bone forming agents in the case of a positive diagnosis, and the number and location of previous osteoporotic fractures. This information was completed with the data obtained from the Hygea registry - an integrated clinical information system documenting the events seen in Primary and Specialized Care.

Results: The incidence of osteoporotic hip fracture in the population over 65 years of age pertaining to Health Care Area 2 was 2.07 males and 5.45 females, and globally 4.19 cases per thousand inhabitants. The estimated incidence according to age groups, gender and thousand inhabitants, is indicated in the following Table 1.

Conclusions: The incidence of osteoporotic hip fracture in Health Care Area 2 is very low before 65 years of age. From this age onwards, the incidence gradually increases and is much greater in women. The peak incidence is recorded after 90 years of age, affecting 17 of every 1000 elderly people.

Table 1

Age (years)	Males (CI)	Females (CI)
40–64	0.12 (0.05–0.20)	0.11 (0.04–0.17)
65–74	0.4 (0.10–0.70)	0.81 (0.45–1.17)
75–79	1.88 (0.89–2.86)	4.22 (3.06–5.39)
80–84	3.05 (1.51–4.59)	7.97 (6.21–9.73)
85–89	5.84 (3.19–9.78)	14.38 (11.4–17.4)
>90	17.29 (10.4–26.9)	17.71 (13.7–21.7)

Conflict of Interest: This research study has been carried out with a grant from Merck Sharp & Dohme (MSD) Spain.

Tu-P384

HIGH CONCENTRATIONS OF SODIUM DEOXYCHOLATE DECREASE INTESTINAL CALCIUM ABSORPTION

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It is known that high concentrations of biliar salt produce carcinogenesis either in colon or in other parts of gastrointestinal tract. The mechanism involves oxidative stress. Due to the fact that we have demonstrated that intestinal Ca absorption is inhibited by oxidative stress, we have hypothesized that the biliar salts might also inhibit intestinal Ca absorption by generation of reactive oxygen species (ROS). To demonstrate this, we have used 4 wk old chicks treated with sodium deoxycholate (NDXC) in the lumen of duodenum at different times and concentrations. We measured intestinal Ca absorption and the expresion of genes involved in the transcellular pathway. The activity of enzymes of the antioxidant system such as superoxide dismutase (SOD), catalase(CAT) and glutathione peroxidase (GPx) and total glutathione content was determined by spectrophotometry. ROS was determined by spin resonance spectrometry. Apoptosis was evaluated via DNA fragmentation and release of cytochrome c. The data revealed that NDXC inhibited the intestinal Ca absorption in 15 min, effect that continued for one hour. The inhibition was dependent of the concentration of NDXC. Levels of mRNA of Ca pump were reduced while the levels of mRNA of sodium/calcium exchanger remained unchanged.DNA fragmentation was enhanced and also the release of cytochrome c from the mitochondria. ROS production was generated by the biliar salt and the total content of glutathione in the enterocytes diminished in 15 min. In conclusion, high concentrations of NDXC inhibits intestinal Ca absorption by oxidative stress, which leads to apoptosis presumably by mitochondrial pathway. The antioxidant system is not sufficient to overcome the effect and the movement of the cation trough the cells is reduced
Conflict of Interest: None declared

Tu-P385

ASSOCIATION OF POLYMORPHISMS IN LOW-DENSITY LIPOPROTEIN RECEPTOR-RELATED PROTEIN 5 AND 6 WITH BONE MINERAL DENSITY AND MARKERS OF BONE TURNOVER

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Heritability studies show, that genetic factors account for more than 50% of the variability in bone mineral density (BMD). Low-density lipoprotein receptor-related protein (LRP) 5 and 6 are among the candidate genes, since their mutations are associated with distinct bone phenotypes. We analyzed the association of two common polymorphisms in LRP5 and LRP6 (A1330V and I1062V, respectively) with BMD and markers of bone turnover in a group of Slovenian postmenopausal women.

443 postmenopausal Slovenian women were enroled in the study. BMD at lumbal spine, total hip and femoral neck were measured by dual energy X ray absorptiometry (DEXA). Osteocalcin (OC), bone alkaline phosphatase (BALP) and C-terminal telopeptide of type I collagen (CTx) were measured by standard biochemical methods.

DNA was extracted from peripheral blood and analyzed for polymorphisms A1330V and I1062V using the ABI TaqMan genotyping assays. Statistical analysis of data was carried out using SPSS 15.0. To test the differences in BMD and bone turnover marker concentrations between genotype groups, analysis of covariance (ANCOVA) was employed.

The distribution of genotypes for both polymorphisms was in Hardy-Weinberg equilibrium. The distribution of A1330V and I1062V genotypes in the study group was as follows: 77.0% AA, 21.4% AV and 1.6% VV; 65.2% II, 31.4% IV and 3.4% VV, respectively. Our results show a statistically significant association between the polymorphism A1330V in LRP5 and the natural logarithm of lumbar spine BMD ($p = 0.004$). BMDs according to genotypes AA, AV and VV were 0.850, 0.826 and 0.957 g/cm^2, respectively. No significant association was found between LRP6 genotypes and BMD. Furthermore, we found no differences in concentrations of bone turnover markers neither between LRP5 genotypes nor between LRP6 genotypes.

Our results suggest an associaton between A1330V polymorphism in LRP5 and lumbal spine BMD in a group of Slovenian postmenopausal women. The minor allele of A1330V seems to be associated with higher lumbal spine BMD, which is contrary to the results of a similar study by van Meurs et al (1). Our results do not support associations between I1062V polymorphism in LRP6 and any of the the parameters studied.

1. van Meurs JB, Rivadeneira F, Jhamai M, et al. Common genetic variation of the low-density lipoprotein receptor-related protein 5 and 6 genes determines fracture risk in elderly white men. J Bone Miner Res 2006;21:141–50.

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Tu-P386

CALCIUM / PHOSPHORUS MAPS OF BONE ARCHITECTURE

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The major high Z mineral components in bone are calcium (Ca) and phosphorus (P) and may provide a sensitive measure of bone mineral changes. Changes in the amounts of Ca and P in biological apatites do not necessarily go hand-in-hand; a decrease in bone density due to a decrease in either Ca or P, or to dissimilar decreases in both cannot be monitored. Thus, the determination of the Ca/P ratio could lead to a greater understanding of the role played by these elements. The mechanical strength of bone depends primarily on the condition of the cortical bone. Hence, the present work uses only cortical bone. The aims of this study were to: 1) compare the bulk Ca/P ratio values for intact bones between sites and animal species, 2) use the Ca/P ratio as step towards understanding its importance as an indicator of bone strength. Ca/P ratio was measured by X-ray absorptiometry and microCT.

For X-ray absorptiometry cortical bone from rear tibias was studied while for microCT, cortical bone samples from the front and rear tibia and femoral neck from rabbits and rats were used. Inflammation-mediated osteoporosis was induced by injections of magnesium silicate in the flanks for 20 consecutive days. The X-ray absorptiometry system uses two energies of 39 and 89 KeV obtained by placing cerium and samarium filters in the beam. The method assumes that bone is a three-component system: Ca, PO4 and water. A significant decrease ($p < 0.01$) in the rear tibia Ca/P ratio in rabbits with osteoporosis compared to Ca/P ratio in controls has been found.

For microCT, multiple 2D slices were reconstructed from the 3D data sets. A full data set typically contained 140 slices. For the analysis all CT values were converted to Ca/P values using results from calibration phantoms. Significant differences ($p < 0.001$) were detected in Ca/P ratios in different bone sites from different experimental animals. In addition, the Ca/P ratio in the same bone site from different species has a significant variation. Different life activities arising from the evolutionary adaptation of these species could be considered an explanation for the observed variation. Also, there is a significant difference ($p < 0.001$) in Ca/P ratios between osteoporotic versus normal bone.

In conclusion, the Ca/P ratio may provide greater insight for diagnosis of bone disorders and X-ray absorptiometry as well as microCT may become valuable techniques to be used during bone therapeutic and diagnostic trials.

Conflict of Interest: None declared

Tu-P387

SELECTIVE SEROTONIN REUPTAKE INHIBITORS AND OTHER ANTIDEPRESSANTS AND RISK OF FRACTURE

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Background: We studied fracture risk in users of various antidepressants (tricyclic antidepressants, selective serotonine reuptake inhibitors, and the group of other antidepressants including monoamine oxidase B inhibitors and drugs with effect on the norepinephrine system) and its relationship with effects on different signalling systems (cholinergic and serotoninergic system).

Subjects and methods: Case control study. Cases were all subjects with any fracture during the year 2000 ($n = 124{,}655$). For each case, three controls ($n = 373{,}962$) matched on age and gender was randomly drawn from the background population. The exposure was use of antidepressants and a number of confounders.

Results: Among the tricyclic antidepressants amitriptyline and clomipramie were associated with a dose dependent increase in fracture risk, while imipramine and nortriptyline were not. Amitryptyline was associated with an increased risk of fractures at low doses, while the other tricyclic antiderpressants were not. Among the selective serotonin reuptake inhibitors, citalopram, fluoxetine, and sertraline were associated with a dose dependent increase in fracture risk, while the increase was borderline statistically insignificant for paroxetine. The group of other antidepressants were not associated with fracture risk. The increase in fracture risk was significantly associated with the pharmacodynamic effect on the serotonine system, but not on other signalling systems.

Conclusion: The effect of antidepressants on the risk of fractures may be linked to their effect on the serotonine system. While selective serotonin receptor uptake inhibitors were associated with an increased risk, tricyclic antidepressants only invariably were associated, and the group of other antidepressants were not associated with fractures.

Conflict of Interest: None declared

Tu-P388

FRACTURE RISK ASSOCIATED WITH DIFFERENT TYPES OF ORAL CORTICOSTEROIDS AND EFFECT OF TERMINATION OF CORTICOSTEROIDS ON THE RISK OF FRACTURES

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Aim: To study fracture risk associated with the use of orally administered prednisolone/prednisone, budesonide, methylprednisolone, and hydrocortisone to assess if the various preparations were associated with different fracture patterns.

Material and methods: Case control study. Cases were all subjects with any fracture sustained during the year 2000 (n = 124,655). For each case, three controls (n = 373,962) matched on age and gender was randomly drawn from the background population. Adjustments were made for concurrent diseases (lung diseases, rheumatic disorders), use of other drugs (inhaled bronchodilators), contacts to hospitals and general practitioners, and social variables.

Results: Oral prednisolone/prednisone was associated with a dose dependent increase in fracture risk starting from around a dose of 6.7 mg/day. Oral budesonide was not associated with an increase in overall fracture risk, but the doses in general were low (< 3 mg/day). Oral hydrocortisone was only associated with an increase in overall risk of fractures at a dose of > 40 mg/day. Oral methylprednisolone was only used intermittently and was not associated with an increase in overall fracture risk at the low doses used. After termination of oral prednisolone/prednisone it took more than one year for fracture risk to return to the levels of the background population. There was a positive relationship between fracture risk and daily prednisolone/prednisone dose and duration of use.

Conclusions: Oral prednisolone is associated with a dose dependent increase in overall fracture risk. High doses of oral hydrocortisone may be associated with an increase in fracture risk. Budesonide at low doses did not seem to be associated with fracture risk. It may take a year from last use of prednisolone/prednisone before fracture risk returns to that of the general population.

Conflict of Interest: None declared

Tu-P389

ANXIOLYTICS AND SEDATIVES AND RISK OF FRACTURES—EFFECTS OF HALF-LIFE

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Aim: To study the risk of fractures associated with anxiolytics, sedatives, and hypnotics

Subjects and methods: Case control study. Cases were all subjects with any fracture during the year 2000 (n = 124,655). For each case, three controls (n = 373,962) matched on age and gender was randomly drawn from the background population. The exposure was use of any anxiolytic, sedative, or hypnotics. Adjustments were made for a number of potential confounders.

Results: Most anxiolytics, sedatives, and hypnotics were associated with a limited increase in the risk of fractures. There was a dose response relationship, and drugs with a half-life longer than 24 hours were associated with a trend towards a higher relative risk of fractures than drugs with a shorter half-life. Both current use (last use less than one year ago) and past use (last use more than one year ago) were associated with an increased risk of fractures.

Conclusion: Anxiolytics, sedatives and hypnotics are associated with a limited increase in the risk of fractures. For most drugs a dose response relationship was present, and drugs with a half-life larger than 24 hours tended to be associated with a higher risk of fractures than drugs with a shorter half-life. This points to an increased dose dependent risk of i.e. falling leading to fractures. However, the increased risk of fractures with past use may point at an effect of the condition for which the drug was prescribed rather than the drug per se (confounding by indication).

Conflict of Interest: None declared

Tu-P390

INCIDENCE OF OSTEOPOROTIC FRACTURES IN A SPANISH COHORT OF PATIENTS WITH RHEUMATOID ARTHRITIS

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Aims: To evaluate incidence of clinical osteoporotic fractures (OF), both vertebral and peripheral, in rheumatoid arthritis (RA) patients and to analyse the influence of factors possibly related.

Methods: The population of the study was the EMECAR cohort: a prospective cohort of Spanish RA patients randomly selected from 34 hospitals, with a follow-up period of 4 years. OF were defined as those produced spontaneously or by a low impact trauma. Only clinical fractures were evaluated. Statistical analysis: incidence rates were calculated by survival analysis. The related factors (demographic and disease characteristics and treatments) were evaluated using linear generalized models, following Poisson distribution. A multivariate regression model was estimated including all variables that were significant in univariate analysis.

Results: 789 patients were included (72% women). Population characteristics (mean ± DE): age of 61 ± 13 years, evolution of 10 ± 7.9 yrs, age at disease onset 48 ± 14.7 yrs, BMI of 26.7 ± 4.3, DAS28 of 4.2 ± 1.4, HAQ of 1.2 ± 0.8, Larsen score of 54.5 ± 26.5 and diagnostic delay of 3 ± 5.5 yrs. 95% were rheumatoid factor positive and 83% were treated with glucocorticoids for more than 3 months. 81% of women were post-menopausal. A total of 72 fractures occurred in 69 patients. Incidence of clinical OF, stratified by groups of age and sex, is shown in the table 1. The global incidence of fractures (95% CI) was 2.8 (2.2–3.5) per 100 person-yrs. The incidence of the different types of fractures was: vertebral 1.4 (0.9–1.9), hip 0.1 (0.04–0.4), Colles 0.2 (0.06–0.4) and rest of non vertebral fractures 1.1 (0.7–1.6) per 100 person-yrs. Factors independently associated with fracture occurrence were: age [IRR: 1.03 (1.0–1.05)], female sex [IRR: 2.13 (1.05–4.32)], glucocorticoid treatment [IRR: 2.08 (1.14–3.78)] and previous fractures [IRR: 2.92 (1.55–5.51)].

Conclusion: The incidence of clinical OF found in the EMECAR cohort was similar to the rates described in other cohorts of RA patients and was independently associated with age, sex, glucocorticoids and previous fractures.

Table 1

Age (yrs)	Men Person-yrs	N	I (95%CI)	Women Person-yrs	N	I (95%CI)
20–44	72	1	1.4 (0.2–9.8)	184	1	0.5 (0.08–3.8)
45–49	42	0	0 (−)	123	2	1.6 (0.4–6.4)
50–59	123	0	0 (−)	324	6	1.8 (0.8–4.1)
60–69	198	2	1.0 (0.2–4.0)	516	21	4.1 (2.6–6.2)
70–79	218	2	0.9 (0.2–3.6)	449	25	5.5 (3.7–8.2)
≥ 80	63	3	4.7 (1.5–14.5)	148	6	4.0 (1.8–8.9)

N: number of fractured patients. I: OF incidence

Conflict of Interest: None declared

Tu-P391

TOWARDS AN UNDERSTANDING OF THE MECHANISM OF FEMUR STRENGTH IMPROVEMENT BY ALENDRONATE IN POSTMENOPAUSAL WOMEN

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Alendronate (ALN) treatment reduces hip fracture rates compared to placebo (PBO) among osteoporotic women, but appears unlikely to affect fall risk suggesting that ALN improves femur strength.

However, the mechanism underlying this association is uncertain. Potentially ALN may alter geometry to diminish load stresses and/or may improve tissue stress resistance. Some effect on the latter is evident from biopsy studies showing increased mean tissue mineralization in subjects treated with ALN. To examine the effect of ALN on bone geometry parameters separate from its mineralization effects, we compared differences in changes in geometric parameters with ALN treatment as defined by 2 different methods in 803 post-menopausal women with low bone mass (400 on PBO and 403 treated with ALN 5–10 mg/d) enrolled in the FIT trial at Minneapolis and Pittsburgh sites. Method 1 derived from Hip Structure Analysis (HSA) assumed a fixed mineralization and Method 2 utilized cortical margin dimensions reliable in only thick cortex regions, but did not require a mineralization assumption.

Annual rates of change after 36 months of treatment in bone cross section analysis (CSA), section modulus, mean cortical thickness and buckling ratio for the two treatment groups by both methods were analyzed. Among women taking PBO, mean rates of change in section modulus, cortical thickness and buckling ratio as defined by both methods were similar, although the increase in CSA was greater using method 2.

Among women assigned to ALN, average rates of change in CSA were similar using both methods, but rates of change in section modulus, cortical thickness, and buckling ratio were smaller in magnitude using method 2. Except for method 2 CSA, differences in rates of change in parameters between ALN and PBO groups reached significance, irrespective of method ($p < 0.05$).

Our findings suggest that ALN treatment improves femur shaft geometry independent of tissue mineralization effects, but geometric improvement is overestimated by 50–75% if mineralization effects are not taken into account. Bone strength improvement by ALN appears to combine geometric changes with greater tissue stress resistance.

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Tu-P392

COMPARATIVE EPIDEMIOLOGIC STUDY OF MEASUREMENT OF BONE DENSITY WITH ULTRASOUND IN THE PROVINCE OF IPATI

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Key words: Osteoporosis, risk factors, ultrasound (US).

Background: Osteoporosis is a growing healthcare crisis affecting millions of women and men worldwide. The most common sites of fracture are the bones of the spine, the hip and the wrist. Examinations which are required for initial diagnosis are biochemical markers from urine and blood, simple radiographs and DXA (Dual Energy X Ray Absorptiometry) measurements; other more specific methods include ultrasound of the os calcis 4. Aim of the study is to assess the epidemiology of osteoporosis in the region of Ipati, based on the age of the populations and various risk factors. Methods. Were examined 350 individuals (323 women and 27 men, mean age 55 years, age range 33–80 years), which have visited Loutra Ipatis. First detailed history was taken from each participant. Subsequently, every participant underwent a densitometric measurement of os calcis by SAHARA US instrument. T-scores were calculated for each patient according the standards of WHO for osteoporosis.

Result. With the base the history of patients, the 350 individuals received that from these the 40 from them they have done again measurement of bone density and the rest not. According to statistical regrouping the 84% do not know for the disease and have not made any measurement. The rest 16 % from the individuals they have again making measurement from which 6% receipt treatment. From the statistically history showed that the 34% were smoked, the 18% took treatment, 22% had the dyslipidemia and 26% had other disease. Then afterwards the measurement of bone density and the result of T-score, we separated the individuals per six age period (32–39, 40–47) and removed them T-score, It was realised that the individuals of age (65–85) had osteopenia or osteoporosis. Furthermore are realised that the individuals of age (45–65) did not almost have by no singularly osteopenia. Finally, became measurement in the 27 men that arrived, and are realised that it was found in physiologic levels T-score.

Conclusion. With base the results of statistics, it is realised that they had ignorance about the disease in the residents of province. Higher appearance of illness was in women that had in higher age menopause. In men the T-score they were in physiologic limits. Furthermore, higher T-score was realized in the individuals that they were smokers. Moreover, the lack of briefing for disease is not realised particular sickliness, something that shows that in provincial areas are smaller the risk factors.

Conflict of Interest: None declared

Tu-P393

OSTEOPOROSIS: MORE THAN BONE TURNOVER?

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Osteoporosis, characterized by bone mineral density loss and bone fragility, has long been attributed to an imbalance of bone turnover. Other factors, perhaps unrelated to bone turnover, are likely to play a role in the pathogenesis of osteoporosis, such as reports of elevated homocysteine levels in patients at risk for fracture. In cell cultures and animal models, homocysteine induces changes in the organic matrix that may or may not be accompanied by changes in turnover. The goal of the present study was to determine whether changes seen in collagen cross-links may be attributed solely to bone turnover changes. Iliac crest biopsies from normal premenopausal women ($n = 12$), women diagnosed with either high turnover ($n = 6$), or low turnover ($n = 6$) osteoporosis, as well as from patients diagnosed with either

surgical or autoimune hypoparathyroidism (HypoPT:n = 21), or primary hyperparathyroidism (PHPT:n = 51) were examined by means of Fourier Transform Infrared Imaging to determine the spatial distribution of collagen cross-links in the area of trabecular bone. The last 2 groups served as controls since both cases are characterized by changes in bone turnover. Analyses were focused on trabeculae containing bone packets with primary mineralization evident on 1 surface. At least 3 such trabeculae were analyzed in each section and the results averaged and treated as a single statistical unit. The results indicate that the patients with PHPT show a signif. lower (2.11 ± 0.0.3) collagen cross-link ratio (Pyr/deH-DHLNL) as compared to normal controls (2.56 ± 0.61), while patients with HypoPT have a higher cross-link ratio (3.57 ± 0.04). Interestingly, both high (5.11 ± 0.72) and low (4.07 ± 0.28) turnover osteoporotic patients exhibited a higher ratio than the normal controls. Although the low turnover patient results may be explained by the known alteration in bone turnover, this is not the case for the high turnover osteoporotic patients. These data suggest that in osteoporosis, the organic matrix is modified and the changes in bone turnover are not solely responsible for the observed changes. On the other hand, based on animal models, these changes would be consistent with the anticipated changes induced on the collagen cross-links by elevated homocysteine levels. In conclusion, the results of the present study suggest that in addition to the well established bone turnover imbalance in osteoporosis other factors may be contributing to collagen alterations, thus fracture risk.

Conflict of Interest: None declared

Su-P394

SUBTROCHANTERIC FEMUR FRACTURE IN PATIENTS TREATED WITH ORAL BISPHOSPHONATES: A NATIONAL REGISTER STUDY

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Recent reports have found bisphosphonate use to be common in patients with subtrochanteric fracture of the femur, but it is unclear if this fracture is a manifestation of osteoporosis itself or perhaps indicative of excessive suppression of bone turnover with anti-resorptives.

All patients who presented with fractures at key osteoporotic sites - except hip fractures - in the period 1996–2005 were identified in national registers. We extracted information on subsequent bisphosphonate use and incident fractures and assigned two controls (N = 14624) to each bisphosphonate exposed patient (N = 7312). Cox proportional hazards models were used, incorporating age, sex, comedications and Charlson comorbidity score.

Fracture patients who had begun bisphosphonates exhibited a significantly decreased risk of forearm fracture, but did not differ significantly from untreated fracture controls in terms of risk of subtrochanteric or humerus fracture. Bisphosphonate users had an increased risk of hip and spine fractures.

Conclusion: The slightly increased risk of spine and hip fractures despite bisphosphonate treatment suggests that bisphosphonates are prescribed to fracture patients with particular morbidity at these locations - probably as a consequence of BMD at these two sites being used in clinical decision-making - and that the increased risk is incompletely offset by therapy. Subtrochanteric fractures should be attributed to osteoporosis and not to bisphosphonate treatment.

Table 1

	Odds ratios (Cox proportional hazards model)			
	Rate in treated VS untreated group (1000 person years)	Unadjusted	Adjusted for sex and age	Adjusted for sex, age, number of comeds and Charlson index
Subtrochanteric femur	1.8 vs 13	1.33 (0.87–2.02)	1.32 (0.87–2.01)	1.33 (0.87–2.03)
Hip fracture	27.0 vs 18.2	1.53 (1.37–1.71)	1.52 (1.36–1.70)	1.45 (1.29–1.62)
Spine fracture	7.1 vs 5.1	1.43 (1.15–1.76)	1.44 (1.16–1.78)	1.32 (1.06–1.64)
Humerus fracture	12.6 vs 12.0	1.08 (0.93–1.26)	1.08 (0.93–1.25)	1.04 (0.89–1.21)
Forearm fracture	19.6 vs 242	0.81 (0.72–0.91)	0.81 (0.72–0.91)	0.81 (0.72–0.91)

Conflict of Interest: BA: Advisory board member: Nycomed. Research grants: Roche and the Novo Nordisk Foundation.

Su-P395

INITIATION OF ANTI-OSTEOPOROTIC MEDICATIONS IN PATIENTS WITH RECENT FRACTURES

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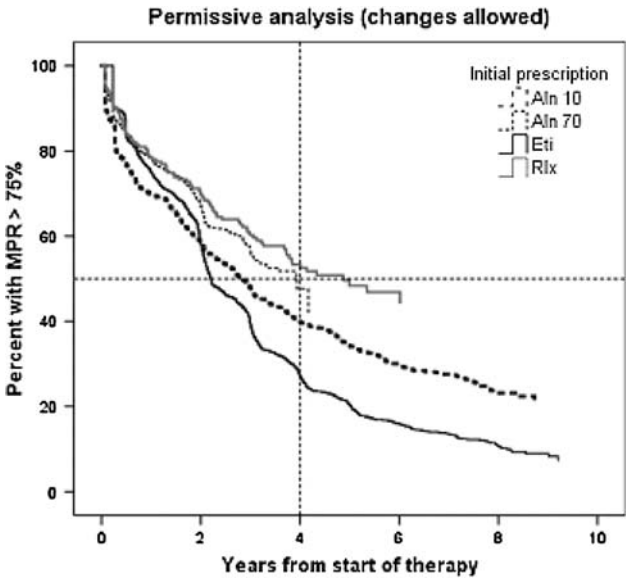
Aim: Assess prescription rates and persistence for anti-osteoporotic therapy (AOT) after fracture.

Methods: We used national registers to identify all patients born 1945 or earlier who sustained a fracture 1997–2004. We found 152,777 first fractures: spine (9,496), humerus (24,478), hip (64,466) and forearm (54,337). Prescriptions (1996–2005) were assessed, and initiation of AOT defined as redemption of prescriptions for a bisphosphonate, SERM, PTH or SR. Persistence was defined by MPR > 75%.

Results: Treatment initiation within 1 y was highest after spine fracture: 39.6% of women began therapy in 2004 compared with 19.5% in 1997. In men, 16.5% began therapy in 2004 vs 8.0% in 1997. Following hip fx, 9.2%(F) and 4.1%(M) began therapy in 2004 vs 3.4%/0.7% in 1997.

Median persistence was 2.8 y for aln 10 mg, 3.8 y for aln 70 mg, 2.5 y for eti, 4.7 y for rlx. The risk of discontinuing/changing AOT increased with age(Cox, $p < 0.001$), spine fracture ($p < 0.001$), humerus fracture ($p < 0.05$) but was independent of sex.

Conclusion: Prescription rates for AOT are low, especially in hip fracture - where nine out of ten did not begin treatment - and in men. Persistence has improved with almost 2/3 of patients who began aln70 or rlx now obtaining a treatment duration equalling that of the licensing trials.



Conflict of Interest: BA: Advisory board member: Nycomed. Research grants: Roche and the Novo Nordisk Foundation.

Su-P396

SIGNIFICANT REDUCTION IN NON-VERTEBRAL FRACTURES WITH INTRAVENOUS IBANDRONATE INJECTION: POST-HOC ANALYSIS OF THE DIVA TRIAL

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Monthly oral and quarterly IV ibandronate (IBN) are licensed for postmenopausal osteoporosis. Daily oral IBN (2.5 mg) significantly reduced vertebral fracture risk by 62% (vs PBO, $p = 0.0001$) at 3 yrs. In a subgroup at high risk of fracture (baseline Tscore < -3.0) IBN reduced non-vertebral fracture risk by 69% ($p = 0.012$).[1] Non-vertebral fracture efficacy was not shown in the overall population who were at low risk.[1] In the randomised, double-blind DIVA study, IBN i.v. injections 2 mg every 2 months and 3 mg every 3 months were superior to daily oral IBN in terms of BMD gains ($p < 0.001$).[2] Bone turnover markers were also reduced.[2] These surrogate marker data suggest that licensed quarterly IV IBN 3 mg may have significant antifracture efficacy.[3] Here we present a post-hoc analysis of individual patient data from DIVA. Both i.v. regimens in DIVA delivered the same annual cumulative dose of IBN (12 mg), therefore, data for these regimens were pooled for this analysis. The high dose was compared with daily oral IBN (annual cumulative dose 5.5 mg; low dose), maintaining randomisation. Osteoporotic non-vertebral fractures were captured as adverse events and confirmed by X-ray. Time-to-event analysis was conducted using Kaplan-Meier methodology and hazard ratios (HRs) were derived from a Cox model (adjusted for prior clinical fracture, age, baseline BMD; full model, then stepwise).

In DIVA, the non-vertebral fracture rate was significantly reduced with i.v. IBN vs low dose daily oral. Fracture incidence was 3.1% vs 4.8%, respectively; a 43% relative risk reduction for non-vertebral fractures with i.v. IBN ($p = 0.0489$; adjusted HR 0.569 [95% CI: 0.324–0.997]). Time to non-vertebral fracture was also extended for high vs low dose IBN ($p = 0.048$). A significant non-vertebral fracture risk reduction was seen when high i.v. IBN doses were compared with low dose daily oral IBN. These data indicate improved fracture efficacy for the licensed 3 mg quarterly IV injection vs daily oral. DIVA is the first study to show fracture efficacy for a licensed intermittent bisphosphonate compared with daily oral treatment.

1. Chesnut CH, et al. J Bone Miner Res 2004;19:1241–9; 2. Eisman J, et al. J Rheumatol 2007;In press; 3. Papapoulos S, Schimmer R. Ann Rheum Dis 2007;66:853–8

Conflict of Interest: Barr,Roche employee Adachi,Consultant/Speaker, Amgen, AstraZeneca, Lilly, GSK, Merck, Novartis, Pfizer, P&G, Roche, Sanofi Aventis, Servier. Clinical Trials, Amgen, Lilly, GSK, Merck, Novartis, Pfizer, P&G, Roche, Clinical Trials Sambrook, Research Support/Consultant, Roche. Papapoulos, Research Support, MSD, P&G. Consultant, MSD, Novartis, P&G, Roche/GSK

Su-P397

SAFETY AND TOLERABILITY OF BAZEDOXIFENE IN POSTMENOPAUSAL WOMEN WITH OSTEOPOROSIS: RESULTS FROM A 3-YEAR, RANDOMIZED, PLACEBO- AND ACTIVE-CONTROLLED CLINICAL TRIAL

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Bazedoxifene (BZA) is a novel selective estrogen receptor modulator (SERM) selected for clinical development based on preclinical evidence of tissue-selective estrogen agonist activity on the skeletal system and lipid metabolism and estrogen antagonist activity on breast and uterine tissues. A total of 7,492 evaluable postmenopausal women with osteoporosis (mean age ± SD, 66.4 ± 6.7 years) received ≥1 dose of BZA 20 mg, BZA 40 mg, raloxifene (RLX) 60 mg, or placebo (PBO) daily. At 36 months, BZA exhibited a statistically significant decrease in new vertebral fracture compared with PBO (primary efficacy endpoint to be reported elsewhere). We report here the 3-year safety and tolerability data from this study. After 3 years of treatment, BZA was well tolerated overall and exhibited a favorable safety profile. Findings of selected safety/tolerability analyses are shown below. No safety concerns related to the cardiovascular and gynecologic systems, including breast, were observed in the BZA treatment groups. There was a higher incidence of deep vein thrombosis in the BZA groups compared with the PBO group. The incidence of breast cancer and cystic/fibrocystic breast disease was lower in women receiving BZA than in those receiving PBO. Transvaginal ultrasonography examinations revealed that BZA had no significant effects on the endometrium or ovaries compared with PBO. In conclusion, BZA had a favorable safety and tolerability profile in postmenopausal women with osteoporosis. The safety and tolerability profile was similar between the BZA 20- and 40-mg groups. BZA is a promising new SERM for the prevention and treatment of postmenopausal osteoporosis.

Table 1

	BZA 20 mg (n = 1336)	BZA 40 mg (n = 1372)	RLX 60 mg (n = 1349)	PBO 20 mg (n = 1335)
Discontinuations (%)	632 (33.5)	643 (34.3)	597 (32.3)	629 (33.4)
Discontinuations due to adverse events (%)	269 (14.3)	270 (14.4)	262 (14.2)	240 (12.7)
Myocardial infarctions (%)	8 (0.4)	8 (0.4)	6 (0.3)	8 (0.4)
Ischemic stroke (%)	11 (0.6)	15 (0.8)	9 (0.5)	11 (0.6)
Hemorrhagic stroke (%)	1 (0.1)	1 (0.1)	2 (0.1)	5 (0.3)
Deep vein thrombosis (%)	8 (0.4)	11 (0.6)	8 (0.4)	1 (0.1)
Hot flushes (%)	238 (12.6)	243 (13.0)	222 (12.0)	118 (6.3)
Leg cramps (%)	205 (10.9)	204 (10.9)	216 (11.7)	155 (8.2)
Breast cancer (%)	5 (0.3)	4 (0.2)	7 (0.4)	8 (0.4)
Breast cyst/fibrocystic breast (%)	14 (0.7)	13 (0.7)	32 (1.7)	20 (1.1)

Conflict of Interest: J. D. Adachi, Amgen, Astra Zeneca, Eli Lilly, GSK, Merck, Novartis, Pfizer, Procter & Gamble, Roche, sanofi-aventis, Servier, Consultant/Speaker
J. P. Brown, Eli Lilly, Merck Frosst, Novartis, Procter & Gamble, sanofi-aventis, Research Support and Consultant
C. Christiansen, Wyeth Pharmaceuticals, Consultant
C. E. Fernandes, Wyeth Pharmaceuticals, Research Support

Su-P398

RAPID SUPPRESSION OF SERUM CTX WITH ONCE-MONTHLY ORAL IBANDRONATE

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Monthly oral ibandronate (IBN) 150 mg has been shown to increase BMD and substantially suppress bone turnover markers (BTMs) in women with postmenopausal osteoporosis (PMO).[1] The 6-month Rapid Onset study assessed the speed of onset and pattern of reduction of the bone resorption marker, serum CTX (sCTX), in women taking monthly oral IBN. Rapid Onset was a randomised, double-blind, placebo-controlled study in women with PMO (diagnosed for $\leq$12 months). All patients had no more than 3 months' exposure to daily or weekly bisphosphonate in the 5 years before screening. Participants received 150 mg monthly oral IBN or placebo for 6 months + calcium and vitamin D. Levels of sCTX were measured at baseline and 3 (1st month only), 7, 14, 21 and 28 days after each dose. Primary endpoint: relative change (%) in median sCTX from baseline to day 3 and change in sCTX in the 1st month of treatment. Responder analyses (defined as sCTX decreases $\geq$50% and $\geq$70%) were also performed (Independent Data Monitoring Committee). 67 women participated; 1 did not take study drug, 49 received IBN and 17 received placebo. Mean baseline sCTX levels were the same for both groups (0.63 ng/mL). Within 3 days of IBN administration median sCTX levels were reduced by almost 70% from baseline ($p < 0.0001$ vs placebo) and remained low at day 28 (median decrease of 43% from baseline; $p = 0.0014$ vs placebo). After IBN dosing, the maximum decrease in sCTX from baseline was 74% on day 7 ($p < 0.0001$ vs placebo). For women receiving placebo, median sCTX was reduced by approx 6% on day 3 with a maximum decrease from baseline of 22% on day 14 (vs 61% for IBN, $p < 0.0001$). At day 3, 71% of patients on IBN had $\geq$50% decreases in sCTX and 47% had $\geq$70% decreases. No patients receiving placebo were classified as responders. Monthly IBN rapidly reduced sCTX levels within 3 days of dosing and maintained suppression throughout the 1st month. The majority of women receiving IBN achieved a response to treatment. Reductions in BTMs are known to correlate with reduction in fracture risk.[2] The current findings suggest that measuring BTMs may allow clinicians to make an early assessment of treatment effect in women with PMO.

1. Reginster JY, et al. Ann Rheum Dis 2006;65:654–61;
2. Hochberg M, et al. J Clin Endocrinal Metab 2002;87:1586–92

Conflict of Interest: J. Boisdron, Roche employee; S. Adami, Roche, Consultant/Speaker; S. Silverman, Speaker's Bureau: Lilly, Merck, Procter&Gamble, Pfizer, Roche. Consultant: Merck, Novartis, Procter&Gamble, Pfizer, Roche, Wyeth. Research Support: Novartis, Lilly, Wyeth, Roche, Procter&Gamble, Merck Board of Directors: Compumed

Su-P399

EFFECT OF CHRONIC STRONTIUM ADMINISTRATION ON BONE MINERAL CONTENT AND DENSITY

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Strontium malonate (SM) is a novel promising pharmaceutical agent for the treatment of osteoporosis. Strontium is known to reduce the numbers of fractures in humans. We assessed the effect of chronic Sr treatment in rats on bone strength and mineral content. SPF Wistar rats were given the following doses of SM 0 (placebo), 100, 300 and 1000 mg/kg/day for 26 weeks. There were 20 males and 20 females in each dose group. At study termination the incisors, femurs and serum were extracted for determination of elements (Mg, P, Ca and Sr content) by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) after microwave oven acid digestion. All determinations were subjected to quality assurance (QA) and reference controls were included in each analytical run. The results of the mineral determination in bone and teeth were correlated mutually and to bone densitometry measurements (BMD) of femur.

A dose dependant content of Sr was observed in serum, with a gender difference 24 h after administration. A dose dependant incorporation of Sr was also observed in both incisors and femurs where the content in femurs was 30% less than Sr found in the incisors. A significant difference ($P < 0.001$) in Sr content between genders was observed in both mineralized tissues with BMD values of 0.19 g/cm^2 and 0.22 g/cm^2 in females and males, respectively. Calcium, phosphorous and magnesium decreased as a function of Sr dose. BMD increased significantly ($P < 0.001$) with SM treatment, with the highest treatment group as the one with the highest BMD value close to 0.24 g/cm^2. The BMD results were correlated to the Sr/Ca mole ratio of the femur and hence needs to be corrected for the influence of Sr.

The study of 6 month SM treatment in rats showed, that there was a clear dose dependent increase in Sr content of plasma and mineralized tissue, and a highly significant correlation between plasma and bone levels. In the highest dose group, substitution of calcium by Sr in teeth (Sr/Ca = 0.10) and to a lesser extent bone (Sr/Ca = 0.07) was observed. No difference in Sr content was found between genders of the placebo group. However, in teeth, the content of Sr increased by a factor of 400 and a factor of 500 in female and in male groups, respectively. A corresponding difference and increment of Sr by a factor of 300 and 400 were found in femur. A linear correlation was observed between Sr femur contents and that of incisors.

Conflict of Interest: None declared

Su-P400

PREFERENCES FROM 500 POST-MENOPAUSAL OSTEOPOROSIS PATIENTS FOR ADMINISTRATION OF PTH TREATMENT

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Background/aims: To measure patients' preferences for administration features of parathyroid hormone (PTH) treatment.

Methods: Choice-format conjoint (CFC) is a technique which is commonly applied to measure patients' preferences. To our knowledge, a CFC has never before been applied to measure Post-Menopausal Osteoporosis (PMO) patients' preferences for different aspects of PTH-treatment in Europe.

Based on an extensive literature review and in-depth interviews with clinical experts, a CFC survey instrument was developed. Subsequently, the CFC survey instrument was pre-tested using one-on-one verbal-protocol interviews with twenty European PMO patients.

In addition to the CFC, the final CFC survey instrument contains items related to the severity of osteoporosis and socio-demographic variables. Furthermore, the patients were asked to fill in the Oswestry Disability Index (ODI).

By applying the CFC survey instrument in face-to-face interviews, data from 500 European PMO patients across five countries has been collected in the period from August 2007 to December 2007.

Results: A preliminary analysis of the collected data from 102 German PMO patients was conducted. 44.4% of the enrolled patients were diagnosed less than five years ago, 27.3% were diagnosed between five and ten years ago and 28.3% were diagnosed more than 10 years ago.

The mean BMD T-score was −3.09. 53.5% of the enrolled patients had a BMD T-score between −2.5 and −2.9 whereas the remaining 46.5% of the enrolled patients had a BMD T-score at −3.0 or below. 50.0% of the enrolled German patients had experienced an osteoporosis-related fracture in the past. The mean ODI-score was 30.9% which according to the ODI-scoring is categorized as moderate disability.

The CFC identified the most important administration features influencing the PMO patients' preferences for administration of PTH treatment as "storage requirements", "reusability of the pen", "number of doses in the pen" and "dexterity/easiness for PMO patients to use the pen".

Conclusion: CFC is the conceptually correct method for measuring valid patient preferences for PTH administration features. The preliminary data analysis indicates that PMO patients' preferences are influenced by certain administration features of PTH treatment.

Conflict of Interest: Mikael Asmussen, Employee at Nycomed

Su-P401

SECONDARY OSTEOPOROSIS AND TEMPOROMANDIBULAR JOINT DISORDER—A THREE-YEARS FOLLOW UP REPORTED CASE

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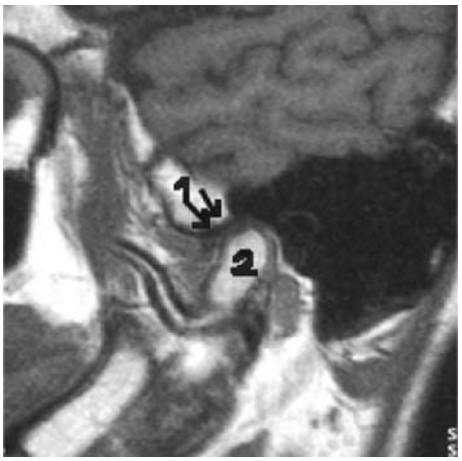
Osteoarthritis of temporomandibular joint (TMJ) associated with osteoporosis may disturb the functional harmony of stomatognathic system and may increase the possibility for TMJ disorder. The case presented is of a 36 year-old male patient treated for secondary osteoporosis. Hyperthyroidosis has been diagnosed and treated since 1999. He underwent radioactive iodine therapy from 2002 until April of 2003. During 2002 he had physical therapy due to back pain. Due to bone, spine and joint pain densitometry was performed in 2004 (femoral osteoporosis (Tscore:-2.51) and spinal osteopenia (L1–L4) (Tscore:-2.00)). Approximately in 2004 symptoms in the left TMJ appeared. Osteoarthritis of TMJ was diagnosed by a clinical examination and manual functional analysis - crepitations and joint pain of 4.5 on the visual-analogue scale (VAS). Osteoarthritis was confirmed by magnetic resonance imaging (Figure: 1 - articular tuberculum, 2 - condyle, arrows - subchondral sclerosation in the articular tuberculum). Occlusal splint treatment since October 2006 reduced the pain significantly and TMJ crepitation during mouth opening does not disturb the patient (VAS = 0.5). The densitometry in 2006 showed a significant improvement after biosposphonates (Fosamax T) treatment: femoral osteopenia (Tscore:-2.16) and spinal osteopenia (L1–L4) (Tscore:-1.14). Three years later, the patient only has painless crepitation in the TMJ (AVS = 0). The findings of the present case report suggest that the symptoms of osteoarthritis of temporomandibular joint are concurrent with osteoporosis. Like osteoarthritis, osteoporosis has multifactorial etiology, and all possible relationships between osteoporosis and pathological changes in the orofacial system are unknown.



Conflict of Interest: None declared

Su-P402

A NATIONAL CLINICAL AUDIT OF STANDARDS IN THE MANAGEMENT OF FALL-RELATED FRAGILITY FRACTURES PRESENTING TO TRAUMA UNITS IN ENGLAND, WALES AND NORTHERN IRELAND

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Background/aims: Previous organisational audit involving 90% of acute hospital trusts in England, Wales and Northern Ireland had indicated that standards in the management of osteoporosis lagged behind those for falls. Further clinical audit was conducted to evaluate documented standards at a patient level in the management of fallers who had sustained a fracture. Criteria for quality ortho-geriatric care and secondary osteoporotic fracture prevention were defined to measure care against national guideline standards. The indicators related to those criteria are reported here.

Methods: The notes of consecutive patients over 65 presenting to A & E units with a fracture following a fall throughout the three nations were reviewed and performance against criteria defined by the study steering group was submitted to an on-line database by local units.

Results: 157 hospital trusts (91% of those eligible) took part and data was acquired on 5642 non-hip and 3184 hip fracture patients. Of the non-hip fracture patients, 1/3 were admitted to hospital and 2/3 were wrist fractures. After 3 months, a minority (19% of non-hip and 35% of hip fracture patients) had received an osteoporosis risk assessment. Most (96%) had not had a previous DXA scan but only 19% of non-hip and 18% of hip fracture patients 65–74 were referred for DXA. About 50% of those scanned had osteoporosis. Three months later, hip fracture patients were more likely (52% v 23%) to be receiving calcium + vitamin D3 or specific bone protective therapy (43% v 20%). 80% hip fracture patients spent >2 hours in casualty. 35% had surgery within 24 hr, 69% within 48 hr and 90% within 4 days. Delays were largely for clinical reasons, 29% of delays being due to organisational issues. Immediate management met most quality standards but exceptions included low levels of assessment for pressure ulcer risk (46%) or of cognitive function (29%). Only 28% received pre-op medical review by registrar grade or above.

Conclusion: Older patients who fall and fracture, in particular those with peripheral fracture, receive sub-optimal care in respect of secondary prevention. It is necessary to ensure a changed systems-wide approach, including fracture liaison services, is aimed at ensuring the care pathway includes evidence-based interventions and is delivered.

On behalf of the Steering Group for the National Clinical Audit of Falls and Bone Health

Conflict of Interest: None declared

Su-P403

MANAGING OSTEOPOROSIS IN UK GENERAL PRACTICE: RESULTS FROM A NATIONAL EVALUATION USING THE QRESEARCH DATABASE

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Background: Little is known about standards in osteoporosis management in primary care. This study reports the first nationally representative evaluation in UK general practice of care for older people at risk of osteoporotic fracture.

Methods: Criteria and indicators were derived from national guidelines by a multi-disciplinary steering group. Read code datasets were constructed and cross-sectional data from the QRESEARCH database (3,386,134 patients in 487 practices using the EMIS™ clinical software) was analysed.

Results: 39,538 patients (1.17%; 95% confidence intervals 1.16–1.18) had a recorded osteoporosis diagnostic code and 23,462 (59.3%; 58.9–59.8) were on specific therapy or had a review in the last 15/12. Of 46,113 on osteoporosis therapy 23,354 (50.6%; 50.2–51.1) had an appropriate diagnostic code and 25,104 (54.4%; 54.0–54.9) were co-prescribed calcium + Vit D3. Long term glucocorticoid users were rarely coded but 17,280 patients > 65 had 2 steroid prescriptions in 6/12 of which 7,256 (42.0%; 41.3–42.7) were on osteoporosis therapy. 3,418 > 75 yrs were coded as resident in care homes of which 1,248 (36.5%; 34.9–38.2) were receiving calcium + vit D3. 31,094 women > 75 yrs had a prior fragility fracture (closed fracture after 45 years at an osteoporotic site). 7,860 (25.3%; 24.8–25.8) were on therapy. 15,025 women aged 65–74 years had a prior fragility fracture. 1,476 (9.8%; 9.4–10.3) had evidence of a DXA referral. Of 2,551 with a prior fracture and osteoporosis 1,862 (73.0%; 71.2–74.7) were on therapy. Only 14,651 males > 65 yr with a fragility fracture were identified and only 261(1.8%; 1.6–2.0) had evidence of a DXA referral. Of the 700 men that had an osteoporosis diagnosis and a prior fracture 305 (43.6%; 39.9–47.3) were on licensed therapy. 41,606 females > 65 had strong risk factors for osteoporosis but only 1,143 (2.8%; 2.6–2.9) had evidence of assessment or DXA in the previous 3 years. However 5,232 had an osteoporosis diagnostic code and 3,255 (62.2%; 60.9–63.6) were on treatment. Insignificant numbers of fallers over 75 had a recorded osteoporosis assessment or those with a prior fracture or osteoporosis a falls assessment.

Conclusion: Despite national guidelines aimed at reducing osteoporotic fracture rate, documented management is suboptimal in UK general practice. An alternative approach aimed at implementing systematic care is urgently needed. Standards can be monitored through large scale electronic audit.

Conflict of Interest: None declared

Su-P404

STRONTIUM ADSORPTION AND DESORPTION ON HYDROXYAPATITE CRYSTALS IN VITRO

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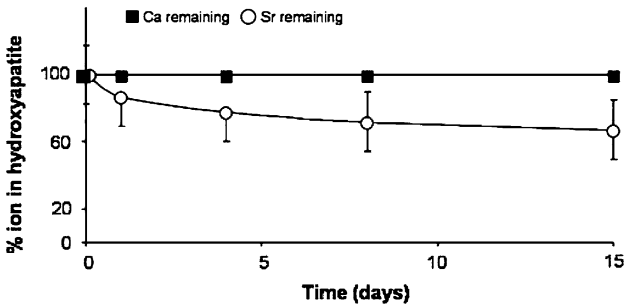
Strontium ranelate is currently used for the treatment of post-menopausal osteoporosis. Sr ranelate acts as an activator of osteoblast progenitor replication and differentiation, reduces the differentiation of osteoclasts and their activity. Sr bounds hydroxyapatite (HA)crystals by ionic exchanges. Little is known about Sr physico-chemical desorption in the human body. We have investigated the effects of Sr on HA crystal formation and its release in vitro.

Pellets of carboxymethylated poly(2-hydroxyethyl) methacrylate were used as a mineralization template. Pellets were incubated 1 week in a body fluid (BF) to induce mineralization, then 2 weeks in BF containing 0, 20, 40 or 60 μg/L of Sr ions to allow the growth and maturation of HA crystals. Ca and PO_4 were measured by spectrophotometry; Sr by mass spectroscopy after dissolution of HA crystals in HCl. Sr desorption was studied by transferring additional pellets in saline during 2 weeks; the saline was collected at regular intervals, ions were dosed in these saline samples and the fraction of ions remaining in calcospherites was computed. HA formed on the pellets was characterized by SEM and EDX and X-ray diffraction.

After 2 weeks in BF ± Sr, Ca and PO_4 were deposited as rounded shaped nodules (calcospherites). Sr addition had not effect on calcospherites shape or size. The Sr adsorbed in calcospherites was proportional of the Sr content in the BF. Desorption study shows that Sr was progressively released in saline, a new ionic balance being created after each saline collection, and the slope of Sr elution was similar whatever the initial concentration added in BF; about

30% Sr was released after 15 days in saline. These data were confirmed by EDX and XRD: Sr was adsorbed on the HA of calcospherites but was loosely bound and was released progressively and quickly.



Conflict of Interest: Non declared

Su-P405

THE SKELETAL RESPONSE TO ZOLEDRONIC ACID IS NOT AFFECTED BY RENAL IMPAIRMENT

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In patients with mild to moderate renal impairment [creatinine clearance (CrCl) > 20 mL/min and <60 mL/min], pharmacokinetic studies show a moderate 30–40% increase in serum levels of zoledronic acid 5 mg (ZOL). To test whether this difference had any impact on the tissue level response to ZOL infusion, we compared skeletal responses to ZOL in patients with baseline CrCl ≥60 mL/min (n = 4222) to the responses observed in patients with a baseline CrCl <60 mL/min (n = 3514) in the 3 year HORIZON-Pivotal Fracture Trial. Patients with baseline CrCl <30 mL/min were excluded. The analysis comprised bone histomorphometric parameters, bone mineral density (BMD), biomarkers of bone turnover, and morphometric and clinical fractures. Comparison of histomorphometry results showed that the reduction of bone turnover, as shown by activation frequency and mineralizing surface, was similar in the two groups (63% in patients with CrCl <60 mL/min vs. 56% in patients with CrCl ≥60 mL/min, and 88% in patients with CrCl <60 mL/min vs. 91% in patients with CrCl ≥60 mL/min, respectively). Microcomputed tomography indices related to bone structure were also unaffected by baseline CrCl. There was no association between treatment and baseline CrCl in the reduction of biochemical markers of bone formation (serum bone alkaline phosphatase and serum procollagen type I intact N-terminal propeptide) and bone resorption (serum C-telopeptides) over time (6–36 months) after treatment with ZOL (interaction P values = 0.09–0.67). Increases in total hip and spine BMD over time were independent of baseline CrCl (interaction P values = 0.10–0.63). Both subgroups showed highly significant relative risk reductions greater than 60%. However, there was a marginally significant treatment by baseline CrCl interaction for the reduction in morphometric vertebral fractures, with patients with renal impairment showing a lesser response (P = 0.0504). For hip fractures, there was no significant interaction with baseline CrCl (P = 0.58), with risk reduction of 34% in the <60 mL/min group and 46% in the ≥60 mL/min group. In conclusion, the 30–40% increase in bone exposure to ZOL in patients with mild to moderate renal failure has no significant impact on the skeletal response to ZOL, as assessed by histomorphometry, biomarkers of bone turnover, BMD or antifracture efficacy. The most frequent adverse events in patients receiving ZOL were pyrexia, myalgia, and bone and musculoskeletal pain.

Conflict of Interest: S. Boonen, Novartis, Grant/Research Support, Consultant, Speakers Bureau

Su-P406

RESPONSIVENESS OF BONE TURNOVER MARKERS TO TERIPARATIDE IS GREATER AND EARLIER FOLLOWING TREATMENT WITH RISEDRONATE COMPARED WITH ALENDRONATE: THE OPTAMISE STUDY

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Background: Previous studies have demonstrated differences in bone turnover marker (BTM) responsiveness to teriparatide (TPTD) following treatment with different antiresorptives. Alendronate (ALN) has been the primary comparator for these studies. Based on known pharmacologic differences, we investigated the responsiveness of TPTD in postmenopausal women treated with risedronate (RIS) or ALN for at least 2 years.

Methods: Prior RIS (n = 146) and prior ALN (n = 146) patients were stratified by duration of prior bisphosphonate therapy, discontinued their bisphosphonate and were treated with TPTD (20 mcg/d SQ) for 12 months. We measured N-terminal propeptide (P1NP, primary endpoint change at Month 3), osteocalcin (OC), bone-specific alkaline phosphatase (BAP), serum CTX and urine NTX in post-RIS and post-ALN groups at 0.5,1,2,3,4,5,6, and 12 months.

Results: Baseline characteristics were comparable between prior therapy groups, including duration of prior therapy, except for baseline BTMs which were higher in the prior RIS group (P < 0.05). Absolute changes in P1NP were significantly greater for prior RIS than prior ALN groups at 3 months, the primary endpoint of the study. Overall BTM changes were greater in the prior RIS group. Significant differences were observed as early as 2 weeks with NTX and through 6 months with OC. Results were consistent regardless of duration of prior therapy or baseline BTMs. TPTD was well-tolerated with a similar incidence of adverse events between groups.

Conclusions: In response to treatment with TPTD, subjects previously treated with RIS showed greater increases in BTMs compared with those previously treated with ALN. Our findings suggest differences between these bisphosphonates in which RIS allows the skeleton to be more responsive to the anabolic properties of TPTD.

Conflict of Interest: This study was supported by The Alliance for Better Bone Health (Procter & Gamble Pharmaceuticals and sanofi-aventis). The data were analyzed by John Stewart (sanofi-aventis, Laval, Canada), however all authors had complete access to the data. S. Boonen, Alliance for Better Bone Health, Grant/Research Support, Consultant; P.D. Delmas, Alliance for Better Bone Health, Grant/

Research Support, Consultant; R. Lindsay, Alliance for Better Bone Health, Grant/Research Support, Consultant; N.B. Watts, Alliance for Better Bone Health, Grant/Research Support, Consultant; P. Miller, Alliance for Better Bone Health, Grant/Research Support, Consultant; J. Stewart, Employee, sanofi-aventis; J.P. Bilezikian, Alliance for Better Bone Health, Grant/Research Support, Consultant

Su-P407

ASSESSMENT OF OSTEONAL POROSITY IN PAIRED ILIAC CREST BIOPSIES OF POSTMENOPAUSAL WOMEN WITH OSTEOPOROSIS BY 3D MICRO-CT: EFFECT OF RISEDRONATE TREATMENT

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Measurements of cortical porosity include 1) Haversian canals (diam. <50 μm), 2) the remodeling spaces of the osteons (diam. ~100 to 300 μm) in the intra-cortical envelope, and 3) larger cavities (diam. > 380 μm) primarily in the endocortical envelope. In this study, we evaluated the effect of risedronate on cortical porosity in transiliac biopsies from osteoporotic women. Paired biopsies taken at baseline and after 5 years of treatment with risedronate (5 mg/day, n = 28) or placebo (n = 21) were imaged by micro-CT at 8 μm isotropic resolution. The cavities were stratified by size, as determined by the minor axis length of the holes in the 2D slices. Porosity was averaged over 10 to 14 slices ~300 μm apart and was calculated as the area of cavities expressed as a percentage of the total cortical bone area. The porosity at baseline was not statistically different between placebo and risedronate for any cavity size. Five years treatment with risedronate significantly reduced porosity (median decreased by ~19%) relative to baseline for cavities of ≤320 μm diameter This effect is likely to be the result of reduced osteonal remodeling. In the placebo group, the median porosity from cavities in the same size range was not significantly different from baseline. The between group difference was significant for cavities ≤160 μm diameter. When cavities > 400 μm were included, the porosity was reduced in the risedronate group, but not significantly relative to baseline. These larger cavities were the result of trabecularization of the inner cortex leading to cortical thinning. The reduction in porosity was likely to be the result of reduced osteonal remodeling, but variability in the age-related changes in endocortical porosity make assessment of porosity difficult in this region of the cortex. The results suggest that risedronate reduces osteonal porosity by reducing the birth rate of new osteons or by filling in the remodeling spaces in osteons that existed prior to treatment.

Conflict of Interest: Borah, Dufresne, Nurre, Chmielewski, Phipps, Lundy, Wagner - Employees of Procter & Gamble.
Prof. Ego Seeman has consulting agreement with P&G
Dr. Mary Bouxsein acts as consultant to P&G.

Su-P408

OSTEOPOROSIS THERAPY FOLLOWING BONE DENSITOMETRY - PATIENT EXPECTATIONS AS DETERMINANTS OF DRUG INITIATION AND PERSISTENCE

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Persistence with osteoporosis therapy has been disappointingly low in the past, possibly related to poor patient perception of treatment benefits. We assessed health beliefs among patients with osteoporosis in relation to initiation of and persistence with bisphosphonates (BP).

We sent a mailed questionnaire to 1000 consecutive patients, who had been referred to DXA-scanning in a Danish osteoporosis clinic one year earlier to assess osteoporosis treatment, persistence and beliefs. Patients and GPs had received written information on diagnosis and treatment recommendations shortly after the DXA-exam, and patients were advised to discuss the implications of DXA-results and recommendations with their GP.

Of the 1000 patients, 717 responded to the questionnaire (72%, mean age 63.5 SD10.3). Initiation of pharmacotherapy had been recommended to 146 patients with osteoporosis. 83% began treatment, of these 90% with a BP. The self-reported persistence with BP one year after DXA assessment was 86%.

The likelihood of initiating a recommended BP treatment was assessed by multivariate logistic regression demonstrating significant association with beliefs in treatment benefits (OR 1.6, 95%CI 1.2–2.2, $p < 0.01$) but not with treatment barrier beliefs, sex or age of the patient. By contrast, no significant association with persistence and beliefs, sex or age could be established for discontinuation of treatment. Side effects were reported as the main reason for discontinuing treatment (7 of 9 patients).

Conclusion: While the encouragingly low rate of discontinuation among respondents limited the information available on factors associated with persistence, the study suggest that initiation of BP treatment is linked to patient perception of drug benefits, whereas this could not be shown for persistence. This highlights the need to improve communicative strategies, perhaps especially related to conveying the message of anti-fracture efficacy.

Conflict of Interest: None declared

Su-P409

FACTORS INFLUENCING THE DIAGNOSIS AND THE TREATMENT OF OSTEOPOROSIS FOLLOWING A FRAGILITY FRACTURE

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The objectives of this study were to determine predictors of osteoporosis (OP) diagnosis (DX) and treatment (TX) 6 to 8 months after fragility fracture.

At phase 1, women were recruited at cast or outpatient clinics 0 to 16 weeks after fracture. Consenting patients were administered a short questionnaire to classify them as having either experienced a fragility or traumatic fracture. At phase 2, 6–8 months following fracture, women were contacted again by phone to complete a questionnaire on demographic features, clinical characteristics and risk factors for OP. The DX (informed of OP and/or BMD measurement with diagnosis of OP) and TX (bisphosphonates, raloxifene, nasal calcitonin or teriparatide) rates of OP were determined via this questionnaire. This analysis included only women with a fragility fracture who were not receiving OP TX at phase 1.

Of the 1273 women who completed phase 1, 1001 sustained a fragility fracture; 818 were untreated at phase 1 and completed the phase 2 questionnaire. Overall, 79% of these participants had not received a DX of osteoporosis or were without OP TX at phase 2. The highest rate of DX and TX of OP occurred between 0–5 months following fracture and decreased thereafter. In multivariate analyses, the results of BMD tests before or after the fracture event ($p < 0.0001$) and mobility problems ($p = 0.03$) were the only variables that influenced the DX of OP. The BMD test result was the strongest predictor ($p < 0.0001$) of TX followed by the fracture site (hip, femur and pelvis; $p = 0.015$) and vitamin D supplements at the time of fracture ($p = 0.035$). No other risk factors for OP such as age, fracture history after age 40, family history of OP, and comorbidities had an influence the DX or TX rate. No demographic and clinical features or OP risk factors were significantly associated with the decision to initiate BMD testing following fracture.

Despite the evidence showing that the occurrence of a fragility fracture represents a greater risk of future fragility fracture than a low BMD, physicians based their decision to treat on BMD results and not on the clinical event (fragility fracture).

Conflict of Interest: LB, sanofi-aventis, P&G, Merck Frosst, Eli Lilly, Novartis, Amgen, Research support and/or consultant; JPB, Merck Frosst, P&G, sanofi-aventis, Novartis, Eli Lilly, Research support and/or consultant; KD, Consultant, Servier; SJ, none declared; LGSM, Glaxo-Smith Kline, Hoffmann-Laroche, Merck Frosst, P&G, sanofi-aventis, Novartis, Eli Lilly, Servier, Research support and/or consultant.

Su-P410

OUTCOMES IN ELDERLY WITH PROXIMAL FEMORAL OR HUMERAL FRACTURES TREATED IN AN ORTHOGERIATRIC REHABILITATION UNIT

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The benefits of orthogeriatric interventions on the recovery after a fracture of the proximal femur in elderly are well documented. Though fractures of the proximal humerus are associated with a marked decrease in functional independence, the influence of an orthogeriatric intervention in elderly with this type of fracture is still poorly documented. We performed a retrospective observational study in patients admitted in an orthogeriatric unit between 2002 and 2006, with a diagnosis of extra- or intracapsular hip fracture (HIP#, $n = 291$; mean age 83 ± 7 yrs) or proximal humeral fracture (HUM#, $n = 73$; mean age 81 ± 7 yrs). The rehabilitation program was administered by an interdisciplinary team, integrating walking balance exercises, muscle strengthening and activity of daily life training. The functional capacity during rehabilitation was evaluated by the functional independence measure (FIM) at admission (T0), after two weeks (T1) and just prior to discharge (T2: median 33 days). To further evaluate the functional outcomes, we separated motor items: (MOTOR FIM), upper and lower limb items (UPPER FIM, LOWER FIM). Values are means (SD) and differences tested using ANOVA and Student's t-test. At admission, age and motor functional independence scores were significantly higher in patients with HIP# (83.3 ± 6.9 vs. 81.5 ± 6.9 yrs; $p = 0.04$), MOTOR FIM 38.2 ± 11 vs. 33.6 ± 11; $p = 0.0017$). After a similar improvement in MOTOR FIM in both groups during the first 2 weeks (14.7 ± 13.6 vs. 11.7 ± 11.1; NS), there was a further increase in the score in HUM# elderly, whereas the change in HIP# was less (8 ± 10 vs. 11.8 ± 10; $p = 0.01$), so that at discharge, similar scores were observed for FIM and MOTOR FIM in both groups. The upper (5.3 ± 4.5 vs. 8.7 ± 5.4; $p < 0.0001$) and lower FIM (6.5 ± 4.6 vs. 9 ± 4.6, $p = 0.003$) improved in both HIP# and HUM# during the rehabilitation program. This study indicates that an orthogeriatric rehabilitation program improves functional performances in elderly with hip or humeral fractures, with different kinetics in the second part of the recovery period.

Conflict of Interest: None declared

Su-P411

OUTCOMES FROM A PATIENT EDUCATIONAL PROGRAM IN POSTMENOPAUSAL OSTEOPOROTIC WOMEN TREATED WITH TERIPARATIDE

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Background: In the last few years, multiple therapies and different ways of administration have emerged for osteoporosis treatment but adherence is often poor in clinical practice. Reasons seems to be multifactorial, so it is probably necessary to design strategies to improve long-term adherence regardless of the type of medication administered.

Material and Methods: A specific patient support program for monitor and help patients on treatment with teriparatide, was designed and conducted in Spain by Internationals SOS, supported by Lilly S.A. and managed by qualified nurse personnel trained in osteoporosis and the handling of the drug. Physicians interested in having their patients participating in the programme offered them the possibility of receiving a series of telephone calls. Patients, who agreed to participate in this program, received around 22 calls over the 18 months of treatment period, with the first four intended to review in detail the handling of the device and how the medication is administered. The other calls verify how the system is being used, announce the dispatch of informative material on the subject of osteoporosis and receive the patients' feedback on the programme (2 types of survey are carried out: one on the skill in using the device and the other on their satisfaction with the programme).

Results: Since July 2005 to August 2007, 6049 postmenopausal women have participated in the program. Regarding the use of the pen, 92% considered themselves to be skilful or very skilful. The persistence of patients followed up at least 3 months ($N = 5183$) was 90%; in patients followed up 6 months ($N = 4210$) was 85.4%; in patients followed up 12 months ($N = 2827$) was 80.3%; and in patients followed up 18 months ($N = 1656$) was 77.7%. 1211 patients (20%) discontinued treatment prematurely and the main reason, according reasons declared by the patient was due to medical events and physicians' decisions (69%). Regarding satisfaction with the programme 97% stated they are totally or quite satisfied with the programme.

>Conclusions: A regular interaction of health-care personnel with the patient, based on telephone contact, providing help with the pen device and dispatching information regarding their disease, results on high persistence with teriparatide treatment and as well skilfulness in the use of the pen.

Conflict of Interest: Mariluz Rentero and Marta Casillas are full-time employees of Lilly SA Spain. Alicia Lopez is full-time employee of SOS Assistance España

Su-P412

THE CATHEPSIN K INHIBITOR AAE581 INDUCES MORPHOLOGICAL CHANGES IN OSTEOCLASTS OF TREATED PATIENTS

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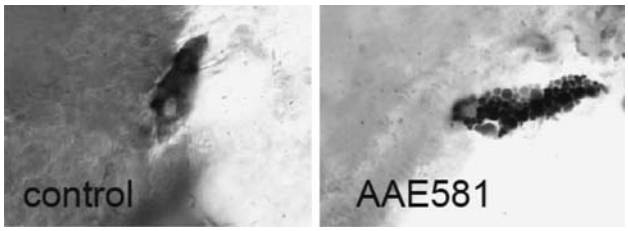
Cathepsin K is a cysteine protease inhibitor that is highly expressed in osteoclasts (OC) and it plays a key role in bone resorption. The enzyme has the capacity to degrade several key molecules of the bone matrix and particularly type I collagen. The enzyme is secreted under the ruffled border and accumulates in the resorption chamber where it degrades demineralized collagen. Tartrate resistant acid phosphatase (TRAcP) is an intracellular enzyme which terminates the degradation of collagen internalized in OC phagosomes. The cathepsin K inhibitor AAE581 was found to inhibit bone resorption in phase II studies and to reduce serum CTX-I bone marker in groups of treated patients.

675 postmenopausal osteoporotic patients received AAE581 (at 0, 5, 10, 25 or 50 mg/D) during one year. 11 patients had a transiliac bone biopsy, studied undecalcified after polymethylmethacrylate embedding. Histochemical detection of TRAcP was used to identify and count OC number. The histomorphometrist was not aware of the patient randomization at the time of analysis (Number of patients 0 mg: 2, 5 mg: 2; 10 mg: 4; 25 mg: 1 and 50 mg: 2). OC were unstained in one patient due to a failure in the fixation protocol. In the 10 remaining patients, OC were easily observable. Regardless of dose, treated patients exhibited a characteristic aspect of the OC cytoplasm which appeared filled of deeply stained brown vacuoles that make the cells look like bunches of grape. These round vacuoles could only be observed on TRAcP stained sections and seemed due to the accumulation of intracytoplasmic TRAcP. AAE581 did not induce OC apoptosis at any dosage but modified the OC morphology. We hypothesized that cathepsin K inhibition (inhibiting the extracellular collagen breakdown) is associated with an accumulation of intracellular TRAcP that could not be used to complete protein degradation.



Conflict of Interest: D. Chappard, Novartis Pharma AG, Grant Research Support
L. Mindeholm, Novartis Pharma AG, New Products Medical Director

Su-P413

EFFECT OF THREE DOSES OF STRONTIUM MALONATE (NB S101) ON MARKERS OF BONE TURNOVER AND BONE MINERAL DENSITY: THE STRONG STUDY

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Background/aim: Strontium ranelate (Protelos®, Servier) reduces fracture risk and is approved in Europe for the treatment of postmenopausal osteoporosis. The exact mechanism whereby strontium salts act on bone is not fully understood. NB S101 (strontium malonate, Osteologix, Inc.), a once-daily tablet formulation of strontium malonate, was evaluated in this phase II study. Methods: 289 postmenopausal women, age > 50, with low BMD were randomized to 5 groups and treated for 12 wks. The primary endpoint was 12-wk % change in CTX-1 with 3 daily doses of NB S101 (0.75 g, 1 g or 2 g) vs placebo or Protelos (2 g). 2° endpoints included: bone formation markers and spine and hip BMD. Results: Baseline (mean) age = 65 yr; spine BMD T-score = −1.9. The table 1 shows the mean percent change over 12 wks as compared to placebo (*$p < 0.05$, ** $p < 0.01$, ***$p < 0.001$). Adverse events included diarrhea, nausea, fatigue, back pain, headache, and muscle spasms. Potentially-related serious AEs with NB S101 included one event each of DVT, TIA, and allergic reaction. Conclusions: All 3 doses of NB S101 were well-tolerated and safely suppressed bone resorption while improving BMD. NB S101 was well tolerated and was effective on bone turnover and BMD in the short-term; it merits development as a treatment for postmenopausal osteoporosis.

Table 1

Treatment	NB S101 0.75 g	NB S101 1 g	NB S101 2 g	Protelos 2 g
Serum CTX %	13***	−16***	−22***	−9*
Serum PINP %	−7*	−8*	−13***	−3
Serum BSAP %	−4	−1	+7.2	+5.3
Spine BMD %	+2.3*	+2.0*	+2.7**	+2.0*
Total hip BMD %	+1.5*	+1.8**	+1.7*	+2.0**

Conflict of Interest: M. Weis, Osteologix, Employee
S. Christgau, Osteologix, Consultant

Su-P414

DIETARY CALCIUM, PHOSPHORUS, PROTEIN AND BONE METABOLISM IN KOREAN POSTMENOPAUSAL WOMEN

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There is a consensus that adequate calcium intake helps to prevent bone resorption and osteoporosis especially in person with low calcium diets. Even though people are concerned about bone health and are encouraged to take calcium supplementation, we believe that most people do not have enough calcium in their diets. The purpose of this study was to determine the nutritional status, urine calcium, bone markers and their relationship in Korean postmenopausal women. The subjects were 80 healthy female with postmenopausal osteopenia and osteoporosis (mean age, 57.8 y). Dietary calcium, phosphorus and protein were measured by 24 hrs recall method and urine calcium, bone markers were measured in fasting. The mean (SD) daily dietary intakes of Ca, P, protein were 616 (211.9) mg, 1002.6 (258.6) mg, 64.8 (16.7) g respectively. Only 6.5% of the participants had a calcium intake of more than 1000 mg. 55.4% of the subjects showed 25-OH D of less than 30 ng/mL. The subjects showing hypercalciuria (> 300 mg/d) was 25%. The percentage of hypercalciuric subjects was decreased into 8% after 6 months supplementation of 500 mg of elemental calcium and 400 IU of vitamin D. Multiple regression analysis showed that urinary calcium excretion was associated with serum P, CTX, 25-OH D but not dietary Ca. Serum Mg was negatively correlated with osteocalcin ($r = -0.286$, $p = 0.013$), CTX ($r = -0.289$, $p = 0.01$). CTX, alkaline phosphatase were significantly decreased and spine BMD significantly increased after 6 months of supplementation of calcium and vitamin D. Calcium and vitamin D supplementation is safe and important for bone health, but is not still enough in most postmenopausal women for prevention of osteoporosis.

Conflict of Interest: None declared

Su-P415

ACUTE AND CHRONIC EFFECTS OF PTH(1–84) TREATMENT ON HEART RATE AND CARDIAC REPOLARIZATION IN POSTMENOPAUSAL WOMEN WITH OSTEOPOROSIS

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Background: PTH(1–84) is a recognized treatment option for osteoporosis. Potential elevation in serum calcium, which may affect cardiac function, is an anticipated consequence of PTH therapy. Endogenous and exogenous PTH have additionally been shown experimentally to influence cardiac function independently of changes in serum calcium.

Material and Methods: A pharmacokinetic acute calcemic response study was performed as part of the CAP study to investigate consequences of PTH therapy on cardiac function. 106 postmenopausal osteoporotic woman were randomized to either Group 1-calcium 700 mg (N = 35) daily; Group 2-PTH(1–84)100 μg daily (N = 36); or Group 3-PTH(1–84) + calcium daily (N = 35). All subjects received 400 IU vitamin D daily. ECG intervals, heart rate (HR), serum calcium and PTH levels were determined before and 1 month after initiation of treatment, at which time PTH levels, serum calcium and complete ECG tracings were obtained at frequent intervals within a 24 hour period after treatment with a single dose of PTH (immediate effects). Results: Chronic effects were reflected in the differences between baseline and predose measurements made after 1 month of treatment. QTcF (Fridericia rate correction) measured at the same pre-treatment morning time point decreased by 9 msec from baseline to month 1. Regression modeling indicated that this decrease was significant and inversely related to calcium level, but not to treatment with PTH. While there was no further decrease in QTcF during treatment at month 1, regression modeling of data collected during 24 hours at month 1 continued to show a significant relationship between QTcF and serum calcium (immediate effects). We also observed an increase in HR on ECG at one hour in subjects receiving PTH (+6 BPM in Group 2 and +5 BPM in Group 3, as compared to Group 1, which coincided with the maximal increase in serum PTH and minimal change in serum calcium. There were no changes in the other measured ECG numerical data (QRS, PR and QRS axis), acutely or chronically. These results suggest that PTH has a, modest, direct, immediate effect on heart rate, but its effects on cardiac repolarization manifest both immediately and chronically, are indirect, resulting from increased serum calcium concentration.

Conclusion: The data suggest that the direction and extent of change in ECG parameters are not of clinical concern or relevance, a conclusion that is consistent with the cardiac safety profile for PTH(1–84).

Conflict of Interest: J.O. Clausen, Employed by Nycomed

Su-P416

EARLY AND LATE CLINICAL AND INSTRUMENTAL RESULTS OF ADJUVANT THERAPY WITH TERIPARATIDE IN A SUBCLINICAL CASE OF OSTEOPOROTIC VERTEBRAL FRACTURES

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Background: Teriparatide (TPTD)-treated women have reduced risk for vertebral fragility fractures up to 18 months (1) respectively after discontinuation of treatment. This is due to for 30–41% by increases in lumbar spine BMD. The remaining fracture risk reduction is caused by improvements in non-BMD determinants of bone strength (2).

We report the case of adjuvant use of TPTD after osteosynthesis of a lumbar vertebrae that has been useful for a care of subclinical osteoporotic dorsal fractures.

Pre-treatment: 63 years old presenting a traumatic fracture of L1 without any anti resorptive therapy. She was undergone to decompression and osteosynthesis with instrumentation of three levels. Then she was remain protecet in a brace for three months. In contemporary she assumed TPTD daily. At third month she began the physiochinesitherapy. But at a distance of six months she started to feel a back pain for which she was undergone to a new radiographic scans with the evidence of dorsal fractures.

Diagnostic tests: PTH, osteocalcin, bone alkaline phosphate, calcium, phosphorus, 25-hydroxyvitamin D and CTX serum levels and urinary calcium, phosphorus, cross-links were measured at the beginning, 1,3, 6, 12, 18 months later. Bone mineral density measurements at the lumbar spine and hip, as well as lateral thoracic and lumbar spine X-rays were obtained at baseline and after 3, 6 ,12, 18 months.

Treatment: Daily subcutaneous TPTD injection (20 microg), adisterolo 3 gtt per day and Calcium carbonate 1g per day for 3 months after operation and then from 6th months for another 15 months.

Follow up: During the follow up reduction of pain was determined at 3rd month and the normality of biochemical markers suggested to suspend TPTD. The vertebral height and endplate angles were measured to assess the restoration of the sagittal alignment. But at 6th month a recidivant painful symptomatology of the back obliged to a new X-rays that made in evidence two dorsal fractures. The biochemical markers revealed an unsuspectable increasing of resorption parameters. Also the BMD was modified. So for 6 weeks she dressed the brace and in the meanwhile she began to assume TPTD without further modification of therapeutic program. After 18 months densitometric and biochemical parameters were changed positively and the patient had recovered a good quality of life. Ref. 1Lindsay R Arch Int Med 2004 2Chen P JBMR2006

Conflict of Interest: None declared

Su-P417

EFFECTS OF TERIPARATIDE ON DISTAL RADIAL FRACTURE HEALING IN POSTMENOPAUSAL WOMEN: A RANDOMIZED DOUBLE-BLINDED STUDY

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The purpose of this randomized, double-blind, placebo controlled trial was to compare the effect of treatment with teriparatide (TPTD), recombinant human parathyroid hormone (1–34), 20 or 40 mcg/day vs. placebo on time to radiographic healing in women with distal

radial fractures. Postmenopausal women, 45–80 years of age, who had sustained a unilateral, dorsally angulated fracture of the distal radius and had received conservative treatment, were randomized within 10 days of fracture to treatment: placebo (n = 34), TPTD20 (n = 34), or TPTD40 (n = 34). The 8-week treatment period was followed by 8 weeks of follow-up, and a 36-week safety extension. One hundred one patients were followed to complete healing. The estimated median healing time from fracture to first radiographic evidence of complete cortical bridging in at least 3 of 4 cortices was evaluated using nonparametric survival analyses and bootstrap method: placebo, 9.1; TPTD20, 7.4; TPTD40, 8.8 weeks, overall p = 0.01. Hypotheses for TPTD40 vs. placebo and TPTD20 vs. placebo were tested sequentially using a gate-keeping strategy. Median time to healing was not different for point estimates (95% CI for difference) between TPTD40 and placebo (−1.1 to 0.6 weeks) p = 0.4. Although time to healing was shorter for TPTD20 than for placebo (−2.8, to −0.6 weeks) p = 0.003, the gate-keeping design for statistical analysis used to control for type I error precludes making an inference of relevance. These results warrant further study. No safety issues were identified. This study was supported by Eli Lilly and Company.

Conflict of Interest: P. Aspenber, Eli Lilly and Company, Grant Research Support H. Genant, Eli Lilly and Company, Grant Research Support A. Nino, Eli Lilly and Company, Full-time employee T. Johansson, Eli Lilly and Company, Grant Research Support K. See, Eli Lilly and Company, Full-time employee K. Krohn, Eli Lilly and Company, Full-time employee P. Garcia, Eli Lilly and Company, Grant Research Support C. Recknor, Eli Lilly and Company, Grant Research Support T. Einhorn, Eli Lilly and Company, Grant Research Support G. Dalsky, Eli Lilly and Company, Full-time employee M. Lakshmanan, Eli Lilly and Company, Full-time employee

Su-P418

RELATIONSHIP BETWEEN SERIAL BIOCHEMICAL MEASUREMENTS AND BMD RESPONSE TO PARATHYROID HORMONE THERAPY

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Response to PTH is usually assessed by measuring BMD pre and post treatment. Our aim was to examine whether certain biochemical variables were predictors of the BMD response to treatment with PTH, in 50 consecutive patients. Baseline levels of GFR,intact PTH, 25(OH)D, P1NP and Osteocalcin (OC) were measured. We also measured changes in P1NP and OC from baseline to 3 and 12 months respectively. These variables were correlated with the percentage change in BMD at the lumbosacral spine. Variables were also divided into quartiles, and comparisons made between the highest quartile and lowest quartile in terms of BMD gain. The mean age was 72; 46 patients were female. The baseline levels of GFR, intact PTH and 25(OH)D were not related to the BMD response. Neither did age have an effect. Baseline levels of P1NP and OC correlated positively and significantly with BMD change at the spine (r = 0.435, p < 0.002 and r = 0.34, p = 0.01 respectively). Patients in the highest quartile of baseline P1NP (mean 76 mug/L) had a twofold greater gain in BMD at the spine than patients in the lowest quartile(mean 13 mug/L). Patients in the highest quartile of baseline OC (mean 41 mug/L) had a fourfold greater gain in BMD than patients in the lowest quartile (mean 11 mug/L). The % increase from baseline to 3 month P1NP & to 12 month OC were not correlated with BMD gain.

The level of bone turnover at baseline is strongly predictive of BMD gain at the lumbosacral spine in patients treated with PTH.

Table 1

	Highest Q % BMD inc	Lowest Q % BMD inc	p	r vs % BMD inc	P
P1NP baseline	16.3	6.9	0.04	0.435	0.0018
%change P1NP 0–3	11.9	8.7	NS	0.099	NS
OC baseline	19.0	4.8	0.001	0.34	0.01
%change OC 0–12	4.86	13.7	NS	−0.04	NS
PTH baseline	10.87	9.96	NS		
25(OH)D baseline	10.3	11.3	NS		
GFR ml/min	11.0	7.6	NS		
Age	12.6	11.6	NS		

Conflict of Interest: None declared

Su-P419

THE EFFECT OF PREVIOUS BISPHOSPHONATE USE ON BONE MINERAL DENSITY RESPONSE TO TREATMENT WITH PARATHYROID HORMONE

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Parathyroid hormone is the first anabolic agent available for the treatment of severe osteoporosis. Bisphosphonates, which had previously been the mainstay of treatment, act by shutting down bone turnover. We aimed to compare the bone mineral density(BMD) benefit of PTH in patients previously treated with bisphosphonates (BIS+) and treatment naive patients (BIS-)

We measured total hip and lumbar spine BMD, at 0 and 18 months, in 52 consecutive patients treated with teriparatide.

Mean age was 74; 48 were female, 4 were male.

20 were BIS+ (mean 20 months treatment(range 4–60))

32 were BIS−

Baseline BMD was comparable in both groups.

BMD improved in both groups, however the increase in BIS- patients BMD was significantly greater. See table 1.

Treatment naive patients made highly significantly greater BMD gains than previouosly bisphosphonate treated patients. Patients with severe established osteoporosis should be considered for initial treatment with PTH, before staring bisphosphonates, to achieve maximal BMD benefit.

Table 1

	BIS + Mean (95%CI)	BIS - Mean (95%CI)	p
Baseline BMD Spine (g/cm2)	0.729	0.742	NS
Baseline BMD Hip (g/cm2)	0.646	0.691	NS
%increase in Spine BMD	4.0 (1.6–8.0)	14.6 (10–19)	0.004
%increase in Hip BMD	1.6 (0.4–3.1)	6.4 (3.8–9.2)	0.006

Conflict of Interest: None declared

Su-P420

COMPARISON BETWEEN THE EFFECTS OF DAILY AND MONTHLY TREATMENT WITH IBANDRONATE IN OSTEOPENIC MALE RATS DUE TO ANDROGEN LACK

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Ibandronate (IBN) is a highly potent bisphosphonate which is approved in many countries for the treatment and prevention of postmenopausal osteoporosis and metastatic bone disease. However, little is known about the effect of IBN in male osteoporosis due to androgen deficiency. In particular, the clinically attractive mode of intermittent administration of IBN has not yet been investigated in male osteoporosis.

The aim of this work was to study the ability of IBN, administered daily (d) or monthly (m), to revert the deleterious effects on bone produced by orchidectomy. Forty, 9 month-old, male Wistar rats were sham-operated (SHAM) or orchidectomized (OQX). All animals were left untreated for 6 months after surgery, and subsequently submitted to 4 groups, which were administered subcutaneously over a duration of 20 weeks with either placebo (SHAM; $n = 10$) and (OQX; $n = 10$) or with two regimens of IBN (Roche Diagnostics GmbH, Germany): 1 μg/Kg/day (OQX + IBNd; $n = 10$) or 28 μg/Kg/28 days (OQX + IBNm; $n = 10$).

After sacrifice, bone mineral density (BMD) was determined in the lumbar spine and in the whole left femur by DEXA in situ. Computerized microtomography (μCT) in femur by Skyscan 1172 was also performed.

OQX group presented values of lumbar and femoral BMD significantly lower than SHAM group ($p < 0.05$). Both treatments, IBNd and IBNm, restored the loss of BMD due to orchidectomy. Results from μCT showed a significant decrease in BV/TV ($p < 0.05$), trabecular number (Tb.N; $p < 0.01$) and an increase in trabecular separation (Tb.Sp; $p < 0.001$) in the OQX vs SHAM. IBNm treatment restored significantly (OQX + IBNm vs. OQX, $p < 0.01$; OQX + IBNm vs. SHAM, n.s.) all these changes observed by μCT in OQX group. IBNd treatment restored completely Tb.Sp (OQX + IBNd vs. OQX, $p < 0.001$; OQX + IBNd vs. SHAM, n.s.) and partially BV/TV (OQX + IBNd vs. OQX, n.s.; OQX + IBNd vs. SHAM, n.s.), but not Tb.N (OQX + IBNd vs. OQX, n.s.; OQX + IBNd vs. SHAM, $p < 0.05$).

The above results suggest that monthly treatment is more effective than daily treatment in order to restore changes in bone quality due to androgen lack in the rats, in spite of the fact that the same cumulative total dose per animal is administered.

Conflict of Interest: F. Bauss, Roche Diagnostics GmbH; Scientific Director, Assoc. Prof. for Exp. Pharmacology.

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Su-P421

RESPONSIVENESS TO TERIPARATIDE IS GREATER IN PATIENTS PREVIOUSLY TREATED WITH RISEDRONATE THAN IN THOSE PREVIOUSLY TREATED WITH ALENDRONATE: THE OPTAMISE STUDY

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Background: The anabolic response to teriparatide (TPTD) is blunted or delayed in patients previously treated with alendronate (ALN). It is not known if this effect is the same with other bisphosphonates. We evaluated the anabolic effect of TPTD in postmenopausal women previously treated for at least 2 years with ALN or risedronate (RIS).

Methods: Prior RIS ($n = 146$) and prior ALN ($n = 146$) subjects were stratified by duration of prior therapy, discontinued their bisphosphonate and received TPTD (20 μg/d SQ) for 12 months. We measured bone turnover markers (BTM) and BMD by DXA and QCT, and investigated the relationship between early changes in P1NP with 12-month changes in QCT.

Results: BTMs were higher in the post-RIS group at baseline ($P < 0.05$). The prior treatment groups were comparable for all other key baseline characteristics including BMD and duration of prior therapy. Prior RIS changes in BTMs were significantly greater from 2 weeks through 6 months ($P < 0.05$; primary endpoint of P1NP change at Month 3, $P < 0.001$), and for BMD by DXA at Month 12 at the spine ($P < 0.05$) and hip ($P < 0.01$). Results were not related to duration of prior bisphosphonate therapy, baseline BTMs, or baseline BMD. Prior RIS subjects also showed a greater increase in QCT of trabecular bone at the spine at 12 months. Spine QCT changes correlated with P1NP changes at 3 months ($r = 0.47$). TPTD was well-tolerated with a similar incidence of adverse events between groups.

Conclusions: When switched to teriparatide, subjects previously treated with risedronate showed a greater QCT response that correlated with a more pronounced, early increase in P1NP. Our findings support differences between these bisphosphonates that affect subsequent response to the anabolic effects of TPTD.

Conflict of Interest: This study was supported by The Alliance for Better Bone Health (Procter & Gamble Pharmaceuticals and sanofi-aventis). The data were analyzed by John Stewart (sanofi-aventis, Laval, Canada), however all authors had complete access to the data.

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Su-P422

BONE MINERAL DENSITY AND BIOCHEMICAL MARKER RESPONSE RATES IN POSTMENOPAUSAL WOMEN AFTER TREATMENT WITH ZOLEDRONIC ACID

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Clinical monitoring of responses to treatment is very important, and the choice of response variable depends on the ability of that given variable to detect clinically relevant responses. In the Health Outcomes and Reduced Incidence with Zoledronic Acid Once Yearly—Pivotal Fracture Trial (HORIZON-PFT), 7736 women were treated with three annual infusions of zoledronic acid 5 mg (ZOL), resulting in significant reduction of osteoporotic fractures of the spine, hip and appendicular skeleton. In this study, bone mineral density (BMD) was measured annually in all women. In a subset of women, bone turnover markers [C-telopeptides (CTX), bone alkaline phosphatase (bone ALP) (n = 605) and procollagen type I intact N-terminal propeptide (PINP) (n = 1248)] were measured in serum at baseline, 12, 24 and 36 months. CTX and bone ALP were also measured at 6 months. We used these measurements to assess the response rates to treatment in two ways: 1) fraction of patients showing the expected deviation from baseline and 2) fraction of patients showing a deviation from baseline in excess of the least significant change (LSC) for the given biomarker. LSC is the least change needed to be 95% certain that a change has truly occurred. Lumbar spine BMD was more sensitive than total hip BMD, and showed increases over baseline in 88.8%, 92.4%, 93.2%, and 94.8% of patients at 6,12, 24, and 36 months respectively. Assuming an LSC of 3.0%, the response rates at the same time points were 50.4%, 61.9%, 83.4% and 83.6%, respectively. Among the biochemical markers, serum CTX (S-CTX) was the most sensitive. The fractions of patients showing a decrease from baseline were 96.0%, 94.5%, 89%, and 86.2% at 6, 12, 24, and 36 months, respectively. Assuming an LSC for CTX of 60%, the response rates were 72.7%, 52.5%, 46.6% and 44.0%, respectively. In conclusion, assessment of S-CTX at 6 months provides excellent early monitoring of patient responses to ZOL, with 96% showing a decrease and 72.2% showing a clinically significant reduction. BMD increases in the spine were seen in 92.4% of patients at 12 and 93.2% at 24 months, while clinically significant BMD responses were seen in 61.9% at 12 months and 83.4% at 24 months. The most frequent adverse events in patients receiving ZOL were pyrexia, myalgia, and bone and musculoskeletal pain.

Conflict of Interest: PD. Delmas, Novartis, Consultant
I. Reid, Novartis, Consultant
S. Adami, Novartis, Consultant
P. Sambrook, Novartis, Consultant
EF. Eriksen, Novartis, Shareholder
P. Mesenbrink, Novartis, Shareholder
R. Eastell, Novartis, Consultant

Su-P423

BISPHOSPHONATE THERAPY AND HIP FRACTURES WITHIN THE RISEDRONATE AND ALENDRONATE (REAL) COHORT STUDY: SUBGROUP WITH PRIOR FRACTURE

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In a prior obserservational study of 33,830 women 65 and over, initiating weekly dosing of bisphosphonate, the incidence of hip fracture in the first year of therapy for patients on risedronate (0.37%) was lower (adjusted rate ratio = 0.57) than patients on alendronate (0.58%) 1. To further assess the homogeneity of this observation across patients at different levels of fracture risk, we used a subgroup within this study who had a diagnosed fracture in the year prior to initiating bisphosphonate therapy. The original study population was identified within records of health services utilization and included new users of weekly dosing of risedronate or alendronate. In the one year prior to initiating therapy for this population, 6.0% had a diagnosed nonvertebral fracture, 3.1% had a diagnosed vertebral fracture, and 8.4% (n = 2845) had either fracture which defined the subgroup for analyses. Cox proportional hazard modeling was used to compare hip fracture incidence during year one of therapy. Compared to the original study population, the subgroup with a fracture prior to initiating therapy was older and had more risk factors for fracture (table 1). Within this subgroup, during the first year of therapy, the incidence of hip fracture for patients on risedronate (0.73%) was lower (adjusted rate ratio 0.34, 95% CI 0.13−0.92) than patients on alendronate (1.93%). Regardless of fracture history, patients receiving risedronate had lower rates of hip fractures during their first year of therapy than patients receiving alendronate. 1Silverman et al.OI 2007 18:25.

Table 1 Baseline Data Original Study vs Subgroup with Fracture

Subjects	Orginal Study RIS 12,215	Population Aln 21,615	Subgroup w/ RIS 1,057	Fracture Hist ALN 1,788
Age	75	75	79	78
Meds, 6 Mos History - Mean	4.0	3.6	4.8	4.5
GI Medication %	26.2	20.1	33.4	28.7
Estrogen %	17.2	16.5	8.3	10.6
Steroid %	10.3	8.5	13.7	11.8
Office Visits Mean	5.6	5.1	8.2	7.7
Hospital Visits (%)	8.2	8.2	33.2	35.6
RA Diagnosis (%)	2.7	2.7	4.0	3.2

Conflict of Interest: PD Delmas, S Silverman, NB Watts, R Lindasy; consultants P&Gt

Su-P424

EFFICACY OF 18 MONTHS TERIPARATIDE TREATMENT IN POSTMENOPAUSAL WOMEN WITH 2 OR MORE PRIOR VERTEBRAL FRACTURES AND A FEMORAL NECK FRACTURE: BMD, OCCURRENCE OF NEW FRACTURES AND BACK PAIN EVALUATION

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Osteoporosis is a cause of significant morbidity and mortality in postmenopausal women. It causes a progressive bone loss and qualitative alterations in the macro and micro architecture of bone: vertebral and neck femoral fractures are the most common osteoporotic fractures.

Objective: the aim of this study was to evaluate the efficacy of teriparatide in plurifractured postmenopausal women monitoring the BMD and the occurence of new fragility fractures. Teriparatide, a bone forming agent for the treatment of osteoporosis, increases BMD, improves both cortical and trabecular bone microarchitecture and reduces the risk of fracture in women with osteoporosis.

Materials and Methods: Inclusion criteria: postmenopausal women (since at least 5 years), with spine or hip BMD T-score $< -2,5$, with the following previous fragility fractures: 2 or more atraumatic vertebral fractures and 1 or more femoral neck fractures. Exclusion criteria: ipersensibility to teriparatide, primitive hyperparathyroidism, radiotherapy, diagnosis of neoplasia at time of enrollement. Forty (40) patients were recruited and treated with teriparatide 20 μg/day self administered subcutaneously once daily. We recruited 25 patients with 3 severe vertebral fractures and 15 patients with 2 severe fracture and a femoral neck fractures. Patients were evaluated by Dexa (Lunar-DPX-P) analysis to asses the lumbar spine and femoral neck BMD, by X-ray of the spine and by VAS scale to evaluate back pain at baseline and at 6 and 18 months of the treatment.

Results: 6 patients were lost during the study and the remaining patients were evaluated after 18 months of treatment the mean BMD increase was 9.7% at spine and 2.8% at femoral neck. New fragility fractures occurred in 5 percent of the patients and the reduction of back pain was observed in 85%of the patients.

Conclusion: Teriparatide increases bone mineral density, reduces back pain and the occurrence of new fragility fractures.

Conflict of Interest: None declared

Su-P425

EFFECTIVENESS OF ONE HOME VISIT BY AN OCCUPATIONAL THERAPIST IN THE PREVENTION OF FALLS: A QUASI-RANDOMIZED CONTROLLED TRIAL IN ELDERLY WOMEN WHO SUSTAINED A HIP FRACTURE

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Aim: Given the high risk for falls and new fractures, hip-fracture survivors are a main target for fall-preventive interventions. However, the implementation of preventive programs in this group of individuals at high risk is low, and few studies have focused on hip-fracture patients to optimize fall prevention strategies. Our aim was to assess the effectiveness of one home visit by an occupational therapist in the reduction of fall risk after hip fracture in elderly women.

Methods: 95 of 119 women aged 60 years or older, living in the community, who sustained a fall-related hip fracture were included in this quasi-randomized controlled trial. The women were allocated to intervention or control groups alternately. All the women underwent a multidisciplinary programme targeted at fall prevention during in-patient rehabilitation. Additionally, the intervention group received one home visit by an occupational therapist at a median of 20 days after discharge. Falls were recorded at a six-month follow-up.

Results: Thirteen of the 50 women in the control group sustained 20 falls during 9231 days whereas six of the 45 women in the intervention group sustained nine falls during 8970 days. After adjustment for observation periods and functional ability assessed by using Barthel Index scores, we found a significantly lower proportion of fallers in the intervention group: the odds ratio was 0.260 (95% CI 0.074 to 0.914, $p = 0.036$).

Conclusion: One home visit by an occupational therapist after discharge from a rehabilitation hospital significantly reduced the risk of falling in a sample of elderly women following a fracture of the hip.

Conflict of Interest: None declared

Su-P426

SERUM CALCIUM VALUES IN POSTMENOPAUSAL WOMEN WITH PRIMARY OSTEOPOROSIS AFTER ONE MONTH OF TREATMENT WITH PTH(1–84)

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Background: Full-length human recombinant parathyroid hormone represents one member of a class of potent anabolic agents currently used in the treatment of primary osteoporosis in postmenopausal women. Physiological PTH increases serum calcium. We report the total serum calcium values after one month of treatment with PTH(1–84) in the PEAK (Preotact after a brEAK) trial.

Methods: The PEAK study is an open label, international multi centre, parallel group, phase III b, randomised trial, investigating lumbar spine BMD changes in postmenopausal women with primary osteoporosis. In the first year of the trial all patients are treated with PTH(1–48). Total serum calcium levels are measured at month 1, 6, and 12 at least 20 hours after prior PTH(1–84) injection. 390 postmenopausal women aged more than 50 years with primary osteoporosis with a lumbar spine T-score < -3.0 SD without abnormalities of calcium metabolism will be enrolled into the study. Women with serum calcium values > 2.55 mmol/l at baseline are excluded. By 1 January 2008 we expect all patients to be enrolled in the study.

Results: By 10/2007 248 patients had reached one months of treatment with PTH (1–84). The majority (86%) of patients had a serum calcium below the upper limit of normal. 9% had mild elevations in serum calcium (> 2.55–2.67 mmol/l) and only 5% had elevations of serum calcium > 2.67 mmol/l. Mean pre-PTH serum calcium was 2.3 mmol/l in those who had serum calcium levels $<$ or $=$ 2.67 mmol/l and 2.4 mmol/l in those whose serum calcium exceeded 2.67 mmol/l.

Conclusion: In this interim analysis the majority of postmenopausal women with primary osteoporosis treated with PTH (1–84) for one month had a normal total serum calcium level.

Table 1 Total s-calcium levels after one month of PTH treatment

Total s-calcium	≤2.67 mmol/l	> 2.67 mmol/l
Number of patients (%)	235 (95)	13 (5)
Mean (mmol/l)	2.4	2.7
Min, Max. (mmol/l)	2.15, 2.67	2.68, 3.05

Conflict of Interest: M. Diaz-Curiel, Nycomed consultant, Roche/GSK, Servier and Eli Lilly Lecture fee

Su-P427

WOMEN LIVING IN NURSING/RESIDENTIAL HOMES: OSTEOPOROSIS TREATMENT, SURVIVAL AND INCIDENCE OF HIP AND OTHER FRACTURES

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Patients in institution tend to have a high prevalence of osteoporosis, but treatment is infrequently started, possibly because survival is thought to be limited.

We have studied survival in Nursing/Residential Homes (N/RH) and whether forearm bone density (BMD) would identify those who might benefit from osteoporosis treatment, and whether risk factors are important in predicting treatment initiation.

123 women (84.8 ± 7.3 yr) had forearm scanning in 2000–02. A bisphosphonate (BP) was suggested if BMD was low (<0.34 g/cm^2, Jones & Davie 1998). Women were re-visited 6.9 ± 0.4 yr later to ascertain survival, whether a BP was started and hip fracture incidence. Statistical analysis for logistic regression, ANOVA, Chi squared or binomial theorem were calculated using SPSS vs14.

N/RH residents had a low BMD z score (-0.335, $p < 0.05$), BMD in 65% being <0.340 g/cm^2. In 6 survival bands (<1 yr, 1–2 yr, 2–3, 3–4, 4–5 and > 5 yr) women with BMD > 0.34 g/cm^2 had survival rates of 95%, 95, 90, 90, 87.5 and 42.5%; in those with low BMD survival was 97.1%, 90, 84.3, 84.3, 78.6, 65.7%. Backward logistic regression analysis showed residents' survival 3 yr post admission to N/RH was associated with younger age at entry (OR 1.33, 95% CI 1.10–1.61) and a tendency to a higher BMD z score (39, 0.97–1636.04).

Initiation of a BP was investigated in 83 women. 6% of women (61% with low BMD) had started on a BP after the bone scan. Younger age at bone scan (OR 1.30, 95% CI 1.02–1.66) was associated with initiation. Previous fracture and weight were not associated with starting a BP.

Fracture history was collected for 73 women. Hip fracture occurred after entry to N/RH in 7% of women with low BMD at yr < 1, 1–2, > 5 yr (1 each) and in 1 woman in the middle BMD group between 1–2 yr. No hip fracture patient had previously been given a BP. Death occurred 0.5 yr (median) post hip fracture. Twelve fractures (10 osteoporotic) occurred in 9 women (27%) with low BMD, after a median 4 yr stay. 13% of women with high BMD sustained fractures.

Over 80% of women in N/RH survive up to 3 yr. Two thirds have osteoporotic BMD, but few began treatment. Of those with low BMD, 6% had hip fractures and 21% other fractures, mostly after a time long enough for BP treatment to have positive effects. As most subjects in N/RH survive long enough for BP treatment to influence hip and other fracture, greater emphasis on treatment is needed in N/RH subjects.

Conflict of Interest: None declared

Su-P428

INTERACTION OF THE ACTIVE SITE THREONINE 201 SIDECHAIN WITH THE NITROGEN OF NITROGEN-CONTAINING BISPHOSPHONATES IS NOT ESSENTIAL FOR SLOW TIGHT INHIBITION TO FARNESYL PYROPHOSPHATE SYNTHASE

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The major molecular target of nitrogen containing bisphosphonates (N-BPs) is the mevalonate pathway enzyme Farnesyl Pyrophosphate Synthase (FPPS). However the exact mechanism of inhibition has not been determined. Recent crystallographic and kinetic studies have shown that potent N-BPs inhibit the enzyme by initially competing with one of the substrates, geranyl pyrophosphate (GPP), for a binding site, followed by a slow isomerisation of the enzyme structure to form a tightly bound complex. This tightly bound complex is presumably maintained by the hydrogen bonding interactions of the bisphosphonate side chain nitrogen with a threonine hydroxyl residue and also the carbonyl oxygen of the adjacent lysine residue (Lys200/Thr201) in the active site of the enzyme. In our present study we aimed to further investigate the nature of the interactions of the bisphosphonate nitrogen and these highly conserved residues. We constructed a mutant of FPPS, replacing the threonine 201 with an alanine residue (T-A Mutant). The mutant had an increased affinity (Km) for GPP with 1.5 μM for T-A compared to 2.1 μM in the wildtype. The Km for the second substrate, Isopentenyl Pyrophosphate was increased from 1.8 μM in the wildtype to 17.7 μM in the TA mutant. The maximal speed of the reaction was also reduced with Kcat of 0.34 S^{-1} for T-A compared to 0.42 S^{-1} for wildtype. Replacement of the Thr with an Ala increased the final inhibition constant (Ki) of zoledronate from 0.07 nM to 2.17 nM and also reduced the isomerisation constant (ICON), a measure of the reversibility of the inhibition, from 1224 to 37.6. Conversely, the effect of this mutation on the inhibition by risedronate was to decrease the Ki from 0.34 nM to 0.16 nM and increase the ICON from 237 to 1000. The inhibition of FPPS by NE58022 (Ki = 302 nM), an analogue of risedronate with no sidechain nitrogen was unchanged in the T-A mutant. Similarly, the inhibition of FPPS by ibandronate, an N-BP which is not predicted to make an interaction with Thr201 was only slightly affected with the final Ki changing from 3.6 nM in the wild type to 5.5 nM in the T-A mutant. In conclusion, we show that the interaction of the N-BP sidechain nitrogen with Thr 201 is not essential for inhibition, but can be responsible for the higher potency of many analogs. It may be that the interaction of the sidechain nitrogen with the carbonyl oxygen of Lys200 is the more important of the two possible hydrogen bonding interactions with FPPS for N-BPs.

Conflict of Interest: None declared

Su-P429

EFFICACY OF FOSAVANCE 5600® (ONCE WEEKLY ALENDRONATE 70 MG & VITAMIN D3 5600 IU COMBINATION) RELATIVE TO RISEDRONATE 35 MG WEEKLY FOR THE PREVENTION OF FRACTURES IN POSTMENOPAUSAL OSTEOPOROSIS: RESULTS OF A MIXED-TREATMENT INDIRECT COMPARISON META-ANALYSIS

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To evaluate the efficacy of Fosavance 5600® relative to risedronate in the prevention of fractures in women with postmenopausal osteoporosis and women with a history of vertebral fractures.

A systematic review of articles identified using MEDLINE and EMBASE search up to June 2007 was performed. Randomized controlled trials that assessed once weekly alendronate 70 mg, once

weekly risedronate 35 mg, and daily vitamin D (800 IU cholecalciferol) supplementation in women aged 60 years and older were included. Fracture endpoints of interest were hip, vertebral and wrist fractures. Findings from individual randomized clinical trails (RCTs) were synthesized using an indirect mixed treatment comparison meta-analysis. The efficacy of Fosavance 5600® was derived by adding the efficacy of alendronate and vitamin D3 800 IU per day together.

Five RCTs studied effect of vitamin D 800 IU/day on the fracture risk reduction among the elderly and 6 RCTs evaluating alendronate once weekly and 4 RCTs evaluating risedronate were identified. Compared to risedronate, treatment with Fosavance 5600® lowered the risk of hip fractures for women with osteoporosis aged 50, 60 and 80 [odds ratio (OR) = 0.26; 95% credible interval (95% CrI):0.10, 0.86]. Also for women with osteoporosis aged 70 [OR 0.44; 95% CrI: 0.16, 1.65] and women with history of vertebral fractures [OR = 0.42; 95CrI:0.20, 1.02], results showed greater trend of fracture risk reduction with alendronate/Vitamin D3 5600 IU than risedroante. Treatment with Fosavance 5600® resulted in a trend of lower risk of vertebral fractures compared to risedronate for women with osteoporosis [OR 0.37; 95% CrI: 0.10, 3.10] as well as for women with a history of vertebral fractures [OR 0.35; 95% CrI: 0.10, 1.88] Similar results were found for wrist fractures: 0.57 (95% CrI: 0.28, 1.37) for women with osteoporosis and 0.50 (95% CrI: 0.24, 1.27) for women with a history of vertebral fractures, corresponding to a probability a being the more effective treatment of 88% and 92%, respectively. A mixed-treatment comparison meta-analysis showed favorable results for Fosavance 5600® compared to risedronate in term of efficacy of preventing hip, vertebral and wrist fractures in postmenopausal women with osteoporosis and in women with a history of vertebral fractures. Given that data were pooled from diverse populations, results will have to be validated further in an experimental setting for reproducibility.

Conflict of Interest: SS Sen, Merck & Co., Inc., employee and Shareholders

T Fan, Merck & Co., Inc., employee and Shareholders

Su-P430

COST-EFFECTIVENESS OF ONCE WEEKLY ALENDRONATE PLUS VITAMIN D3 5600 IU COMBINATON THERAPY IN THE PREVENTION OF FRACTURES IN POSTMENOPAUSAL WOMEN WITH OSTEOPOROSIS BUT WITHOUT FRACTURE HISTORY IN THE NETHERLANDS

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Background: Evidence showed efficacy of alendronate 70 mg and Vitamin D3 5600 IU weekly combination therapy (available as Fosavance 5600®) in fracture prevention for osteoporosis patients. This study estimated the cost-effectiveness of alendronate 70 mg and Vitamin D3 5600 IU weekly versus no treatment, and risedronate 35 mg weekly in the prevention of fractures in postmenopausal women with osteoporosis but no fracture history aged 60 years and older in the Netherlands.

Methods: The efficacy of Alendronate 70 mg and Vitamin D3 5600 IU weekly combination therapy was estimated indirectly from mixed treatment comparison meta-analysis, including Vitamin D3 800 IU/day, alendronate and risedronate randomized trials. A comprehensive decision analytical (Bayesian) modelling approach incorporated the mixed treatment comparison with a Markov model to evaluate the cost-effectiveness in terms of cost/QALY gained of the various regimens. Direct medical costs and utilities associated with different health states were derived from existing literature.

Results: At a 10-yr time horizon, Alendronate 70 mg and Vitamin D3 5600 IU weekly combination therapy resulted in more QALYs {6.5 per 100 treated patients [95% Credible Interval (95%CrI: 2.5,8.8)]} and lower cost [−€435 (95%CrI:−965,411)] compared to no treatment among women aged 80 and older. Compared to risedronate, Alendronate 70 mg and Vitamin D3 5600 IU weekly combination therapy was found to reduce more fractures [1.2 (95%CrI:−2.8, 5.5) per 100 treated patients for women aged 60] among patients 60 years and older. Alendronate/Vitamin D3 5600 IU combination therapy economically dominates risedronate with a probability over 95% for women aged over 60.

Conclusions: This economic evaluation showed that Alendronate/Vitamin D3 5600 IU combination therapy was economically dominant versus no treatment for women aged 80 and older, and versus risedronate for postmenopausal women aged 60 years or older. Results are sensitive to prices and efficacy assumptions for the combination therapy.

Conflict of Interest: SS Sen, Merck & Co., Inc., employee and Shareholders

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Mo-P431

EFFECTS OF PROPRANOLOL ON THE SKELETAL SYSTEM OF NON-OVARIECTOMIZED AND OVARIECTOMIZED RATS

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Propranolol, a nonselective beta-adrenergic receptor antagonist, was reported to favourably affect the skeletal system in different animal models.

The aim of the present study was to investigate whether the effects of propranolol on the skeletal system depend on the estrogen status in rats. The effects of propranolol on bones of normal and bilaterally ovariectomized (OVX, estrogen-deficient) rats were compared.

The in vivo experiments were carried out on the following groups of mature Wistar rats (n = 6–11): sham-operated control rats, sham-operated rats receiving propranolol, OVX control rats, OVX rats receiving propranolol, OVX rats receiving estradiol, OVX rats receiving estradiol and propranolol. Propranolol hydrochloride (10 mg/kg p.o.) and/or estradiol (0.1 mg/kg p.o.) were administered daily for 4 weeks. Bone mass, mineral and calcium content, macrometric and histomorphometric parameters, and mechanical properties were examined. In vitro, effects of propranolol (0.1−10 microM) on the formation of mouse osteoclasts and on the activity of mouse osteoblasts were investigated. In some cultures, estradiol (10 nM) was added.

In vivo, administration of propranolol to sham-operated and OVX rats caused similar skeletal effects, increasing bone mass and bone mineral mass. Propranolol counteracted the deleterious effects of estrogen deficiency on the rat skeletal system (impairment of bone mineralization and bone mechanical properties). Administration of estradiol to OVX rats receiving also propranolol did not significantly affect the effect of propranolol.

Propranolol profoundly decreased the number of osteoclasts formed in vitro. Addition of estradiol to the culture media slightly attenuated the inhibitory effect of propranolol on osteoclast formation. Propranolol decreased the RANKL mRNA/OPG mRNA expression ratio in osteoblast cultures in the presence and absence of

additional estradiol. Propranolol did not significantly affect the expression of collagen, alkaline phosphatase and ectonucleotide pyrophosphatase phosphodiesterase 1 mRNA, independently of the estradiol concentration.

Concluding, the effects of propranolol on the skeletal system in vivo seemed to be independent of the estrogen status in rats.

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Conflict of Interest: None declared

Mo-P432

DIFFERENTIAL EFFECTS OF FENOTEROL ON THE SKELETAL SYSTEM OF NON-OVARIECTOMIZED AND OVARIECTOMIZED RATS

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Sympathetic nervous system takes part in the regulation of bone growth and remodeling. The aim of the present study was to compare the effects of fenoterol, a beta2-adrenergic receptor agonist, on the skeletal system of normal and bilaterally ovariectomized (OVX) rats.

The in vivo experiments were carried out on the following groups of mature Wistar rats ($n = 6-11$): sham-operated control rats, sham-operated rats receiving fenoterol, OVX control rats, OVX rats receiving fenoterol, OVX rats receiving estradiol, OVX rats receiving estradiol and fenoterol. Fenoterol hydrobromide (5 mg/kg p.o.) and/or estradiol (0.1 mg/kg p.o.) were administered daily for 4 weeks. Bone mass, mineral and calcium content, macrometric and histomorphometric parameters, and mechanical properties were examined. In vitro, effects of fenoterol (0.1−10 microM) on the formation of mouse osteoclasts and on the activity of mouse osteoblasts were investigated. In some cultures, estradiol (10 nM) was added.

In sham-operated rats, fenoterol induced unfavourable changes in bone histomorphometric parameters (inhibition of bone formation), and worsened bone mechanical properties, not affecting bone mass and mineralization. In OVX rats, estrogen deficiency resulted in the impairment of bone mineralization and bone mechanical properties. Fenoterol in OVX rats increased bone mass, improved mineralization, and increased bone formation, but did not affect bone mechanical properties. Administration of estradiol to OVX rats receiving also fenoterol, counteracted the effect of fenoterol on bone mass and growth, and improved bone mechanical properties.

In vitro, fenoterol decreased the number of formed osteoclasts. However, when estradiol was added to the culture media, fenoterol strongly increased the osteoclast number. Consistently, in osteoblast cultures, fenoterol without estradiol decreased the RANKL mRNA/OPG mRNA expression ratio, whereas in the presence of estradiol the ratio was increased. In the absence of additional estradiol, fenoterol tended to increase collagen mRNA expression and the ratio of alkaline phosphatase mRNA expression to ectonucleotide pyrophosphatase phosphodiesterase 1 mRNA expression.

In conclusion, fenoterol exerted differential effects depending on the estrogen status, both in the rat skeletal system in vivo and in mouse bone cells in vitro.

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Conflict of Interest: None declared

Mo-P433

EFFECT OF CONCURRENT ADMINISTRATION OF OMEPRAZOLE AND ALENDRONATE ON BONE HISTOMORPHOMETRIC PARAMETERS IN OVARIECTOMIZED RATS

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Omeprazole is an inhibitor of the proton pump (H+/K+-ATPase) responsible for HCl production by parietal cells in the stomach. Its effect on V-ATPase in osteoclasts is not well recognized. Alendronate, an antiosteoporotic drug used in postmenopausal osteoporosis, can induce esophagitis and stomach ulcers. The effect of concurrent administration of omeprazole and alendronate on the processes of bone remodeling in estrogen deficiency has not been studied.

The aim of the present study was to investigate the effect of concurrent administration of omeprazole and alendronate on histomorphometric parameters of long bones in bilaterally ovariectomized rats.

The experiments were carried out on 3-month-old outbred Wistar rats, divided into 6 groups: I—sham-operated control rats, II—sham-operated rats, which were administered omeprazole (3 mg/kg), III—ovariectomized control rats, IV—ovariectomized rats, which were administered omeprazole (3 mg/kg), V—ovariectomized rats, which were administered alendronate (3 mg/kg), VI—ovariectomized rats, which were administered omeprazole (3 mg/kg) and alendronate (3 mg/kg). The drugs were administered to the rats by daily oral gavage (alendronate in the morning, omeprazole in the afternoon) for 28 days.

Bone mass, mineral and calcium content, macrometric and histomorphometric parameters (endosteal and periosteal transverse growth, width of endosteal and periosteal osteoid, transverse cross-section area of the cortical bone in the diaphysis and of the marrow cavity in the tibia, width of epiphyseal cartilage, width of trabeculae in the epiphysis and metaphysis in the femur) were studied.

Estrogen deficiency resulted in the development of osteoporosis in ovariectomized rats. In sham-operated rats, omeprazole slightly intensified bone remodeling, whereas in ovariectomized rats it slightly prevented the development of osteoporosis caused by estrogen deficiency. Alendronate inhibited the development of osteoporosis in ovariectomized rats. Omeprazole administered concurrently with alendronate did not significantly affect the antiresorptive effect of alendronate in ovariectomized rats.

Conflict of Interest: None declared

Mo-P434

EFFECTS OF NATURAL PHENOLIC ACIDS ON THE SKELETAL SYSTEM OF OVARIECTOMIZED RATS

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There is increasing interest in the discovery of natural compounds that could be useful in the prophylaxis and treatment of osteoporosis.

Natural phenolic acids are commonly present in plants that are normally consumed in the diet. Recent reports indicate the possibility that representatives of natural phenolic acids are able to inhibit bone resorption and/or to stimulate bone formation.

We were interested in whether natural phenolic acids, at doses moderately exceeding the daily intake with normal human diet, may

affect the skeletal system in rats. The aim of the present study was to investigate the effects of ferulic, caffeic, p-coumaric and chlorogenic acids on the skeletal system of bilaterally ovariectomized (estrogen-deficient) rats.

The experiments were carried out on 3-month old female Wistar Cmd:(WI)WU rats, divided into following groups (n = 8): sham-operated control rats, ovariectomized control rats and ovariectomized rats receiving ferulic, caffeic, p-coumaric or chlorogenic acids. The ovariectomy was performed 7 days before the start of administration of the phenolic acids. The phenolic acids were administered at a dose of 10 mg/kg p.o. daily for 4 weeks. Bone mass, mineral and calcium content, macrometric and histomorphometric parameters, and mechanical properties were examined.

Estrogen deficiency significantly increased body mass gain and induced osteoporotic changes in the skeletal system of the ovariectomized control rats.

Caffeic and p-coumaric acids significantly decreased the body mass gain in ovariectomized rats; ferulic and chlorogenic acids had weaker effect.

Caffeic acid decreased the bone mineral mass/body mass ratio and bone mass/body mass ratio in L-4 vertebra, whereas p-coumaric acid increased the bone mineral mass/body mass ratio and bone mass/body mass ratio in the long bones, in comparison with the ovariectomized control rats.

The phenolic acids slightly improved some bone histomorphometric parameters, impaired by estrogen deficiency. However, they did not increase the ratio of bone mineral mass to bone mass, decreased by estrogen-deficiency, and did not significantly affect bone mechanical properties.

Concluding, the effects of different phenolic acids on the skeletal system of ovariectomized rats were differential.

Conflict of Interest: None declared

Mo-P435

SAFETY AND TOLERABILITY OF BAZEDOXIFENE FOR THE PREVENTION OF POSTMENOPAUSAL OSTEOPOROSIS

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Bazedoxifene (BZA) is a new selective estrogen receptor modulator (SERM) selected for its antagonist activity on endometrial and breast tissues. Here we report on the safety and tolerability of BZA for the prevention of postmenopausal osteoporosis in a 2-year randomized, double-blind, placebo- and raloxifene-controlled phase III trial. Healthy postmenopausal women (aged ≥45 years) with lumbar spine or femoral neck bone mineral density T-scores no less than −2.5 (mean, −1.2) were enrolled if they did not have vasomotor symptoms requiring treatment, bone diseases (other than osteoporosis), previous vertebral fractures, or endometrial hyperplasia at baseline. Subjects were randomized to take BZA 10, 20, or 40 mg; raloxifene 60 mg; or placebo daily for 2 years. Efficacy assessments (bone mineral density, bone markers, and lipids) are presented in detail elsewhere. Safety was evaluated based on adverse event (AE) reporting, laboratory analyses, and physical examination. Endometrial and ovarian safety was assessed by periodic transvaginal ultrasonography and endometrial biopsy. Of 1,583 women (mean age ± standard deviation, 57.6 ± 6.5 years) included in the safety population, 1,113 (70.3%) completed the 2-year study. The rates of treatment-emergent AEs, serious AEs, and discontinuations due to AEs were similar among treatment groups. Vasodilatation was more common with BZA 20 and 40 mg (19.9% and 22.6%, respectively) than with placebo (13.2%; $P < 0.05$ for both) but similar to that with raloxifene (18.3%). The incidence of leg cramps was similar across treatment groups (range, 9.3%−11.6%). The incidence of venous thrombotic AEs with BZA was low (< 1%) and similar to that with raloxifene or placebo. No cases of endometrial hyperplasia or malignancy were diagnosed in women treated with BZA. In conclusion, BZA was well tolerated and had a safety profile similar to that of placebo and raloxifene in a population of relatively young postmenopausal women. BZA is a promising new SERM that could become an important addition to currently available therapies for the prevention of postmenopausal osteoporosis.

Conflict of Interest: H. K. Genant, Synarc stock holder
H. K. Genant, Amgen, Wyeth, GSK, Merck, BMS, Servier, Eli Lilly, SABs with compensation
C. Christiansen, Wyeth Pharmaceuticals, Consultant

Mo-P436

EFFECT OF TWO DIFFERENT TREATMENT REGIMENS OF ALENDRONATE ON BONE MINERAL DENSITY IN POST-MENOPAUSAL WOMEN WITH OSTEOPOROSIS: A RANDOMIZED-CONTROLLED TRIAL

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Objectives: In clinical practice the pharmacological choice for osteoporosis therapy must consider both the cost-benefit ratio and patient compliance. The aim of this study was to compare the efficacy on bone mineral density (BMD) and tolerability of a short-term treatment with oral twice-monthly alendronate 70 mg (ALN-TM) with those of an oral once-weekly alendronate 70 mg (ALN-OW) therapy in a one-year randomised trial of postmenopausal women with osteoporosis.

Methods: Community-dwelling women aged 55 and older with a BMD lumbar spine T-score below −2.5, from 3 osteoporosis centers of Northen Italy, were randomly assigned to receive 70 mg ALN-OW or ALN-TM (70 mg day 1 and 15 of the month). Age, weight and age at menopause were collected at baseline. BMD measurements were made on the lumbar spine (L1–L4) in the antero-posterior position and total hip at baseline and twelve months, by dual-energy X-ray absorptiometry.

Results: Overall 101 women were enrolled: 51 subjects were allocated to ALN-OW and 50 patients received ALN-TM. 91 subjects (90%) completed the 12-months treatment and were included in the analysis. Seven subjects out of 51 (13.7%) in the ALN-OW group and three patients out of 50 (6%) in the ALN-TM group experienced minor gastrointestinal adverse events (nausea, epigastric discomfort) related to the drug ($p = 0.05$) and withdrew from the study. There was no statistically significant difference in baseline characteristics between the 2 groups. After 1 year, BMD at the lumbar spine and total hip had increased significantly from baseline in both group. Relative to baseline, BMD at the lumbar spine increased (mean percentage ± standard deviation, SD) by 5.1 ± 5.7% and 4.1 ± 6.7% in the ALN-OW and ALN-TM groups ($p < 0.001$ per site per group), respectively, the difference between

the groups being not significant ($p = 0.962$). At the total hip, BMD increased from baseline (mean percentage ± SD) by 2.3 ± 4.3% ($p < 0.001$) and 3.1 ± 3.1% ($p < 0.005$) with ALN-OW and ALN-TM respectively, with no significant difference between the two regimen groups ($p = 0.084$).

Conclusion: These results demonstrate that, in postmenopausal women with osteoporosis, ALN 70 mg twice-monthly therapy produce, after 12 months, substantial and significant increases in lumbar spine and total hip BMD, comparable to those induced by ALN 70 mg once-weekly. Furthermore, the twice-monthly regimen is associated with a better patient adherence to therapy compared to that obtained with once-weekly treatment.

Conflict of Interest: None Declared

Mo-P437

Abstract withdrawn

Mo-P438

COMPARISON OF THE EFFECTS OF TERIPARATIDE AND ALENDRONATE ON PARAMETERS OF TOTAL HIP STRENGTH AS ASSESSED BY FINITE ELEMENT ANALYSIS: RESULTS FROM THE FORTEO AND ALENDRONATE COMPARISON TRIAL

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Background: Biomechanical computed tomography (BCT) uses finite element analysis of QCT scans to provide non-invasive measures of femoral strength and density plus a strength:density ratio that can be considered a measure of bone "quality". Teriparatide [rhPTH (1–34), TPTD] 20 mcg/d and alendronate 10 mg/d (ALN) were previously shown to have positive effects on vertebral strength as assessed by BCT during a randomized, double-blind, 18-month study in postmenopausal women with osteoporosis. The present analysis extends these studies to the analysis of proximal femoral strength for a simulated sideways fall.

Methods: Using the QCT scans, volumetric density from QCT and strength from BCT were determined for total hip and for the trabecular and cortical compartments, and a strength:density ratio was calculated.

Results: In the TPTD group, total hip density was not significantly different from baseline at any timepoint, however, at 18 months trabecular density significantly increased 5.1% and cortical density significantly decreased 1.0% from baseline. In the ALN group, density was not significantly different from baseline at any timepoint for any compartment. Total hip strength significantly increased 5.9% from baseline at 18 months in the TPTD group, and strength did not significantly change from baseline at any timepoint in the ALN group. No significant changes were seen in the strength measures associated with isolated changes in the cortical or trabecular compartments. The strength:density ratio significantly increased 4.1% from baseline at 18 months in the TPTD group, whereas no significant changes were seen for ALN.

Conclusion: Total hip strength at 18 months significantly increased in the TPTD group and did not significantly change in the ALN group. This significant biomechanical effect for TPTD was associated with a significant decrease in cortical density and a somewhat larger increase in trabecular density.

Conflict of Interest: Sponsored by Eli lilly and Company

Mo-P439

A RANDOMISED DOUBLE BLIND PLACEBO CONTROLLED TRIAL TO DETERMINE THE MAGNITUDE OF CHANGE IN BONE MINERAL DENSITY IN RESPONSE TO LASOFOXIFENE

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Lasofoxifene is a novel selective estrogen receptor modulator (SERM) currently being developed for the treatment of postmenopausal osteoporosis. The aim of this study was to determine the effects of lasofoxifene on bone mineral density (BMD) at the lumbar spine (LS), total hip (TH) and distal forearm (DF).

This was a 2-year prospective, randomized, double-blind, placebo controlled study. Fifty-two postmenopausal osteopenic women, ages 55 to 77 (mean 63.7) years were recruited from a single centre (Sheffield, UK), 43 of whom completed the 2 years. Subjects were randomized to receive either lasofoxifene (0.25 mg/day) or placebo, in a 1:1 ratio. All women received calcium (1000 mg/d) and vitamin D (400 IU/d) for a lead-in period of 6 weeks and for the duration of the study. Duplicate measurements of BMD at the LS and TH were made by dual-energy X-ray absorptiometry (DXA, Hologic QDR 4500 Acclaim) and at the DF (DTX 200, Osteometer) at baseline, one and two years in all subjects.

There were no significant differences in mean baseline BMI or BMD between the treatment groups.

Percentage change in mean LS BMD, from baseline, was significantly greater in the lasofoxifene group compared to placebo at 1 and 2 years. An increase in TH BMD was not apparent until 2 years in the lasofoxifene group compared to placebo. There was no significant difference in the change in DF BMD in women treated with placebo or lasofoxifene at either 1 or 2 years (Table 1).

We conclude that the use of lasofoxifene therapy leads to significant increases in LS BMD after one year of therapy, whereas a change in TH BMD was not apparent until 2 years of therapy. In this group of postmenopausal women, no significant change in BMD at the DF was observed through 2 years of treatment.

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Table 1 Mean % Change from Baseline at 1 and 2 Years

% Change	1 Year Placebo	Lasofoxifene	2 Years Placebo	Lasofoxifene
LS BMD	−0.89	2.41***	0.24	3.33***
TH BMD	0.73	1.65	0.00	2.20**
DF BMD	−0.34	−0.68	−1.22	0.31

** $p<0.01$, *** $p<0.001$ Lasofoxifene vs Placebo by t test

Conflict of Interest: S. Glover: None Declared
A. Rogers: None Declared
R. Eastell: Pfizer, Grant Research Support and Consultant

Mo-P440

MR-DETECTED BONE MARROW EDEMA IS NOT A PREREQUISITE FOR LONG TERM BENEFIT BY KYPHOPLASTY

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Introduction: Kyphoplasty has been shown to be a safe and effective method for reducing pain in patients with painful osteoporotic vertebral fractures. In fractured vertebral bodies, bone marrow edema detected by MRI is a radiological finding in acute fracture cases which is usually not discernable after 3 months any more. This study investigates the possibility that only patients with acute vertebral fractures as indicated by MR-detected bone marrow edema benefit from kyphoplasty in terms of pain reduction.

Methods: Painful vertebral fractures of 45 patients (primary osteoporosis) with preoperative MR-Images were treated by kyphoplasty. MR-Images were evaluated with regard to the presence or absence of a bone marrow edema. All patients received a pharmacological antiosteoporosis treatment (1000 mg calcium, 1000IU vitamin D3, oral aminobisphosphonate), pain medication and physiotherapy. Pain (visual analog scale (VAS), range 0–100) and radiomorphological measures were assessed at baseline, after 1 and 12 months.

Results: In 27 patients with MR-detected bone marrow edema the pain score (VAS) changed from 72.7 (preoperative) to 46.8 (postoperative) and to 48.0 after 12 months. In 18 patients with no preoperative bone marrow edema the pain score improved from 70.7 (preoperative) to 60.3 (postoperative) and to 50.1 after 12 months. Height restoration was greater in the group with initial bone marrow edema, however the difference was not significant between both groups.

Conclusions: In patients with new vertebral fractures, confirmed by bone marrow edema in MR-Images, kyphoplasty is an effective method for an immediate and sustained pain reduction. After 12 months there was a comparable pain reduction in patients with and without preoperative bone marrow edema. We conclude that MR-detected bone edema is not a prerequisite for the long-term benefit of kyphoplasty in patients with painful osteoporotic vertebral fractures, provided that kyphoplasty was performed at the vertebral bodies which were truly responsible for the back pain.

Conflict of Interest: None declared

Mo-P441

SURVIVAL, FUNCTIONAL OUTCOME, AND COSTS OF CARE AMONG POSTMENOPAUSAL WOMEN WITH AN INTERTROCHANTERIC HIP FRACTURE:

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Objectives: To examine the impact of the type of surgical procedure on survival, functional outcome, and direct costs of medical care during the one year period after hospital discharge among postmenopausal women who sustained an intertrochanteric hip fracture.

Methods: The design was a one-year prospective cohort study reflecting day-to-day clinical practice. Eighty-two women were enrolled on a consecutive basis. Three groups were defined by the time of surgery: sliding hip screw fixation, intramedullary nail fixation, and prosthetic replacement.

Results: There were no significant differences between the three groups for prefracture residence, type and number of comorbidities, and mean age at the time of the injury. The mean age at the time of the injury was 80.8 years, 80.6 years, and 81.3 years for women treated with sliding hip screw fixation, intramedullary nail fixation, and prosthetic replacement, respectively. Survival differed significantly ($P = 0.003$), with one-year mortality rates of 20%, 27%, and 66% for women treated with sliding hip screw fixation, intramedullary nail fixation, and prosthetic replacement, respectively. No differences were found between the treatment groups for functional outcome at one year. The mean direct costs of medical care during the one year period after hospital discharge amounted 12,046 EURO, 18,859 EURO, and 42,767 EURO for women treated with sliding hip screw fixation, intramedullary nail fixation, and prosthetic replacement, respectively ($P = 0.001$).

Conclusions: Among postmenopausal women with an intertrochanteric hip fracture, mortality and direct costs of medical care for patients treated with primary prosthetic replacement are higher than that for patients treated with sliding hip screw or intramedullary nail fixation. At one year, functional outcome is not significantly different. Most importantly, our findings underscore the need to perform an adequately powered, randomized trial to address the critically important question whether differences in outcome for the three groups are the result of the different treatment regimens given or related to, as yet unknown, baseline characteristics of the patients.

Conflict of Interest: None declared

Mo-P442

COSTS OF CARE AFTER HOSPITAL DISCHARGE AMONG POSTMENOPAUSAL WOMEN WITH AN INTERTROCHANTERIC HIP FRACTURE

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Objectives: To identify potential predictors of direct costs of medical care during the one year period after hospital discharge, and to examine the impact of the type of surgical procedure among postmenopausal women having sustained an intertrochanteric hip fracture.

Participants and methods: The design was a one year prospective cohort study reflecting standard day-to-day clinical practice. Sixty-two women 50 years or older with an intertrochanteric hip fracture were enrolled on a consecutive basis. Three groups were defined by the time of surgical repair: sliding hip screw fixation, intramedullary nail fixation, and prosthetic replacement. Direct costs of medical care were documented during the one year period after hospital discharge. Multivariable analyses were done to explore potential predictors of costs.

Results: There were no significant differences between the three groups for prefracture residence, type and number of comorbidities, and age at the time of the injury. The mean age of the women treated with sliding hip screw fixation, intramedullary nail fixation, and prosthetic replacement was 80.8 years, 80.6 years, and 81.3 years, respectively. The mean direct costs of medical care during the one year period after hospital discharge amounted €12,046 after sliding hip screw fixation, €18,859 after intramedullary nail fixation, and €42,767 after prosthetic replacement surgery (ANOVA among the three groups, $P = 0.001$). A multivariable model identified living in an institution at the time of the injury ($P = 0.026$) and prosthetic replacement surgery ($P < 0.001$) as the two significant determinants of increased medical costs during the one year period after hospital discharge.

Conclusions: Among postmenopausal women with an intertrochanteric hip fracture, direct costs of medical care for women treated with primary prosthetic replacement are higher than that for women treated with intramedullary nail fixation. In turn, costs for women treated with intramedullary nail fixation are higher that for women treated with sliding hip screw. Living in an institution at the time of the injury and treatment with prosthetic replacement surgery are strong predictors of increased direct costs of medical care after hospital discharge.
Conflict of Interest: None declared

Mo-P443

LEPTIN RECEPTOR EXPRESSION IN SKELETAL MUSCLE DECLINES WITH AGING: A MECHANISM LINKING ALTERED LEPTIN SIGNALING WITH FRAILTY AND SARCOPENIA

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Skeletal muscle atrophies with age (sarcopenia), and the muscle weakness and postural instability that accompany sarcopenia are major contributors to falls and osteoporotic fractures. The role of altered leptin signaling in the sarcopenia of aging is not well understood; however, leptin binding to its receptor in skeletal muscle promotes cell survival through an Akt pathway, and the functional characteristics and contractile properties of skeletal muscle in leptin-deficient ob/ob mice are noted to resemble those of aged animals. In a previous study we found that aging in mice was associated with a decline in serum leptin levels, loss of bone mass (BMC) and density (BMD), and decreased physical activity and muscle mass. It is known that leptin receptor expression in the hypothalamus declines with age in mammals, and here we tested the hypothesis that leptin receptor levels in muscle were also altered with age. Quadriceps femoris muscles were harvested from mice 6–12 months of age and from mice 24–29 months of age. Specimens were embedded in paraffin and stained with a polyclonal antibody recognizing both short and long forms of the leptin receptor (Ob-R). The average size of quadriceps muscle fibers decreased by approximately 30% between 6 and 29 months of age ($P < .001$), and image analysis indicated strong positive staining for the leptin receptor along the sarcolemma of muscle fibers in both young and old mice; however, approximately 30% of muscle fibers in younger mice showed positive (Ob-R) staining whereas less than 20% of muscle fibers showed positive Ob-R staining in aged mice ($P < .001$). These findings suggest that age-associated declines in leptin sensitivity may be implicated in the muscle atrophy that occurs with aging, and that therapeutic approaches for increasing peripheral leptin sensitivity might attenuate the increasing frailty and risk for falls observed among elderly adults.
Conflict of Interest: None declared.

Mo-P444

EFFECTS OF PAMIDRONATE (APD) IN OSTEOPOROTIC PATIENTS. ANALYSIS OF THE EFFECTS ON BONE REMODELING, BONE MINERAL METABOLISM, RENAL FUNCTION, BONE MINERAL DENSITY (BMD) AND FRACTURES

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Background/Aims: Treatment with intravenous (iv) bisphosphonates(BP)could be effective in osteoporotic patients (pt.) with intolerance to oral intake; however studies that analyze the efficacy and safety of this treatment are needed. We analyze the effects of APD treatment on bone remodeling, BMD, fractures and parameters of mineral metabolism, as well as the adverse events (AE) in pt.with osteoporosis that showed intolerance and/or have any contraindication to oral BP.

Methods and Results: We analyzed prospectively 17 osteoporotic pt.(age 66.8 ± 9.4 yrs), 64.7% women, 81.2% with prevalent vertebral fractures. All pt. started APD therapy (30 mg/iv every 3 months) and were followed-up for 1 year. We analyzed: PINP and NTX (as markers of bone formation and resorption), Ca, P, PTH, 25-OH vitamin D, creatinine (creat.) and clearance of creatinine (Cl. cre.) prior to starting treatment (baseline), one week after starting APD therapy (1-wk) and thereafter for every 3 months (prior to infusion) during 1 year. We also analyzed lumbar and femoral BMD at baseline and after 1 year, the incidence of new fractures, and treatment-related AE. One week after APD treatment, a significant decrease of NTX (32.4%) as well as an increase of PTH (66%) were observed. However, no significant differences were observed thereafter. No differences were observed in the other parameters analyzed during the study, nor impairment of renal function. 10 pt. suffered new vertebral fractures. 29% of pt. showed flu-like syndrome after APD infusion and 1 pt. withdrawn treatment due to AE.

Conclusion: APD treatment (30 mg iv/ every 3 months) does not produce renal impairment. However, this treatment does not appear to reduce the incidence of new fractures, nor does it produce significant changes in markers of bone turnover during the follow-up, suggesting that APD, at this doses, is not effective for treating osteoporosis.

Table 1

	Baseline	1-wk	6-months	1-year
PINP ng/ml	28 ± 19	33 ± 21	34 ± 27	42 ± 37
NTX nM/mM	37 ± 22	25 ± 24*	47 ± 49	50 ± 40
PTH pg/ml	45 ± 17	76 ± 43*	55 ± 31	56 ± 29
25-OH D ng/ml	30 ± 13	27 ± 10	24 ± 10	22 ± 8.7
Creat. mg/dl	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.8 ± 0.1*
Cl cre. ml/mi	61 ± 20	59 ± 18	62 ± 21	72 ± 24*
BMD lumbar	0.781 ± 0.137	–	–	0.792 ± 0.135
BMD hip	0.729 ± 0.151	–	–	0.720 ± 0.162

(mean ± SD) * $p < 0.05$ to baseline

Conflict of Interest: None declared

Mo-P445

INCIDENT FRACTURES PREDICT MORTALITY: THE CANADIAN MULTICENTRE OSTEOPOROSIS STUDY (CAMOS)

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Osteoporosis is the most common metabolic bone disease in the elderly. Although the clinical consequences of osteoporosis and fracture are well-recognized, their impact on mortality remains unclear. The Canadian Multicentre Osteoporosis Study (CAMOS) involves nine sites across Canada and presents an opportunity to examine mortality rates in a cohort of patients randomly selected from the Canadian population. Participants (both men and women) 50 years of age and older were classified to 6 incident fracture groups: hip, spine, wrist, pelvic, rib, and other fractures and compared with patients without these fractures. The fracture groups were divided according to the time of the new fracture (fracture occurred between baseline and year 1, fracture occurred between year 1 and year 2, fracture occurred between year 2 and year 3, and fracture occurred between year 3 and year 5). The fracture cohort was followed for 5 years from baseline to either the end of the study period or to the date of death. A Cox proportional hazards modeling analysis was conducted to determine the association among new fractures and mortality. Results were adjusted for potential confounding factors. Hazard ratios and 95% confidence intervals (CI) were calculated. Of the 7753 participants that were evaluated 85, 100, 199, 23, 147, 305 subjects had hip, spine, wrist, pelvic, rib, or other fractures during the course of the study. Hip fractures that occurred between baseline and year 1 were associated with increased mortality (hazard ratio: 2.8; 95% CI: 1.22, 6.6). Spine fractures that occurred between baseline and year 1, and year 1 and year 2 were associated with increased mortality (hazard ratio: 2.8; 95% CI: 1.0, 7.6; hazard ratio: 2.9; 95% CI: 1.2, 6.9). Other fractures that occurred between year 3 and 5 were associated with decreased mortality (hazard ratio: 0.14; 95% CI: 0.02, 0.98). In general, participants with incident fractures had higher mortality rates as compared with patients without fractures. However, the rates varied depending on fracture type and the time of the new fracture.

Conflict of Interest: None declared

Mo-P446

TREATMENT INCREASES IN PATIENTS WITH OSTEOPENIA AND PRIOR FRACTURES FOLLOWING THE CANADIAN QUALITY CIRCLE (CQC) NATIONAL PROJECT

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The Quality Circles (QCs) project is a disease management process that involves a small group of people who identify and analyze work related problems and recommend solutions. The project was developed to improve family physicians' (FPs) management of osteoporosis in accordance with the Osteoporosis Canada (OC) 2002 guidelines and consists of five phases: baseline (BASE) data collection, 1st educational intervention, follow-up I (FOL-I) data collection, 2nd educational intervention, and follow-up II (FOL-II) data collection. This analysis evaluated the change in treatment administration in high risk patients with bone mineral density (BMD) t-scores in the osteopenia range and prior fragility fracture at the hip, wrist or spine. Therapy included alendronate, calcitonin, etidronate, hormone replacement therapy, PTH, raloxifene, and risedronate. A total of 340, 301 and 162 FPs formed 34, 34 and 28 QCs, during BASE, FOL-I and FOL-II, respectively. For each phase, FPs gathered data from different patients via chart reviews and a standardized collection form. A total of 8376 (BASE), 7354 (FOL-I) and 3673 (FOL-II) patient records were selected at random and analyzed. All patients were women 55 years and older. To adjust for possible clustering within a physician, generalized estimating equations (GEE) approach assuming an exchangeable correlation structure was used to evaluate differences in appropriate treatment in patients following the educational interventions. Odds ratios (OR) and 95% confidence intervals (CI) were calculated. Of those who had a BMD measurement, 3.0% (169/5554), 5.3% (290/5457) and 4.5% (135/3020) of patients had osteopenia and a fracture during BASE, FOL-I and FOL-II, respectively. Of the high risk patients, 64.5% (109/169) at BASE, 79.0% (229/290) at FOL-I, and 83.0% (112/135) at FOL-II were treated. Compared with baseline values, the odds of a patient receiving treatment increased during FOL-I (OR: 2.1; 95% CI: 1.3, 3.2) and FOL-II (OR: 2.7; 95% CI: 1.5, 4.8). In conclusion, the use of QCs is an effective knowledge translation approach that increases FPs treatment utilization in high risk patients with osteopenia and fracture. Appropriate treatment, in accordance with the OC guidelines, may reduce the future fracture risk of these high risk patients.

Sponsored by: Alliance for Better Bone Health, and Ontario College of Family Physicians.

Conflict of Interest: None declared

Mo-P447

FAMILY PHYSICIANS USE OF NON-PHARMACOLOGICAL INTERVENTIONS INCREASES FOLLOWING THE CANADIAN QUALITY CIRCLE (CQC) NATIONAL PROJECT

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The CQC Project was designed to improve family physicians' (FPs) adherence with the Canadian osteoporosis guidelines (2002). This analysis examined the rate that FPs prescribed non-pharmacological interventions to their patients. The Canadian osteoporosis guidelines suggest that all patients should be prescribed calcium (CAL), vitamin D (VIT) and physical activity (PHYS). The non-pharmacological interventions were assessed as binary outcomes (CAL: $\leq$ 1500 mg/day, $>$ 1500 mg/day; VIT: $\leq$ 800 IU/day, $>$ 800 IU/day; and PHYS: None, Yes). A total of 340, 301 and 162 PCPs formed 34, 34 and 28 QCs, during BASE, FOL-I and FOL-II, respectively. For each wave, PCPs gathered data from different patients via chart reviews and a standardized collection form. A total of 8376 (wave I), 7354 (wave II) and 3673 patient records were selected at random and analyzed. All patients were women 55 years and older. The generalized estimating equations (GEE) approach was used to evaluate differences in prescribing patterns for

non-pharmacological interventions. The cluster variable for the GEE model was physician. An exchangeable correlation matrix was used for the analysis. Odds ratios (OR) and 95% confidence intervals (CI) were calculated. Compared with baseline values, the likelihood that FPs prescribed higher doses of calcium and vitamin D increased during FOL-I (CAL OR 1.8; 95% CI: 1.4, 2.3; VIT OR 2.0; 95% CI: 1.6, 2.4) and FOL-II (CAL OR 2.6; 95% CI: 2.0, 3.5; VIT OR 2.8; 95% CI: 2.2, 3.6). In addition, more patients were prescribed physical activity during FOL-I (OR 1.8; 95% CI: 1.5, 2.0) and FOL-II (OR 2.0; 95% CI: 1.6, 2.4). The use of QCs is an effective knowledge translation approach that increases the appropriate use of prescribed non-pharmacological interventions. These interventions may improve patient outcomes.

Sponsored by: Alliance for Better Bone Health, and Ontario College of Family Physicians.

Conflict of Interest: honoraria or consultancies- Eli Lilly and Company, Merck Frosst, NPS-Allelix, Zelos Therapeutics, Servier, Pfizer Pharmaceuticlas USA, Novartis Pharmaceuticals Corporation, The Alliance for Better Bone Health (Procter & Gamble Pharmaceuticals and sanofi-aventis), GlaxoSmithKline Consumer Healthcare

Mo-P448

COMPARATIVE EFFICACY OF SEVERAL OSTEOPOROSIS MEDICATIONS IN POSTMENOPAUSAL WOMEN WITH OSTEOPOROSIS

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Although a number of drugs are available for the treatment of osteoporosis, there are few studies of the comparative efficacy of several medications. This study was conducted to assess the comparative effectiveness of several medications on bone mineral density (BMD), biochemical bone markers, and the incidence of vertebral fractures, and to investigate the difference in the efficacy of several medications according to the age, bone turnover, and the prevalence of vertebral fractures at baseline in postmenopausal women with osteoporosis. The analysis was based on combined data from three randomized controlled trials. A total of 717 postmenopausal women, aged 50 to 85 years, were analyzed on an intention-to-treat basis. Treatment groups were: 1) alendronate, 2) risedronate, 3) etidronate, 4) hormone replacement therapy (HRT), 5) eel calcitonin, 6) alfacalcidol, 7) vitamin K2 (menatetrenone), and 8) control (no treatment). Thoracic and lumbar spine radiographs, BMD at distal 1/3 radius, and markers of bone turnover (Bone ALP, NTX) were assessed at baseline and at 3, 6, 12, 18, and 24 months of treatment. Mean changes in BMD relative to baseline after 2-year treatment were 2.3% for alendronate, 1.9% for risedronate, −0.5% for etidronate, 2.0% for HRT, 1.6% for calcitonin, −3.6% for alfacalcidol, −1.9% for vitamin K2, and −3.3% for control. During the 2-year treatment period, 25.6% of control patients developed new vertebral fractures. Compared with controls, the fracture incidence was reduced by 69% ($P = 0.02$) in alendronate, 66% ($P = 0.01$) in risedronate, 56% ($P = 0.04$) in etidronate, 65% ($P = 0.02$) in HRT, 59% ($P = 0.03$) in calcitonin, 44% ($P = 0.13$) in alfacalcidol, and 56% ($P = 0.049$) in vitamin K2. We observed significant reductions in the incidence of vertebral fractures with HRT, alendronate, risedronate, etidronate, calcitonin, and vitamin K2, and significant improvements in BMD with HRT, alendronate, risedronate, and calcitonin. With regard to the magnitude of increase in BMD and the magnitude of the reduction in the risk of vertebral fractures, alendronate, risedronate, and HRT have shown significantly greater efficacy than that in other medications. In addition, the magnitude of these effects appears to be independent of the age, bone turnover, and the prevalence of vertebral fractures at baseline.

Conflict of Interest: None declared

Mo-P449

CONTINUED INCREASE IN HIP BMD AFTER 36 MONTHS OF PTH(1–84) TREATMENT IN POSTMENOPAUSAL WOMEN

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Introduction: PTH(1–84) has proven to be efficacious and safe in clinical trials for 18 months of treatment. We report results from 36 months of treatment with PTH(1–84).

Methods: The Treatment of Osteoporosis with PTH(TOP) study, an 18-month, randomized, double-blind, placebo-controlled trial, assessed the effect of PTH(1–84) on vertebral fracture incidence in osteoporotic women. The women receiving placebo in TOP could participate in two consecutive extension studies(Open-label Extension Study(OLES) and the Treatment Extension Study(TRES)). Placebo treatment in TOP was followed by 18 months of PTH(1–84) treatment in OLES and additional 18 months PTH(1–84) treatment in TRES. Subjects in TOP included postmenopausal women with low BMD without($n = 2056$) or with($n = 471$) prevalent vertebral fracture. 1681 patients from TOP continued in OLES (781 from the PTH (1–84)-group in TOP and 900 from the placebo-group). 103 women participated in TRES. There was a median approximately 2 months break in PTH(1–84) treatment between end of OLES and start of TRES.

Results: In subjects administered with PTH 100 μg daily for 36 months mean lumbar spine BMD increased rapidly and progressively (8.0%) during the first 18 months of treatment. There was a small decrease in lumbar spine BMD between OLES and TRES; at the beginning of TRES the increase over the OLES baseline was 7.0%. This was an expected consequence of the median approximately 2 months break in PTH treatment between the two studies. Upon initiation of PTH(1–84) treatment in TRES, there was another rapid increase in lumbar spine BMD such that mean BMD increased to 8.5% above OLES baseline at 24 months. There was no further change in lumbar spine BMD between 24 and 36 months. Total hip BMD increased progressively throughout the 36 months of PTH treatment. Total hip BMD increased by 2.0% at Month 18, decreased slightly to a 1.7% increase during the break between OLES and TRES, before continuing to increase to 3.2% above the OLES baseline at Month 36. The pattern of increase in femoral neck BMD was similar to that of total hip with an overall increase above the OLES baseline of 3.4% at Month 36.

Conclusion: Treatment with PTH for 36 months resulted in an initial rapid increase in lumbar spine BMD during the first 12 months of treatment(OLES), followed by a slower but steady increase for the next 12 months. Total hip BMD and femoral neck BMD increased linearly during the 36 months of treatment.

Conflict of Interest: None delcared

Mo-P450

OVERALL INCIDENCE OF ATRIAL FIBRILLATION, STROKE, AND MORTALITY FROM STROKE IN PATIENTS TREATED WITH RISEDRONATE OR PLACEBO

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Black et al. report a significant increase in the risk of atrial fibrillation, classified as a serious adverse event, among patients treated with intravenous zoledronic acid. In a letter in the same issue of the Journal, Cummings et al. report a trend toward an increased risk of atrial fibrillation among patients treated with oral alendronate.

To determine whether there was a similar effect with oral risedronate, we evaluated the incidence of nonadjudicated adverse events of atrial fibrillation and cerebrovascular accident (stroke) and of death from these events in placebo-controlled, phase 3 clinical trials of risedronate for the treatment of osteoporosis. These trials followed approximately 15,000 patients for up to 3 years (Table 1).

In the risedronate group, as compared with the placebo group, there was no significant difference in the incidence of atrial fibrillation (classified as an adverse event or a serious adverse event), cerebrovascular accident, or death associated with cardiovascular adverse events. The difference in the rate of death from cerebrovascular accident (P = 0.003) was consistent with findings reported previously.

These data do not support a causal association between atrial fibrillation and the use of risedronate.

Table 1 Demographic Characteristics and Incidence of A

Variable	Placebo (N = 5048)	Risedronate 2.5 mg (N = 4998)	Risedronate 5 mg (N = 5020)	P value
Age - yr	73.4 + 9.2	73.6 + 9.1	73.5 + 9.3	0.44
Exposure in Months	23.8 + 13.2	21.5 + 12.6	24.0 + 13.2	0.50
Afib – AEs no. (%)	70 (1.4)	66 (1.3)	70 (1.4)	1.0
Afib – SAEs no. (%)	24 (0.5)	24 (0.5)	29 (0.6)	0.49
Stroke AEs	77 (1.5)	71 (1.4)	70 (1.4)	0.62
Mortality from Stroke	24 (0.5)	15 (0.3)	7 (0.1)	0.003
Cardiovasc. Mortality	96 (1.9)	83 (1.7)	80 (1.6)	0.25

Conflict of Interest: None declared

Mo-P451

VITAMIN D SUPPLEMENTATION IN DAILY PRACTICE. 10.000 IU WEEKLY—TOO HIGH OR TOO LOW?

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Background: Due to high prevalence of vitamin D insufficiency/deficit in elderly patients supplementation with vitamin D represents an essential background of both prevention and treatment of osteoporosis.

A frequent problem encountered in clinical practice regards the most appropriate dose of vitamin D and dosing frequency.

Our goal was to evaluate if target level of serum vitamin D of 80 nmol/L can be achieved with less frequent dosing (once weekly) 8.000–14.600 IU vitamin D.

Patients and methods: 25(OH)D status was assessed in the outpatient setting in osteoporotic patients during 3 months period (September-November 2007). We evaluated 67 patients. 46 patients were supplemented with cholecalciferol at doses ranging from 8.000 IU to 14.600 IU once weekly for at least 3 months. 21 patients had no previous vitamin D supplementation.

25(OH)D levels as indicator of vitamin D status were measured by Roche Elecsys analyzer and expressed as nmol/L.

Results: Results are expressed separately based on weekly supplementation dose.

The mean 25(OH)D level was 77 nmol/L (range 48–98 nmol/L) in patients taking 8.000 IU weekly (n = 19, mean age 69 years), 83 nmol/L (range 54–112) in patients taking 11.300 IU (n = 20, mean age 69 years), 72 nmol/L (range 64–103 nmol/L) in patients taking 14.600 IU weekly (n = 7, mean age 70 years).

In 22 (48 %) of these patients levels of 25(OH)D reached at least 80 nmol/L.

In 21 patients (mean age 61 years) without vitamin D supplementation mean 25(OH)D level was 60 nmol/L (range 26–93 nmol/L).

Conclusion: Supplementation with vitamin D 8.000 IU to 14.600 IU once weekly during three-months period leads to target levels of 25(OH)D in about half of patients. The highest level observed was 112 nmol/L, i.e. within safety margin. With respect to our results we consider routine use of vitamin D doses higher than those usually recommended (weekly cummulative dose 5.600 IU) administered once weekly daily beeing safe. Once-weekly approach might be more convenient particularly for older patients. Data from extended supplementation period are desirable.

Conflict of Interest: None declared

Mo-P452

AN ENVIRONMENTAL SCAN OF OSTEOPOROSIS MANAGEMENT IN CANADIAN NURSING HOMES

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The incidence rate of hip fractures in nursing home residents is approximately 4 times higher than age-matched community-dwelling elders. Up to 80% of female residents have a T-score -2.5 SD or less. Despite these numbers, osteoporosis and fractures are under treated in nursing homes in part because of uncertainty regarding management in this medically complex group. A Long Term Care, Osteoporosis Strategy Project is currently underway in Ontario, Canada to address appropriate management of fractures and osteoporosis in nursing home residents. To determine the current rate of osteoporosis-related prescribing in Ontario nursing homes, we performed an environmental scan of a nine nursing homes (N = 1728 beds) from several regions that are serviced by a large, centralized pharmacy provider. Consultant pharmacists completed a pharmacy database audit for all residents residing in the respective facilities they served. The mean age of individuals on any osteoporosis therapy was 83.0 (SD = 9.8) years. The overall rate of prescribing for a bisphosphonate was 17.9%. Only 20.5% of residents were taking 800 IU or greater of Vitamin D daily, and 16.3% of residents were taking 1000 mg or greater of calcium daily. This is the first study in Canada to examine

a large, unselected group of nursing home residents. As compared with other types of data (administrative, chart audit), there is a high degree of accuracy for these records given the database represents every prescription filled in a facility. Our results indicate that the rate of osteoporosis-related prescribing remains sub-optimal, and confirms the need for our community of practice initiative to educate and improve osteoporosis management in nursing homes. Baseline focus group data including barriers and facilitators to osteoporosis management in nursing homes will also be presented.

Conflict of Interest: JD Adachi: Current or past consultant for the following: Amgen, Astra Zeneca, Aventis, Eli Lilly, Glaxo Smith Kline, Merck, Novartis, Pfizer, Procter & Gamble, Roche, Servier, Wyeth.

Alexandra Papaioannou: Competing interests include Eli Lilly, Merck Frosst Canada Ltd., Novartis, Procter & Gamble Pharmaceuticals Canada, and sanofi-aventis.

Mo-P453

EFFICACY OF RISEDRONATE FOR NON-VERTEBRAL FRACTURES IS CONSISTENT ACROSS AGE GROUPS

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Age is one of the most important risk factors for fracture among untreated patients. With age, fracture risk increases. In this study, we examined relationships between age and fracture risk using data from randomized, placebo-controlled clinical trials. Risedronate anti-fracture efficacy (5 mg daily vs.placebo) was examined among and across a wide range of age groups. The analysis population included 3229 postmenopausal women from 4 risedronate phase III randomized placebo-controlled clinical trials. Average age was 68 yrs and Femoral Neck (FN) T-score was -2.2. Fracture was defined as any osteoporosis-related radiographically confirmed non-vertebral fracture. The impact of age was estimated using a Cox regression model, adjusted for FN T-score and treatment group and stratified by clinical trial in order to account for different underlying hazard functions. Interactions among treatment, age and FN T-score were included in the initial model but then removed because it was not statistically significant ($p > 0.05$). The association between increased fracture risk and age did not differ significantly by treatment groups ($p > 0.05$, for the interaction terms). For every decade increase in age, patient risk for any osteoporotic fracture increased by 49% (95% CI 21–88%)among patients in the same treatment group with similar baseline FN T-score. Overall, risedronate reduced non-vertebral fracture risk by 43% (33%,58%) relative to placebo after adjusting for age and BMD. The relative risk reductions for patients with age ≤ 65, 66–75 and ≥ 76 were 51%, 39% and 45% with p-values < 0.03. The therapy is well tolerated in the treatment of women with established postmenopausal osteoporosis (Harris JAMA 1999). Age is an important risk factor for fracture. Risedronate demonstrated consistent efficacy for nonvertebral fractures across a wide range of age groups.

Table 1 Osteoporotic Nonvertebral Fracture Incidence 0–3 Years;

Age Group	≤ 65	66–75	≥76	OVERALL
Number of Patients	1197	1480	552	3229
Non-Vert Fx Incidence				
Placebo	7.5%	9.4%	19.5%	10.5%
Risedronate 5 mg	3.8%	6.3%	11.3%	6.4%
RR in 95% CI	0.49	0.61	0.54	0.57

Conflict of Interest: Bilezikian consultant P&G
Boonen Consultant P&G
Klemes Employee P&G
Zhou Employee P&G

Mo-P454

CONTINUOUS TREATMENT OF POSTMENOPAUSAL OSTEOPOROTIC GREEK WOMEN WITH RISEDRONATE AND ITS EFFECT ON BONE MARKERS

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The aim of this study was to investigate the effect administration of Risedronate to early postmenopausal women for 72 months by measuring Ca,P,creatinine,serum CTX changes, serum osteocalcin and 25(OH)2 D3. Forty early postmenopausal women 48–53 years old (mean 50), 6 months –1 year after menopause, with T score < 2SD on lumbar spine DEXA, without any prior metabolic disorders or fractures.Women were separated in 2 groups: Group A ($n = 30$) daily received 5 mg Risedronate, 1 mcg Alphacalcidol and 1000 mg Calcium carbonate for 12 months and 0,25 Alphacalcidol and calcium for the rest of the study period, while Group B($n = 10$) received the same doses of Alphacalcidol and calcium for the first 12 months and only 0,25 Alphacalcidol and calcium thereafter. Serum bone turnover markers were measured at 0, 6, 12, 24, 36, 48, 60, 72 months intervals by automated electrochemiluminence assay. No premenopausal values were available for comparison. Group A showed a statistically important decrease in sCTX (by −11,69% in 6mon., −9,63% in 12mon., −9,50% in 72 mon., with $p < 0.0005$). In Group B sCTX was increased (+8,9% in 6 mon., +13,3% in12 mon., +16,5% in 72 mon., with $p < 0.0005$) ,while the rest of the markers showed a statistically important decrease for the same period, no values fell below normal. Changes in the measured markers, especially sCTX, demonstrate that Risedronate effectively decreases the turnover as early as 6 months after treatment and the effect is maintained without further changes from the end of the first year until the end of the 72 months period provided that vitD is sufficient. The fact that no values fell bellow normal excludes the untoward presence of frozen bone. Quality of life was also improved in the Risedronate group without any untoward effects from upper gastrointestinal tract and musculoskeletal system.

Conflict of Interest: None declared

Mo-P455

A RANDOMIZED CONTROL TRIAL TO EVALUATE THE IMPACT OF RISEDRONATE ON BONE LOSS CAUSED BY A SINGLE FLARE-UP OF INFLAMMATORY BOWEL DISEASE

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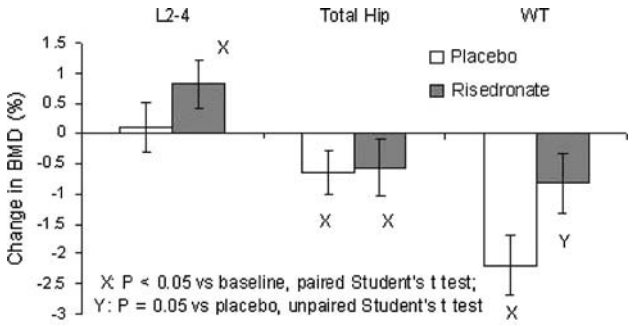
Inflammatory bowel disease (IBD) is associated with an increased risk of fracture, presumably reflecting the accumulative effect of multiple disease flare-ups, and concomitant glucocortocioid (GC) therapy, on the skeleton. Strikingly, in patients receiving GC therapy for a flare-up of Crohn's disease (CD), in the absence of calcium and vitamin D supplementation or any other bone protective therapy, 4% bone loss at Ward's Triangle (WT) was observed in our previous study after only 8 weeks. Here, we report a randomized control trial to examine whether equivalent bone loss occurs in patients with a flare-up in IBD administered calcium and vitamin D supplements with or without bisphosphonates. Participants had a DXA scan of the lumbar spine (L2–4) and both hips within one week of commencing GC therapy for a flare-up in IBD. Calcium and vitamin D supplements were given to all subjects, and in addition, patients were randomized to receive placebo or risdedronate 35 mg once weekly for 8 weeks, a repeat DXA was then performed. 78 patients completed the study (mean age 42.5, 57.7% male), of whom 39 received placebo (16 CD and 23 ulcerative colitis (UC)), and 39 risedronate (17 CD and 22 UC). Change in BMD over the 8 week study period is shown in the figure (results show mean + SEM). These results indicate that even when calcium and vitamin D supplements are taken, IBD patients sustain considerable bone loss at the hip during a disease flare-up, mainly at WT. As well as increasing spinal BMD, concomitant treatment with risedronate in this context reduces bone loss at WT, but does not prevent bone loss at the hip as a whole as reflected by total hip BMD, possibly reflecting an important contribution of defective osteoblast function to IBD-associated bone loss.



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Mo-P456

PROSPECTIVE DATA ON THE FUNCTIONAL OUTCOME OF PATIENTS AFTER HIP FRACTURE

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Aims: To prospectively study the functional outcome following proximal hip fractures

Materials and Methods: Prospectively collected data from sixty-eight consecutive patients who had been admitted to hospital from May 2001 to September 2001 were reviewed. Patients were followed prospectively to determine the functional and social status associated with hip fracture over a 2-year follow-up period. A basic questionnaire and Barthel index of activities of daily living were used to assess the morbidity and social status of the patients.

Results: After fracture, improvement occurred progressively and reached a plateau at 6 months.

The total Barthel index (continuous) was analyzed using 'SAS Proc Mixed' adjusting for ASA, age groups (more than and equal to 80 years old or less), gender of the patients, types of fracture, living situation (home/nursing home), number of co-morbidities and length of stay during acute hospital admission.

The mean total Barthel score at 6 month, 1 year and 2 years is significantly higher than in hospital ($p < 0.0001$). There is no significant difference between the mean total Barthel score of 6 months, 1 year and 2 years. The mean total Barthel score of the patient who survived showed a persistent decrease compared with their mean total Barthel score before the hip fracture.

Conclusions: The functional outcome afetr hip fracture appears to plateau after 6 months. Only about 35% of patients are back to their previous ambulatory status in this 2 years follow-up. About 50% of patients require a step down care facilities upon discharge from hospital but at the end of 2 years, the number of patient requiring nursing home care was not significantly higher than that of pre-fracture.

Conflict of Interest: None declared

Mo-P457

EFFECTS OF PAMIDRONATE, CALCIUM AND VITAMIN D IN TREATMENT OF EXPERIMENTAL OSTEOPOROSIS

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Backgrounds: Bisphosphonates are well known potent inhibitors of osteoclast activity and widely used clinically for postmenopausal osteoporosis.

Aim: Aim of the study was to evaluate the effects of pamidronate (P), calcium (Ca) and vitamin D on osteoporosis include by ovarian hormone (estrogen) deficiency.

Methods: 14-weeks-old female Wistar rats ($n = 21$) were randomized in three groups: OVX + P + Ca + vit D ($n = 7$), OVX (ovariectomized) ($n = 7$) and INT (intact control) ($n = 7$). The accommodation conditions and feeding were the same for all groups temperature 22–24°C. After six weeks the OVX + P + ca + vit D group was treated with pamidronate 0,3 mg/ 100 g body weight intraperitonealy every fourth day, during five weeks, Ca (500 mg) and vit D (400 i.j.) per os during five days in week, for five weeks. At the end of five weeks period the experimental animals (rats) were sacrificed. The biochemical analyses: osteocalcin (OC), alkaline phosphatase (AP), calcium (Ca) and phosphorus (P) were evaluated. The histological analyses of left tibia stained with hematoxillin-eozin (HE) were studied by routine microscopy. Biochemical properties were tested on TOMI-2001.

Results: Statistically significant decrease of OC ($p < 0, 01$), AP ($p < 0, 05$) and PHOS ($p < 0, 01$) was obtained in experimental OVX + P + Ca + vit D group compared to OVX group. Histological analysis showed increased ossification, the trabecules were unequal and irregular, with less connections between themselves, in comparison with untreated control group (OVX). Biomechanical properties of femur from treated group showed statistically significant increase bone fragility in comparison to intact bone.

Conclusion: This study shows that pamidronate, calcium and vitamin D have antiresorptive effect in the treatment of experimental osteoporosis.

Key words: experimental osteoporosis, pamidronate, calcium, vitamin D.

Conflict of Interest: None declared

Mo-P458

PATIENTS WITH NEW COLLES' FRACTURES HAVE HIGH LEVELS OF UNDERDIAGNOSED AND UNDERTREATED PREVIOUS FRACTURES

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Introduction: Older community-dwelling subjects with previous Colles' fractures have a high prevalence of osteoporosis and are under-investigated and under-treated 1. This study aimed to assess the previous treatment of osteoporosis in patients presenting with Colles' fractures. This data gives insight into the bone quality and treatment practices for this population.

Methods: Each patient attending an outpatient trauma clinic with a Colles' fracture in the period 28/04/04 to 1/11/07 was invited to attend a nurse-led specialist clinic to assess the risk of osteoporosis and falls.

Results: Of the 196 subjects, 81 had a previous history of a fracture, 44 of these being Colles' fracture. Fifty-six (69%) of the patients with prior history of fractures, and 35 of the patients (80%) with prior Colles' fracture had osteoporosis. Despite the previous history of fracture only 11(14%) patients were on bone protectants. There were no significant differences (p value 0.12 to 0.55) found in terms of age, gender or mean T score between those with and without a previous fracture.

Conclusions: In this group with Colles' fracture, 41% had had a previous fracture; 69% of these had proven osteoporosis. However, only 14% were on bone protectants. This highlights the lack of import and low level of intervention given to patients with initial fractures.

References: 1. Masud T, Jordan D, Hosking DJ; Age Aging 2001

Conflict of Interest: None declared

Mo-P459

INTAKE OF CALCIUM, MAGNESIUM AND SODIUM THROUGH WATER: HEALTH IMPLICATIONS

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Calcium (Ca2+) intake through diet is mainly obtained from dairy products. However, there are other sources of Ca2+, such as water, which can significantly contribute to its intake. Moreover, water also contains other minerals, such as magnesium (Mg2+) and sodium (Na+), with potential implications for health. Thus, Mg2+ has been associated with a reduction of sudden death, whereas Na+ contributes to the occurrence of hypertension. The rise in the consumption of bottled water in the general population clearly indicates the necessity of knowing the possible effects on health. Indeed, there may be a great variation in the content of these minerals depending on the type of water.

Methods: We obtained the mineral content of Ca2+, Mg2+ y Na+ from tap water of 492 Spanish towns and cities (through data given by autonomous communities, city/town halls or municipal water companies) and from 182 commercially available bottled waters (122 available in Spain, 60 available in Europe). The results were compared with the recommended dietary intake of these minerals Results: There is a great variation in the mineral content among the different bottled waters and also among tap waters. Thus, among bottled waters in our country the Ca2+ concentration ranges between 0.5–672 mg/l; 16% of these waters had a concentration > 100 mg/l and only two > 300 mg/l; some European waters showed high concentrations of Ca2+ (459–575 mg/l); Na+ concentrations ranged between 0.1–2.000 mg/l, and Mg2+ between 0.1–128. In tap water Ca2+ concentrations ranged between 0–337 mg/l, Na+ between 1.0–260 mg/L, and Mg2+ between 0.3–315 mg/L. In 33.4% of the analysed tap waters the Ca2+ concentration was > 100 mg/l, in 4 of them it was > 200 mg/l.

Conclusion: Water, even bottled water or tap water, has a great variability in the concentrations of Ca2+, Mg2+ and Na+. In some occasions, water may even supply the minimum recommended intake of Ca2+ and Mg2+ and can exceed the Na+ content. These data should be considered when selecting one for consumption.

Conflict of Interest: None declared

Mo-P460

SAFETY AND TOLERABILITY OF TERIPARATIDE IN ELDERLY OSTEOPOROTIC ITALIAN WOMEN

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Backgroung: Teriparatide (TPTD)is an osteoanabolic drug for the treatment of osteoporosis in postmenopausal women and in men. Due to pharmacoeconomic reasons, in Italy this drug is used for the treatment of patients non responder to prior antiresorptive drugs or with 3 or more fragility fracture at diagnosis. Therefore TPTD is frequently used in elderly patients with comorbidities and concomitant treatments. In clinical trials various side effects have been described in course of TPTD treatment, the most frequent being represented by nausea, limb pain, headache and dizziness. The aim of our study was verify the safety and tolerability of TPTD in elderly osteoporotic women with 2 or more incident fractures.

Methods: A sample of 150 elderly osteoporotic women (mean age 70 ± 8 yy)was treated with TPTD according to the recommendations of Italian Drug Agency (AIFA). Side effects were recorded during a 12-month observation period. We looked for relationships between side effects and age, comorbidities, concomitant treatment and number, site and severity of incident fractures.

Results: During the observation period only 7 of the 150 TPTD-treated patients reported side-effects (4.7%): 4 pazients reported headache and 3 patients nausea. All the side effects were mild and not required treatment withdrawal. We found no relationships between side effects and age, comorbidities, concomitant treatment and number, site and severity of incident fractures.

Conclusion: TPTD treatment seems to be safe and well-tolerated in elderly osteoporotic women. Patient age, comorbidities and concomitant treatment don't seem to influence side-effects incidence.
Conflict of Interest: None declared

Mo-P461

TERIPARATIDE USE IN THE ELDERLY: REAL LIFE DATA FROM THE UK

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Introduction: The Fracture Prevention Trial (FPT) has previously shown that teriparatide, an anabolic agent, can effectively reduce the incidences of new fractures [Neer et al, NEngJMed 2001]. Further analysis that age did not affect safety and efficacy in postmenopausal women, and that it was well tolerated in elderly patients with no differences in the safety profiles between those younger and older than 75 years [Boonen et al, JAGS,2006]. Medication adherence and persistence are important issues in osteoporosis.

Aims and Methods: The aims were to report the experience of using teriparatide in the elderly (aged over 75 years) in terms of persistence and adverse effects. Data for descriptive analysis were provided by "Healthcare at Home", an organisation through which most teriparatide prescriptions are processed and delivered in the UK. It also teaches patients on administering injections and checks compliance.

Results: Data were available on 1293 patients (62 males) aged 75 years and above registered for teriparatide (47.4% of the total population registered). The numbers in the age ranges 75–79, 80–85, 86–90 and > 90 years were 645 (49.9%), 468 (36.2%), 157 (12.1%) and 23 (1.8%) respectively, with the oldest being 97 years. 440 (34.0%) patients were still on teriparatide therapy and 48 (3.7%) died whilst on therapy. In 7 subjects the initial registration had not been taken up, and in another 8 registration was on hold. The number of patients who completed the full 18 month course and those who stopped before completion were 518 (40.1%) and 272 (21.0%) respectively. The persistence at 18 months was 65.6%. For those "finishing" therapy (completed course or stopped early) the reasons for discontinuing were: clinical decision 138 (17.5%), patient's decision 51 (6.5%), adverse events 51 (6.5%), problems with funding treatment 12 (1.5%), inadequate response 5 (0.6%).

Conclusions: This "real life" data show that in patients aged > 75 years, teriparatide was generally well tolerated and persistence with teriparatide at 18 months (full course) is good compared to oral treatments available for osteoporosis. Age is not a barrier for teriparatide use.
Conflict of Interest: T Masud Educational support from Eli Lilly UK M Goater Company Eli Lilly

Mo-P462

THE IMPACT OF RISEDRONATE ON CLINICAL FRACTURE RISK AMONG PATIENTS WITH PRIOR HIP FRACTURE

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Previous hip fracture is a risk factor for subsequent fractures. In this analysis, the anti-fracture efficacy of risedronate was examined among patients with previous hip fracture at baseline. A total of 339 postmenopausal women between ages 70–79 years with low BMD in HIP trials (McClung,MR et al. 2001 NEJM) had a history of at least one hip fracture prior to study entry. These subjects were treated with either placebo (PLC) or risedronate (RIS) 2.5 mg or 5 mg daily. The incidence of osteoporosis-related clinical fracture, defined by the occurrence of the radiographically confirmed clinical vertebral fracture or radiographically confirmed non-traumatic non-vertebral fracture was calculated using Kaplan-Meier survival estimates over a 3 year period and was compared between placebo- and risedronate-treated subjects. Treatments were compared using log rank test, and the risk ratio and its 95% confidence interval were obtained using a Cox regression model stratified for study. Subjects' mean age was 75 years, and their mean femoral neck (FN) and lumbar spine (LS) T-scores were -3.1 and -3.2 SD, respectively. Baseline characteristics were well balanced between the 3 treatment groups. Over the 0–3 year period, the clinical fracture incidences were 28.4%, 14.9% and 13% for the PLC, RIS 2.5 mg and RIS 5 mg groups, respectively. Relative to the placebo group, RIS 5 mg and 2.5 mg statistically significantly reduced the risk for clinical fracture over 0–3 year period ($p < 0.05$). The risk ratio of clinical fracture was 0.5 for both the risedronate 5 mg and 2.5 daily groups relative to the placebo group. Risedronate treatment was well tolerated in HIP trial. Risedronate significantly reduced the risk of clinical fracture among women with postmenopausal osteoporosis with prior hip fracture.

Table 1 Clinical Fracture Incidence Following Hip Fracture

Treatement Group	Placebo	RIS 2.5 mg	RIS 5 mg	RIS 2.5 &5 mg
Clin Fracture Incidence (%)	27/111(28.4%)	15/122(14.9%)	12/106(13.0%)	27/228(14.1%)
RR		0.5	0.5	0.5
		$p = 0.031$	$p = 0.048$	$p = 0.011$

Conflict of Interest: McClung, Miller, Boonen; Consultants P&G

Mo-P463

THE BMD AND BONE TURNOVER MARKER RESPONSE TO STRONTIUM RANELATE IN WOMEN WITH PRIOR BISPHOSPHONATE EXPOSURE

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Background/aims: The treatment response to Strontium Ranelate (SR) in women with prior bisphosphonates (BP) exposure is not known. SR is predominantly deposited in new bone. BP markedly reduce new bone formation which may impede SR uptake. We investigate whether prior BP exposure affects the subsequent response to SR.

Method: We recruited 120 women (60 women taking BPs, 60 BP naïve) into a 1 year observational study. All women commenced SR 2 g/day and 1 g calcium with 800 iu vitamin D. Follow up was at 3,6 and 12 months with BMD and P1NP.

Results: Before the first follow up visit 8 women discontinued from the prior BP group and 4 discontinued in the BP naïve group leaving 108 women. The prior BP group was older (66.9 vs. 62.5, $p = 0.01$) and had a lower baseline BMD at the spine (0.801 vs 0.836 g/cm^2, $p = 0.03$). Total hip BMD was similar (0.751 vs. 0.780 g/cm^2,

$p = 0.18$). In the prior BP group P1NP was suppressed consistent with BP use (30.1 vs. 54.9, $p < 0.001$). There were no other significant differences between the groups.

To date preliminary results are available on 96 women at 6 months while 65 have completed one year. There was no significant change in P1NP over the first year in the BP naïve group while P1NP increased steadily from 30.1 to 45.5 ($p < 0.001$) in the prior BP group.

Spine BMD increased to a greater extent in the BP naïve women than in the prior BP group at 6 months (0.021 vs. -0.006 g/cm^2, $p = 0.02$) and 12 months (0.044 vs. 0.023 g/cm^2, $p = 0.07$). After 1 year spine BMD had increased significantly by 5.2% ($p < 0.001$) in BP naïve women and by 2.9% ($p = 0.005$) in the prior BP group. Total hip BMD increased to a greater extent in the BP naïve women than in the prior BP group at 6 months (0.014 vs. 0.001 g/cm^2, $p = 0.001$) and 12 months (0.028 vs. 0.004 g /cm^2, $p < 0.001$). After 1 year total hip BMD had increased significantly in the BP naïve group (3.6%, $p < 0.001$) but not the prior BP group (0.5%, $p = 0.21$).

Conclusion: After switching from a BP to SR there is an increase in bone turnover during the first year. However there is no increase in BMD at the hip. At the spine there is no increase in BMD at 6 months and the increase after 1 year is less than that observed in BP naïve women. These results demonstrate mild blunting of the BMD response to SR in women with prior BP exposure however BMD starts to increase after 6 months suggesting the blunting is only temporary.

Conflict of Interest: Our Centre received an educational grant from Servier which part funded this study.

Mo-P464

THE EFFECT OF STRONTIUM RANELATE ON HEEL BMD AND ULTRASOUND

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Background/aims: Strontium Ranelate (SR) is known to cause a marked increase in bone mineral density (BMD) as measured by DXA. When strontium is incorporated into the skeleton there is an artefactual increase in BMD due to the increase in X-ray attenuation by the strontium atoms. SR is also thought to increase the bone volume fraction and improve the microarchitecture of bone. Broadband Ultrasound Attenuation (BUA) is related to both bone volume fraction and the internal structure of the bone. DXA and ultrasound can both be performed at the heel. Direct comparison of these measures may help determine the proportion of the increase in BMD which is attributable to increased bone volume and microarchitectural changes rather than the X-ray attenuation artefact.

Method: We recruited 60 women into a 1 year prospective study. All women were started on SR 2 g/day and 1 g calcium with 800 iu vitamin D and were followed up at 6 and 12 months with heel DXA and ultrasound. Results:60 women, mean age 62.5, were recruited. The women were on average 15 years postmenopausal, had a BMI of 24.9 kg/m2 and were vitamin D replete (72.9 nmol/l). Heel BMD at baseline was 0.391 g/cm^2. Heel BUA was 51.7 dB/MHz and velocity of sound (VOS) was 1568.1 m/s.

At present data is available on 50 women at 6 months and 38 women at one year. Heel BMD increased significantly at both 6 (+2.9%, $p = 0.002$) and 12 months (+4.1%,$p < 0.001$). BUA did not increase significantly at 6 months (+1.3%, $p = 0.21$) although the increase by 12 months was almost significant (+2.9%, $p = 0.06$). VOS did not alter at 6 months (-0.1%, $p = 0.38$) and reduced slightly at 12 months (0.72% $p < 0.001$).

There were significant correlations between the 12 month changes in heel BMD with both heel BUA ($r = 0.47$, $p = 0.007$) and total hip BMD ($r = 0.34$, $p = 0.039$).

Conclusions: With SR therapy BMD at the heel increases progressively during the first year of treatment. This is consistent with the reported increase in BMD at the spine and hip with SR therapy which is partly due to the artefact induced by strontium's high atomic mass. Ultrasound however demonstrates no increase in attenuation at 6 months although BUA almost significantly increased at 12 months. BUA is unlikely to be affected by the high atomic mass of strontium and may provide in-vivo evidence of an increase in bone volume fraction and improved microarchitecture with strontium ranelate.

Conflict of Interest: Our Centre recieved an educational grant from Servier which part funded this study.

Mo-P465

EFFICACY OF BAZEDOXIFENE FOR THE PREVENTION OF POSTMENOPAUSAL OSTEOPOROSIS: RESULTS OF A 2-YEAR, PHASE III, PLACEBO- AND ACTIVE-CONTROLLED STUDY

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Bazedoxifene (BZA) is a novel selective estrogen receptor modulator (SERM) currently in clinical development as monotherapy for prevention and treatment of postmenopausal osteoporosis. In preclinical studies, BZA maintained skeletal mass without stimulation of the mammary gland or endometrial tissue. This 2-year, Phase III study was designed to assess the efficacy and safety of 3 BZA doses compared with placebo (PBO) and raloxifene (RLX) in the prevention of postmenopausal osteoporosis. Healthy postmenopausal women ($N = 1583$; mean age: 57.6 y) with lumbar spine or femoral neck BMD T-scores no less than -2.5 (mean, -1.2) were randomized to 1 of 5 groups: BZA 10, 20, or 40 mg, PBO, or RLX 60 mg. All women received 600 mg elemental calcium. The primary outcome was percent change in lumbar spine BMD at 24 months; secondary outcomes included BMD at other skeletal sites and serum bone turnover markers. All BZA doses and RLX prevented bone loss, whereas PBO was associated with significant reductions in BMD. The difference in percent change of lumbar spine BMD from baseline to 24 months relative to PBO was 1.08%, 1.41%, 1.49%, and 1.49% for 10 mg, 20 mg, 40 mg BZA and 60 mg RLX, respectively ($P < 0.001$ for all comparisons). Comparable BMD responses were observed with BZA at other skeletal sites. Significant decreases in serum osteocalcin and C-telopeptide levels from baseline and relative to PBO were observed as early as 3 months and remained sustained through study end ($P < 0.001$). By Month 24, median serum osteocalcin levels decreased from baseline by 21%, 22%, 22%, and 27% with BZA 10, 20, and 40 mg, and RLX, respectively, and 6% with PBO ($P < 0.001$ vs baseline for each); median serum C-telopeptide levels decreased by 25%, 24%, 22%, and 32% with respective BZA doses and RLX and 13% with PBO ($P < 0.001$ vs baseline for each). Overall, BZA was well tolerated and exhibited a favorable safety profile. In conclusion, this study demonstrated that treatment with BZA, a new SERM, prevented bone loss, reduced bone turnover, and was generally well tolerated in postmenopausal women with normal or low BMD.

Conflict of Interest: Miller, Amgen, Merck, Novartis, P&G, Roche, sanofi-aventis, Grant/Consultant. Christiansen, Wyeth, Consultant.

Kendler, Merck, Eli Lilly, Pfizer, Novartis, Servier, Takeda, Wyeth, Zelos, Amgen, Grant, Consultant/Speakers Bureau. Delmas, P&G, Eli Lilly, Amgen, Research Grants/Consultant/Speaker. Lewiecki, Amgen, Eli Lilly, GSK, Merck, Novartis, Pfizer, P&G, Roche, Wyeth, Grant; P&G, Stock. Woodson, Amgen, Eli Lilly, GSK, Merck, Wyeth, Grant Support; Eli Lilly, Speaker.

Mo-P466

INFLUENCE OF LUMBAR SPINE BMD AT BASELINE ON THE INCIDENCE OF NEW VERTEBRAL FRACTURES AFTER 18 MONTHS TREATMENT WITH PTH(1–84). RESULTS FROM THE TOP STUDY

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Background: Bone mineral density (BMD) measurement is important in the assessment of fracture risk in postmenopausal women. Treatment with PTH(1–84) is indicated in postmenopausal women with a high risk of suffering a new vertebral fracture. This analysis presents results from a stratified analysis of fracture incidence based on baseline lumbar spine BMD.

Materials and Methods: The Treatment of Osteoporosis with PTH (TOP) study was designed to determine the effectiveness of PTH(1–84) (100 μg daily) in preventing vertebral fractures in postmenopausal osteoporotic women during 18 months of treatment. Subjects also received 700 mg calcium and 400 IU vitamin D. The TOP study included postmenopausal women with low bone mass without ($n = 2056$) or with ($n = 471$) a prevalent vertebral fracture. Subjects $> = 55$ years of age with spine, femoral neck, or total hip BMD T-score ≤ -2.5 (or ≤ -2.0 with a prevalent vertebral fracture), or 45–54 years of age with a T-score ≤ -3.0 (or ≤ -2.5 with a fracture) were included. A total of 2532 subjects were enrolled and baseline BMD was available in 1214 women receiving placebo and 1258 receiving PTH(1–84).

Results: The table 1 shows the number of subjects in the different strata of lumbar spine BMD T-scores at baseline and the prevalence of new vertebral fractures during 18 months of treatment.

Conclusion: A wide range of lumbar spine BMD values and risk factors for vertebral fractures were encountered in women recruited into the TOP study. The results shows that PTH(1–84) is effective in postmenopausal women with a high risk of suffering a new vertebral fracture.

Conflict of Interest: None declared

Table 1

Baseline T-score	No. Subject Placebo	No. Subject PTH	Relative risk 95%CI	p-value
>-1.0	25	20	NA	0.444
$\leq -1.0$to>-2.0	58	77	NA	0.429
$\leq -2.0$to>-3.0	471	439	1.43(0.3–6.4)	0.717
$\leq -3.0$to>-4.0	485	525	0.21(0.1–0.7)	0.009
≤ -4.0	207	225	0.33(0.2–0.7)	0.002

Mo-P467

EFFECT OF LIVER TRANSPLANTATION AND HIGH-DOSE ZOLENDRONATE TREATMENT ON BONE MINERALIZATION IN "HEPATIC" BONE DISEASE

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The first period after liver transplantation (LTX) is characterized by dramatic bone loss and increased fracture risk. In a recent clinical trial we could prove the efficacy of zolendronic acid (ZOL) in preventing bone fractures after LTX in patients with hepatic bone disease who had decreased T-scores and higher fracture rates than normal already prior to transplantation. Paired transiliacal biopsies (at and 6 months after LTX) from a subgroup of patients of this clinical trial were measured for effects of ZOL on the bone mineralization density distribution (BMDD) by quantitative backscattered electron imaging. Control (CON, $n = 18$) and treatment group (ZOL, $n = 21$) were treated with calcium and VitD. The ZOL group received iv ZOL at doses of 4 mg/month. The "hepatic" bone disease at baseline displayed a decreased mean and typical calcium concentration (CaMean and CaPeak $= -2.9\%$ and -2.8%, resp. $p < 0.001$), whereas the heterogeneity of mineralization (CaWidth $= +12.2\%$, $p = 0.01$) and the percentage of bone areas undergoing primary mineralization (CaLow $= +32.4\%$, $p < 0.02$) were increased. Moreover, CaMean and CaPeak were negatively, while CaWidth and CaLow were positively correlated with the serum parameters osteocalcin (OC), crosslaps (CTX) and also with intact parathyroid hormone (iPTH) (in part $p < 0.05$). Six months after LTX, the following differences between the ZOL group and the CON group could be found: i) CaLow (-50%, $p = 0.047$) was reduced. ii) The relative mean changes in BMDD-parameters were in the opposite direction: delta CaMean $= +1.9\%$ for ZOL and -1.9% for CON ($p = 0.038$) and delta CaLow $= -11.7\%$ for ZOL and $+92.7\%$ for CON ($p = 0.014$). iii) The differences in CaMean between baseline and 6 months after LTX plotted as a function of baseline CaMean revealed a linear relationship (linear regression $r2 = 0.58$, negative slope, $p < 0.0001$) in the ZOL group, while in the CON group there was no such association. iv) No indication of hypermineralization could be observed. The findings are consistent with an antiresorptive action of ZOL, which is associated with an increase of mineralization towards normal. This effect of ZOL might explain in part the significant decrease in fracture risk after LTX.

Conflict of Interest: None declared

Mo-P468

TERIPARATIDE ADMINISTRATION FOLLOWING INSUFFICIENT BISPHOSPHONATE THERAPY (BBB-STUDY): CLINICAL CHANGES, 3D MICROARCHITECTURE & HISTOLOGIC FINDINGS AFTER 6 MONTHS

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Background: Cases with progressive osteoporosis (OPO) exist despite good compliance/persistence to bisphosphonate treatment (continuous fractures, loss of BMD). Teriparatide (TPD) is a therapeutic option, but there is limited experience about the effects of previous treatments on lab, BMD and bone structural parameters. 6 month data of a prospective study are presented. Methods: 25 women (age 69 ± 9) with progression of severe OPO(mean treatment duration 3.5 (1....7) yrs;12 ALN/13 RIS; new fragility fx $n = 14$, BMD decline $> 3.5\%$ $n = 11$) were recruited for 18 months TPD therapy (+500 mg Ca & 400 IU Vit.D3). BMD of lumbar spine (LS), femoral neck (FN) and total-hip (TH) were measured at M0 and M6 with DXA Lunar Prodigy. Lab (calcium, BALP, CTX) and QoL questionnaire (with VAS for pain) were performed at M0, 1, 3 and 6. Paired bone biopsies by Jamshidi technique from dorsal iliac crest were taken at M0 and M6 at alternating sites and analysed by light microscopy and MicroCT 40. Results: 22 patients for clinical data and 189 paired biopsies were available for analysis. BMD T-scores M0/M6: LS $-2.96/-2.20$($p = 0.05$), FN $-2.21/-2.08$ (NS), TH $-1.94/-1.73$ (NS). Lab M0/M1/M3/M6: s-calcium 2.32/2.36/2.46/2.36 ($p < 0.001$ for M3 vs. M0; normal 2.05...2.65 mmol/l; observation of hypercalcaemia at any time, $n = 4$); BALP 14.4/20.5/20.3/28.4 ($p < 0.001$; ULN = 21.4 mg/l); CTX 239/350/553/850 μg/l ($p < 0.001$; ULN = 573 μg/l). Pain (VAS) was unchanged. Duration of BIS > 3.5 y delayed changes of CTX slightly until M3 (despite trend also at M1). Neither use of ALN or RIS, nor fracture vs. BMD decline as inclusion parameter influenced any of the observed results. Pairwise changes in the degree of tissue mineralization at M6 did not differ from M0, independent of bone turnover status. Pairwise changes in BV/TV and trabecular number improved significantly. The average increase in bone volume fraction was about 2 %. Conclusions: Early and continuous increases of BALP and CTX were observed as well as significant increase of LS-BMD at M6. Type/duration of previous BIS did not influence results. Mean increases of s-calcium were moderate, but 4 of 22 patients had (intermittent and asymptomatic) hypercalcaemia. TPD stimulated or balanced bone turnover. In contrast to previous reports there was no detectable decrease in tissue mineralization, yet. There was a relatively small gain in new mineralized bone at M6. It appears that an early gain in bone volume at M6 was best represented by BV/TV.

Conflict of Interest: Study received Resarch Support Grant from Lilly Germany.

Mo-P469

INHIBITION OF INFLAMMATORY EFFECT OF ALENDRONATE BY SIMVASTATIN IN A RAT MODEL OF ACUTE LOCAL INFLAMMATION

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Background: Nitrogen-containing bisphosphonates (alendronate) are anti-bone-resorptive drugs, but can induce some inflammatory effects. Statins, well known antihypercholesterolemic agents were shown to exert antiinflammatory effects, by interfering with the mevalonate pathway in cholesterol synthesis. Objective: We tested if alendronate may act on acute local inflammation and also if its effect can be modulate by simvastatin.

Methods: Wistar rats (180–220 g) were divided into four experimental groups ($n = 6$,each) The carrageenan-induced rat paw oedema test has been used as an experimental model for acute local inflammation. Experimental groups were given saline (control), alendronate (20 mg/kg), simvastatin (20 mg/kg) or (alendronate 20/mg then 1 hour later simvastatin 20 mg/kg), per os via oral gavage, respectively. Carrageenan was injected into the the right hind paw 1 h after the administration of the agents. Three hours later, footpad volume was measured with a mercury plethysmograph and compared with the pre-injection volume of the same paw. Swelling was then calculated, and in drug-treated animals, percent inhibition was derived through comparison with the control group. Skin biopsies of paw from the control and treated groups were taken for histological examination and stained with HE. Data were compared using analysis of variance.

Results: Alendronate significantly increased inflammatory response induced by carrageenan (87%, $p<0.001$) compared to control. Simvastain produced antiinflammatory effects by decreasing rat paw volume by 57,17% ($p>0.05$) compared to control. Coadministration of alendronate with simvastatin also produced antiinflammatory effect (29.16%, $p<0.01$) compared to control, but simvastatin alone has much stronger effect than its combination with alendronate ($p<0.01$). Histological evaluation of the control and alendronate tissue slices revealed an acute oedema in the dermis with extensive extravasations, mainly polymorphonuclear leukocytes (PMN), at the borderline between the dermis and subcutis. Simvastatin and coadministratin of alendronate with simvastatin reduced PMNL leukocytes infiltration in dermis and minimized histological organ injury.

Conclustion: Simvastatin could prevents some inflammatory side effects of alendronate. The underlying mechanisms could be inhibition of the mevalonate pathway by simvastatin and consequently inhibited production to proinflammatory stimuli involved in the inflammatory actions of alendronate.

Conflict of Interest: None declared

Tu-P470

ADHERENCE TO TERIPARATIDE THERAPY N A CLINICAL SETTING

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Background: Studies have shown that long-term adherence with pharmacological treatment of osteoporosis is generally low, approximately 50% after two years. Randomized studies have shown a high adherence to teriparatide, but only few data on adherence to teriparatide in routine clinical setting have been shown so far.

Aim: Our aims were to examine adherence to teriparatide therapy in a routine clinical setting, as well as the number of consultations, and the need for support for administration.

Participants and Design: Specialist nurses and doctors followed the patients in our routine clinical setting. Clinical guidelines were worked out covering four nurse consultations; introduction to the therapy (V1), initiation of teriparatide (V2), and follow up at one and four weeks (V3 and V4). Four consultations with the doctors took place after 3, 6, 12 and 18 months (V5, V6, V7 and V8). The nurse consultations focused on education of the patient, and checked up the need for support from district nurse. The study comprised 121 patients (24 men and 97 women) aged 71 [45 to 89] years diagnosed with osteoporosis. A total of 17% had one vertebral fracture, 83% had two or more. Visits, telephone calls and use of district nurse were prospectively registered.

Results: Adherence to teriparatide in a clinical setting is very high, 92%, at 18 months. Mean duration of treatment was 16.4 months. Patients came to 3.8 and 3.5 visits at the doctors and nurses, respectively, and 57% had at least one phone consultation. 13%

needed continued supervision and 26% temporary support from the district nurse. A total of 9% of the patients stopped treatment due to side effects and 3% died during follow up.

Conclusion: Teamwork between doctors and nurses is essential for the quality of care of patients with osteoporosis and high adherence to teriparatide. Our patient education programme led by specialist nurses helped the patients to manage the treatment, and the follow up consultation supported adherence.

Conflict of Interest: Mette Rothmann, Eli Lilly, Speakers Bureau
Dorthe Nielsen, Eli Lilly, MSD, Speakers Bureau
Dorthe Nielsen, Eli Lilly, Nycomed, Norvartis, Servier, MSD, Grant Research Support

Tu-P471

FALL ASSESSMENT AND PREVENTION PROGRAM FOR ELDERLY IN ACUTE HOSPITAL

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Background: One hundred forty-eight fall accidents (14%) were reported of one thousand sixty-three total medical accident reports in Japan. There are five death and twenty-one serous impairments in the fallen patients in 2005. Fall prevention is important for the risk management in hospital. We determined the efficacy of fall assessment and prevention program in hospital to decrease the number of fall and faller, injury.

Methods: To add to ordinary incident report we started the fall accident report by doctor and nurse from August, 2005. Fall risk assessment was adapted to all new in-patients from June, 2006. The assessment was consisted of two factors; dementia and Barthel Index. The in-patients were classified to eight groups and indicated the specific methods.We compared the number of fall and faller, the fall incident rate (= # of fall/# of total patient*hospital days), the number of injury and fracture between before and after intervention. We examined the rate of dementia and the implementation of the fall prevention task.

Results: Fall incident rate was significantly decreased 0.32% after intervention from 0.42% before($p < 0.05$). The faller decreased 164 patients after from 204 before. The faller more than three decreased 12 from 29. The rate of dementia in frequent faller was 83.3 percent. Total fractures are decreased 10 to 5, but hip fractures increased 2 to 4, head injury 5 to 10.

Conclusion: Fall prevention program was successed in a short period. But we should use the bed alarm and hip protector, head guard hat in a proper in-patient.

Conflict of Interest: Grant/Research Support
Japan Health, Labor and Welfare Longevity Sciences 17Kou-4

Tu-P472

DENOSUMAB, A FULLY HUMAN RANKL ANTIBODY, REDUCED BONE TURNOVER AND INCREASED CANCELLOUS AND CORTICAL BONE MASS, DENSITY, AND STRENGTH IN OVARIECTOMIZED CYNOMOLGUS MONKEYS

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RANKL inhibition by denosumab decreased bone resorption and increased bone mineral density (BMD) in postmenopausal women. Whether denosumab-related increments in BMD predict improvements in bone strength had not been examined in adult primates with high bone turnover. One month after ovariectomy (OVX), adult cynomolgus monkeys were treated with vehicle (OVX-Veh) or denosumab (25 or 50 mg/kg) SC every 4 weeks for 15 mos (n = 14–20/group). Sham controls were treated with vehicle (n = 17). Denosumab reduced urinary NTx and serum CTx, BSAP, and osteocalcin throughout the study ($p < 0.05$ vs OVX-Veh and Sham). Denosumab significantly increased DXA BMD of the lumbar spine, total hip, and radius ($p < 0.05$ vs OVX-Veh and Sham), and vBMC by pQCT at both the metaphyses and diaphyses of the proximal tibia and distal radius ($p < 0.05$ vs OVX-Veh and Sham). Denosumab markedly reduced cancellous bone turnover at the lumbar spine, iliac crest, and femur neck as evidenced by reduced mineralizing surface, bone formation rate (BFR), and activation frequency ($p < 0.05$ vs OVX-Veh and Sham). Denosumab significantly reduced cortical bone turnover, as shown by significantly lower cortical porosity, mineralizing surface, and BFR at the endocortical and Haversian surfaces of the tibial diaphysis and rib ($p < 0.05$ vs OVX-Veh). Both doses of denosumab were associated with significantly greater peak load for femur neck (19–34%), L3-L4 vertebral bodies (54–55%), and L5–L6 cancellous cores (69–82%) (all $p < 0.05$ vs OVX-Veh). Material properties derived from bending tests of femur diaphyses and cortical beams machined from the humerus were unchanged. Maintenance of normal material properties was also demonstrated by strong linear correlations between peak load and BMC at the femur diaphysis (overall r2 = 0.85), femur neck (r2 = 0.59), L3–L4 bodies (r2 = 0.70), and L5–L6 cores (r2 = 0.81). Bone strength was also strongly predicted by in vivo DXA BMD at the lumbar spine and femur neck (r2 = 0.60 and 0.50, respectively). In summary, denosumab decreased bone turnover to levels that were significantly lower than in OVX or sham controls. Denosumab increased cortical and cancellous bone volume and density in association with improvements in strength parameters, thereby maintaining the strong natural relationship between bone mass and bone strength.

Conflict of Interest: MS Ominsky, J Schroeder, JE Atkinson, WS Simonet, PJ Kostenuik: Amgen, Employees SY Smith, J Jolette,: Amgen, Paid Contractors

Tu-P473

PERFORMANCE OF QUANTITATIVE ULTRASOUND MEASUREMENTS OF BONE FOR MONITORING RALOXIFENE THERAPY

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Bone mineral density (BMD) changes in response to raloxifene therapy are small compared to other therapies. Quantitative ultrasound (QUS) may prove of greater value than BMD for monitoring treatment but to date there is only limited information. We aimed to determine the magnitude of change in QUS variables due to raloxifene therapy, in contrast to those seen in BMD, and to study the offset effect on BMD and QUS variables following treatment withdrawal.

Osteopenic, postmenopausal women (n = 125, age = 50–80 years) participated in a four year randomised, controlled study. At baseline subjects were randomised to receive either raloxifene (60

mg/day) plus elemental calcium (500 mg/day) (n = 100) or no treatment (n = 25). All subjects completing phase 1 of the study (n = 93) were invited to participate in an extension phase. Subjects then either continued on no treatment (Group 1, n = 22), or were randomly assigned from the treatment group of phase 1 to either placebo (receiving 500 mg/day elemental calcium) (Group 2, n = 23), or continued on raloxifene (Group 3, n = 24). We made duplicate measurements, one week apart, of the total hip and lumbar spine using dual energy X-ray absorptiometry (DXA) (QDR 1000W) and QUS of the fingers (DBM Sonic Bone Profiler) and calcaneus (Achilles Plus) at 0, 1, 2, 3 and 4 years.

In Group 1 there was no significant decrease at 4 years in total hip or lumbar spine BMD (THBMD or LSBMD) but finger QUS did decrease (amplitude-dependent speed of sound (Ad-SoS) by 6.2%, p = 0.004 and Ultrasound Bone Profile Index (UBPI) by 32.8%, p = 0.007). No decrease in calcaneal QUS was seen. In Group 2 only calcaneal broadband ultrasound attenuation (BUA) increased significantly by 2 years (2.5%, p = 0.033) but there was no significant decrease after cessation of raloxifene. In contrast there was a significant decrease in THBMD (p = 0.008) by year 4 following treatment withdrawal. In Group 3 there was a significant decrease in Ad-SoS of 3.5% (p = 0.038) by year 4. Any raloxifene therapy during the 4 years resulted in higher levels of LSBMD (p < 0.000), THBMD (p = 0.004), BUA (p < 0.000), Ad-SoS (p < 0.000), UBPI (p = 0.019), speed of sound (SOS) (p < 0.000) and stiffness (p < 0.000) for Groups 2 and 3 compared to Group 1.

Significant differences in group change were seen by QUS that were not detected by DXA. Quantitative ultrasound may be a potential monitoring tool in raloxifene therapy and deserves further evaluation.

Conflict of Interest: M.A. Paggiosi, IGEA s.r.l., Carpi, Italy, Grant/Research Support

Tu-P474

EARLY CHANGE IN A BONE FORMATION BIOCHEMICAL MARKER CORRELATES WITH HISTOMORPHOMETRIC BONE FORMATION ACTIVITY AFTER 2-YEAR TERIPARATIDE TREATMENT IN POSTMENOPAUSAL WOMEN WITH OSTEOPOROSIS

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Teriparatide treatment increases bone formation of subjects with osteoporosis. Recently, significant correlations were observed between early (1–3 months) but not late (6–12 months) changes in biochemical markers of bone formation and in bone microarchitecture parameters. We investigated the correlations between the changes of bone turnover markers and histomorphometric indices of bone formation in patients treated with teriparatide. Sixty-six postmenopausal women with osteoporosis (mean age of 68.0 years, mean lumbar spine BMD T-score -2.8, total hip -1.7 and 62% of patients with prevalent fractures) were treated with teriparatide (20 μg/day, subcutaneously) for 24 months. Paired iliac crest bone biopsies were analyzed by histomorphometry. Serum concentration of intact amino terminal propeptide of type I procollagen (PINP) and type 1 collagen cross-linked C-telopeptide (CTX) were measured at baseline, 1,3,6,12 and 24 months. As shown in Table 1, the percentage changes in main histomorphometric indices of bone formation, double-labeled perimeter (dL.Pm) and activation frequency (AcF) correlated with 0–1 month changes of PINP but not of CTX. Change in mineralizing surface (MS/BS) and bone formation rate (BFR/BV) did not correlate with early changes of PINP or CTX. 0–24 months changes of PINP and CTX correlated with histomorphometric bone formation, except dL.Pm with CTX. In cross-sectional analyses (data not shown), all four histomorphometric indices correlated with biochemical markers at baseline (r-values > 0.5, p-values < 0.001) and after 24 months (r-values > 0.35, p-values < 0.05). Slopes of the regression-lines estimated, from four repeated measures, models between PINP and dL.Pm, MS/BS, AcF and BFR/BV were less steep after 24 months than at baseline (p-values = 0.027, 0.043, 0.021 and 0.006 respectively). Our results indicate that early changes in PINP correlate with histological activity of bone formation after 24-month treatment with teriparatide.

Table 1

%Change, n = 35 0–24 month	0–1month PINP r-/p-values	0–1month CTX r-/p-values	0–24mns PINP r-/p-values	0–24mns CTX r-/p-values
dL.Pm	0.39/0.04	0.17/ns	0.39/0.04	0.37/ns
MS/BS	0.33/ns	0.21/ns	0.48/0.005	0.45/0.009
AcF	0.49/0.003	0.28/ns	0.73/ <0.001	0.69/ <0.001
BFR/BV	0.24/ns	0.15/ns	0.47/0.006	0.44/0.011

Conflict of Interest: D.B. Burr, Indiana University School of Medicine, Grant/Research Support, Shareholder
H. Petto, Eli Lilly and Company, Employee
A. Sipos, Eli Lilly and Company, Employee and Shareholder
I. Pavo, Eli Lilly and Company, Employee and Shareholder

Tu-P475

SAFETY OF PTH(1–84) AFTER 24 MONTHS THERAPY AND A 12 MONTHS FOLLOW-UP PERIOD

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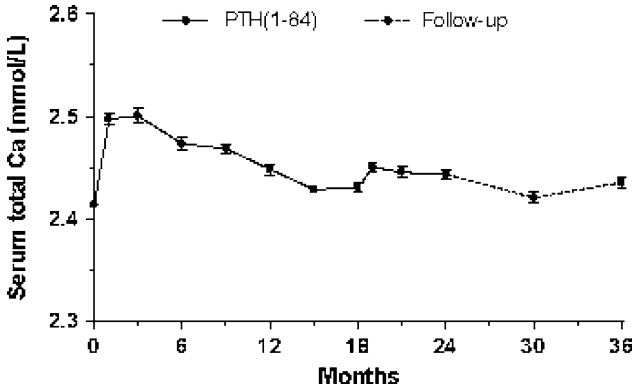
Introduction: PTH(1–84) treatment has proven to be safe in trials of 18 months duration. We report the safty results of PTH(1–84) for an additional 6 months and after 12 months of cessation of therapy.

Methods: Long-term safety of daily PTH(1–84)100 μg was studied in the Open-Label Extension Study(OLES), which included women treated with PTH(1–84) in The Treatment of Osteoporosis with PTH (TOP)study. TOP was an 18-month, randomized, double-blind, placebo-controlled trial that assessed the effect of PTH(1–84) on vertebral fracture incidence in osteoporotic women. PTH was given for a total of 24 months in OLES (18 months in TOP and 6 months in OLES). Patients were followed up for an additional 12 months. TOP included patients with low BMD without(n = 2056) or with(n = 471) prevalent vertebral fracture. 781 PTH-treated patients

from TOP continued into OLES. Demographics of the OLES population did not differ from TOP.

Results: In the TOP study, PTH(1–84) was well tolerated, with some patients experiencing transient episodes of headache, dizziness, and nausea occuring more often in the PTH(1–84)-group than in the placebo-group. In OLES headache(8.5%), dizziness(3.3%) and nausea(6.1%) were the most common adverse events. Prevalence of any hypercalcemia was 5.4% during the 6 months of OLES. The figure shows total serum calcium levels(±SE) during the 24 months of PTH(1–84) treatment and the following 12 months of follow-up.

Conclusion: The consistency of safety of PTH(1–84) throughout 24 months of therapy is supported by the low number of adverse events from 18 months to 24 months of treatment and the normal mean serum calcium level.



Conflict of Interest: L.Pérez-Edo, None declared

Tu-P476

LONGITUDINAL MICRO-CT EXAMINATION DETECTS BONE CHANGES IN OVARIECTOMIZED, ZOLENDRONIC ACID TREATED AND SHAM OPERATED RATS

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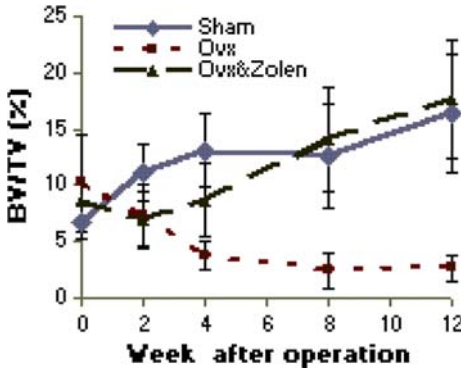
In osteoporosis studies, the ovariectomized (OVX) rat is an accepted model for investigating the effects of induced bone loss over time, together with the efficacy of drugs aimed to prevent this loss. Nowadays, compact in vivo micro-CT systems enable longitudinal studies.

Aim: To monitor variations of the cancellous bone structure in the proximal tibia of rats subjected to different treatments using a longitudinal study design.

Methods: Sprague-Dawley rats, age 9 weeks, were assigned to three groups: sham operated (N = 7), OVX (N = 7), and OVX+zolendronic acid treated group (N = 6). The zolendronic acid treatment began 2 weeks after operation. The rats were scanned in vivo by micro-CT at 0, 2, 4, 8 and 12 weeks after operation. 3D structural parameters, such as bone volume fraction (BV/TV), trabecular number (Tb.N) and trabecular separation (Tb.Sp) were calculated at each time point.

Results: For each structural parameter, the pattern of time related changes differed depending on the treatment (repeated measures ANOVA, $p < 0.01$). The sham group showed consistent increased bone formation (significant increase in BV/TV, Tb.N). The OVX+zolendronic acid group after 6 weeks of treatment, showed a striking increase in BV/TV ($p < 0.01$). After 8 weeks BV/TV remained similar to the sham group (Figure). Conversely, the OVX group showed a general dramatic bone loss in the first 4 weeks after operation (decrease of BV/TV, Tb.N, increase of Tb.Sp, $p < 0.01$).
Conclusion: Recently it was suggested that significant changes in bone microarchitecture are detectable within the first 3 months of ovariectomy, and that evaluation of drug-treatments should be initiated within this period. The outcomes of the present study using in vivo micro-CT support this hypothesis, showing that zolendronic acid treatment restores BV/TV to that of the sham group. This confirms in vivo microCT as a powerful tool for longitudinal examinations in osteoporosis treatment studies.



Conflict of Interest: None declared

Tu-P477

FUNCTIONAL AND RADIOGRAPHIC OUTCOMES OF BALLOON-KYPHOPLASTY IN A PROSPECTIVE TRIAL IN THE TREATMENT OF OSTEOPOROTIC THORACIC AND LUMBAR VERTEBRAL FRACTURES

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Purpose: Balloon-Kyphoplasty is a safe and effective method for reducing pain and improving quality of life in patients with painful osteoporotic vertebral fractures in prospective and randomized trials. The purpose of our study was to investigate the long term functional outcomes and radiographic results.

Material and Methods: 72 patients (49 females and 23 males) with 109 osteoporotic vertebral fractures were treated with Balloon Kyphoplasty. We were able to have a 3 year follow up in 64 patients (44 females and 20 males) with 96 vertebrae treated. Preoperatively conventional radiographs in lateral and a.p. view, CT and / or MRI were preformed. Pre- and postoperatively the clinical parameters VAS (Visual Analogue Scale) and the Oswestry score were evaluated. Radiographic scans were performed pre- and postoperatively and after 3, 6, 12, 24 and 36 months. The vertebral height and endplate angles were measured.

Results: The median pain scores (VAS) improved significantly from pre- to post-treatment as did the Oswestry Disability Score ($p < 0.001$). This improvement was maintained at 3 year follow up. A total of 21 patients out of 64 (32.8%) (14 female, 7 male) suffered

new vertebral fractures, which occurred in 32 out of 96 vertebrae (33.3 %) at 36 months follow-up. 10 patients with symptomatic new fractures were treated again with Balloon Kyphoplasty.

Postoperatively this surgical technique demonstrated a significant restoration and stabilization of the height of the vertebral body ($p < 0.05$) and reduction of kyphotic deformity ($p < 0.05$). During 3 year follow-up Balloon-Kyphoplasty was able to stabilize the vertebral height and avoid further kyphotic deformity.

Conclusion: Balloon-Kyphoplasty provided a safe and effective treatment for pain and disability in patients with vertebral compression fractures secondary to osteoporosis. In addition, restoration of the vertebral height and reduction of the kyphotic angle was possible specifically due to the balloon technique. Balloon Kyphoplasty was able to stabilize the fractured vertebrae in the long-term and was able to prevent an increase of kyphotic deformity.

Conflict of Interest: None declared

Tu-P478

SECONDARY OSTEOPOROSIS IN ADOLESCENCE—CASE REPORT

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Also most cases of osteoporosis are seen in adults, there are some with juvenile or adolescent onset. In adults, many of the osteoporosis forms are considered primary, while in children most cases are secondary to underlying chronic diseases or long-term medication. We present the case of a 19 years old male patient, with type I poly glandular autoimmune syndrome, having a long medical history. At age of 3, he was diagnosed with vitiligo and cutaneo-mucosal candidosis. At 7 years, primary adrenal insufficiency was diagnosed. Treatment with hydrocortisone 60 mg/day and fludrocortisone 0.05 mg/day was administered. Frequent acute episodes due to antifungal medication made necessary temporary increasings of the substitutive doses. At the age of 13, primary hypoparathyroidism was diagnosed based on low PTH levels of 8.75 pg/mL, low total and ionic serum calcium levels with elevated serum phosphate level of 6.6 mg/dL. Therapy with calcium and calcitriol (1 mg/day) was started. Until age of 17, he suffered two fragility fractures (right humerus and left radius). Radiograph examination of the spine revealed compression fractures on 3 lumbar vertebras. Dual energy X-ray absorptiometry (DXA) showed a significant reduction in bone density for age. Bone mineral density (BMD) at lumbar spine (L2–L4) was 0.544 g/cm^2, with a Z-score of -6.6. Low values of bone markers were found: beta CrossLaps of 0.131 ng/mL (range between 0.142–0.522) and osteocalcin level of 4.36 ng/mL (range between 41.7–111.3). Bisphosphonate therapy (risendronate 35 mg/week) was initiated for 2 years, with significant improvement on BMD at value of 0.679 g/cm^2. No height acquisition was seen. Long-term treatment with corticosteroids in childhood induced not only osteoporosis, but also growth failure, due to direct and indirect actions of glucocorticoids on cartilage and bone, as well as delayed puberty. Low BMD is a marker of osteoporosis and a predictor of fracture risk in growing patients, but its clinical usefulness remains a matter of debate. Moreover, the BMD is continuously changing, depending on pubertal spurt, weight, skeletal age, gender and genetic background. The complexity of comorbidities as polyglandular autoimmune syndrome and glucocorticoid-induced osteoporosis causes a poor prognosis and a long-term disability. Unlike adult osteoporosis, where there are well-defined treatment options, in children the literature data are limited.

Conflict of Interest: None declared.

Tu-P479

SECONDARY PREVENTION OF FRAGILITY FRACURES, A STUDY OF OUR PRACTICE AND KNOWLEDGE IN A TRAUMA CENTRE

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Introduction: The prevalence of hip fractures is expected to triple from 1990 to 2020 in the United States because of an ageing population and suboptimal treatment of fragility fractures. In our study we focused on assessing clinical practice and the knowledge of orthopaedic surgeons on current guidelines.

Method: The study was conducted retrospectively and prospectively where we quantified the number of orthopaedic patients discharged on bisphosphonates or referred for a DEXA scan over an eight-month period. Questionnaires were used to check the surgeons' basic knowledge of secondary prevention of fragility fracture and assessed their agreement with starting treatment for osteoporosis and their awareness of the NICE guidelines.

Results: Over eight months of activity in a busy level 1 trauma centre, no patients were started on Biphosphonates or referred for DEXA scans. 56% prescribed Biphosphonates < 1 year ago, 31% were aware of guidelines, 9% felt confident to treat osteoporosis, 42% thought it right but not have confidence and 49% referred.

Discussion: Women who have suffered a previous fragility fracture are at increased risk of further fractures, independent of BMD. Men and women aged 65 years or older with a vertebral fracture have a five year risk of femur or hip fracture of 6.7% and 13.3% respectively. NICE concluded that: Bisphosphonates are recommended as treatment options for the secondary prevention of osteoporotic fragility fractures.

Conclusion: We believe that with persistent education and constant reminders and multidisciplinary treatment we could increase the treatment of osteoporotic patients who have experience fragility fractures.

Conflict of Interest: None declared

Tu-P480

VALUE OF BONE BIOMARKERS IN DETECTING EARLY RESPONSE TO TERIPARATIDE TREATMENT IN OSTEOPOROSIS

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Teriparatide (rh 1–34 PTH) increases bone mass and improves bone micro architecture by preferential stimulation of osteoblastic over osteoclastic activity. Bone formation markers would be expected to show an earlier increase than resorption markers. Recent evidence suggests a rise in PINP by 10 ug/L from baseline at 1–3 months predicts a consequent increase in lumbar spine bone density (1)

The aim of the study was to determine the best bone marker protocol for early detection of response in patients on teriparatide. 18 postmenopausal women and 2 men (63–84 yrs), with severe osteoporosis received 20 ug of teriparatide daily by sc self-injection without any washout period; 15 had received prior alendronate therapy. Blood and urine for routine biochemistry and specific bone markers BALP, C1CP and P1NP (formation markers) and urine NTX (resorption marker) were performed at baseline, 1, 3, 6 and 12 months of treatment. DXA of lumbar vertebrae and hip was performed at 0

and 12 months. Forearm densitometry was performed at 0 and 6 months.

Results were evaluated using a least significant change of > 30% for a serum marker, > 50% for a urine marker, > 3% BMD.

P1NP showed the earliest rise: 18/20 subjects responded at one month (mean increase from baseline 242 %, $p < 0.01$). Levels peaked at 3 months ($p = 0.001$) and remain elevated at 12 months. C1CP also showed an early rise in 16/20 patients at 1 month (mean increase 162%, $p = 0.01$) but subsequently fell thereafter. A significant increase was seen later in BALP (17/20 patients at 3 months) and urine NTX (15/20 patients at 3 months).

Forearm densitometry showed no significant change after 6 months. At the time of submission of this abstract 13 subjects had BMD measurement at hip and spine following 12–14 months of treatment.

10/13 subjects showed a significant increase in spine BMD at 1 year (mean increase 7.5%, $p = 0.001$). In 9 of these 10 cases, a positive response was predicted by an early rise in P1NP. There was no change in hip BMD

Our study supports the use of P1NP for the early conformation of anabolic response to teriparatide treatment.

Reference: 1. Eastell R, Krege JH, Chen P et al Development of an algorithm for using P1NP to monitor treatment of patients with teriparatide. Current Medical Research and opinion Vol 22, no 1: 2006 61–66.

Conflict of Interest: None declared

Tu-P481

ZOLEDRONIC ACID REDUCES FRACTURES AND INCREASES BMD WITH AND WITHOUT CONCOMITANT OSTEOPOROSIS THERAPY

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In HORIZON-PFT, which assessed the effects of annual infusions of zoledronic acid (ZOL) 5 mg in the treatment of postmenopausal osteoporosis, randomization was stratified by whether patients were treated with study medication (ZOL or placebo and calcium + vitamin D) alone (Stratum I; N = 6084) or receiving concomitant non-bisphosphonate (non-BP) antiresorptive therapy (HRT, SERMs, calcitonin, tibolone) with their study medication (Stratum II; N = 1652). Given the uniqueness of the trial design, the hypothesis of whether or not the efficacy of ZOL was affected by concomitant use of other osteoporosis therapies was examined by evaluating the between-treatment differences in the incidences of morphometric and clinical fractures and changes in BMD within and across the two strata. In Stratum I, 3.3% of ZOL-treated patients experienced a morphometric vertebral fracture over 3 years compared with 10.9% of placebo-treated patients, which corresponds to a 70% relative risk reduction (RRR); in Stratum II, 3.9% of ZOL-treated patients experienced a morphometric vertebral fracture compared with 8.8% of placebo-treated patients, which corresponds to a RRR of 56% (both $p < 0.001$). Reductions in the risk of hip fractures were similar between Stratum I and II (41% [$p = 0.007$] and 42% [$p = 0.169$], respectively), as were those for nonvertebral fractures (26% [$p < 0.001$] and 22% [$p < 0.128$], respectively). The risk reduction for clinical vertebral fractures was 83% for Stratum I ($p < 0.001$) and 66% for Stratum II ($p = 0.0035$). No significant treatment-by-stratum interactions were observed for any of the fracture endpoints. Comparison of changes in total hip, femoral neck, and lumbar spine BMD over 36 months indicated that ZOL significantly increased BMD by 4.8% to 6.7% relative to placebo at all sites (all $p < 0.001$) and similarly across strata, although there were smaller increases favoring ZOL 5 mg in Stratum I patients at the lumbar spine (6.7% vs. 6.6%), femoral neck (5.1% vs. 4.8%), and total hip (6.2% vs. 5.4%). Treatment with ZOL had a favorable safety profile and was generally well tolerated, with adverse events rates similar across strata. In conclusion, zoledronic acid significantly increased BMD and yielded similar reductions in vertebral and nonvertebral fracture risk whether given alone or with concomitant non-BP antiresorptive therapy. Thus the use of zoledronic acid can yield significant anti-fracture benefits even in patients on existing osteoporosis therapies.

Conflict of Interest: DM Reid, Novartis, Consultant

Tu-P482

EFFECTIVENESS OF BISPHOSPHONATE TREATMENT ON NONVERTEBRAL FRACTURES: AN OBSERVATIONAL COHORT STUDY OF RISEDRONATE AND ALENDRONATE WITH THE ADDITION OF IBANDRONATE

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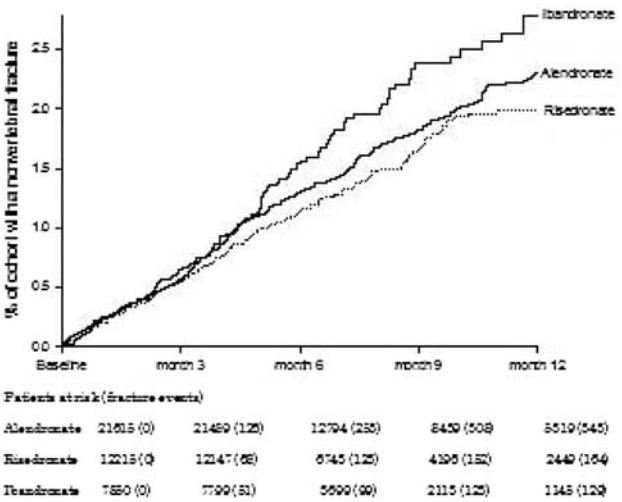
In a prior observational study of 33,830 women aged 65+ who initiated weekly bisphosphonate dosing, patients on risedronate had a lower incidence of nonvertebral fracture during the first year of therapy than patients on alendronate(REAL)1. Monthly dosing of bisphosphonate became available in 2005. In this study we compared nonvertebral fracture incidence during the first year of therapy among patients on monthly ibandronate to patients in the REAL study. The study population of REAL was identified from health services utilization records between 2002–2004 and included new users of risedronate or alendronate. Current study includes new users of ibandronate from the same data source. Cox proportional modeling was used to compare fracture incidence among the 3 patient groups,



adjusting for baseline fracture risk. All 3 patient groups, mean age 75, had similar prevalent fracture status. In REAL, the nonvertebral fracture incidence in year one of therapy was 1.99% for risedronate patients and 2.30% for alendronate patients. In comparison, nonvertebral fracture incidence was 2.79% for ibandronate patients. Relative to ibandronate, the adjusted relative rate of nonvertebral fracture for risedronate patients was 0.76 (95%CI 0.60–0.96) and for alendronate patients was 0.92 (95%CI 0.74–1.13). These results do not appear to be explained by baseline differences in fracture risk between cohorts. As with all cohort studies, interpretation of results are limited by the nonrandomized study design. In this study, patients on risedronate had a lower incidence of nonvertebral fractures during their first year of therapy than patients on ibandronate. 1 Silverman OI 2007 18:25
Conflict of Interest: DT Gold, JD Ringe, consultant P&G, JL Lange employee P&G.

Tu-P483

EFFECTIVENESS OF BISPHOSPHONATE TREATMENT ON HIP FRACTURES: AN OBSERVATIONAL COHORT STUDY OF RISEDRONATE AND ALENDRONATE WITH THE ADDITION OF IBANDRONATE

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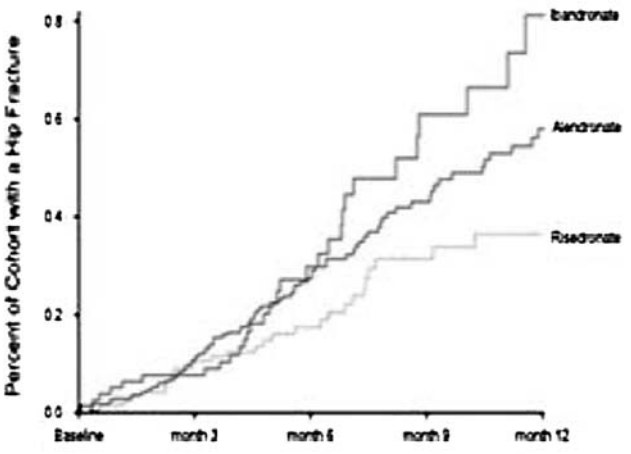
In a prior observational study of 33,830 women aged 65+ who initiated weekly bisphosphonate dosing; patients on risedronate had a lower incidence of hip fracture during the first year of therapy than di patients on alendronate (REAL). Monthly dosing of bisphosphonate became available in 2005. In this study, we compared hip fractures during the first year of therapy among patients on monthly ibandronate to patients in the REAL study. The study population of REAL was identified within records of health services utilization between 2002–2004 and included new initiators of risedronate or alendronate. The current study included new ibandronate users from the same data source. Cox proportional modeling was used to compare hip fracture incidence in the 3 patient groups, adjusting for baseline fracture risk. All 3 patient groups, mean age 75, had a similar prevalent fracture status. In REAL, hip fracture incidence in year one of therapy was 0.37% for risedronate patients and 0.58% for alendronate patients. In comparison, hip fracture incidence was 0.81% for ibandronate patients. Relative to ibandronate, the adjusted relative hip fracture rate for risedronate patients was 0.51(95% CI 0.30–0.87)& 0.88(95% CI 0.57–1.36)for alendronate patients. These differences in hip fracture rates don't appear to be due to baseline fracture risk differences among cohorts. As with all cohort studies, interpretation of results are limited by the non-randomized study design. In this study, risedronate patients had a lower incidence of hip fracture during their first year of therapy than ibandronate patients. Silverman,OI 2007 18:25
Conflict of Interest: Abelson Ringe Gold P&G Consultant



Patients at Risk (fracture events)

Alendronate	21615 (0)	21550 (25)	12993 (54)	8677 (69)	5582 (80)
Risedronate	12215 (0)	12202 (11)	6847 (19)	4319 (27)	2584 (29)
Ibandronate	7850 (0)	7844 (5)	3745 (19)	2151 (27)	1163 (30)

Tu-P484

BISPHOSPHONATE IS SELECTIVELY INTERNALISED BY PERIPHERAL BLOOD MONOCYTES; IMPLICATIONS FOR THE ACUTE PHASE RESPONSE

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The major side-effect of intravenously administered nitrogen-containing bisphosphonates (N-BPs) is a flu-like syndrome called the acute-phase response, which is caused by indirect activation of Vgamma9Vdelta2 T cells by N-BPs. N-BPs can indirectly activate Vgamma9Vdelta2 T cells through inhibition of FPP synthase and the intracellular accumulation of the upstream mevalonate pathway intermediates IPP and DMAPP, which are agonists of the Vgamma9Vdelta2 T cell receptor. However, no studies to date have demonstrated IPP/DMAPP accumulation in N-BP-treated peripheral blood mononuclear cells (PBMCs), nor identified the major cell type responsible for the IPP/DMAPP production. Human PBMCs were obtained by density gradient centrifugation, and treated with the pharmacologically-relevant concentration of 1 microM of the N-BP zoledronate (ZOL) for 2 hours, followed by a further incubation in drug-free medium for 22 hours. Isoprenoid lipids were extracted with acetonitrile, and IPP/DMAPP was detected by ion-pairing HPLC-ESI-MS. ZOL selectively induced the accumulation of IPP/DMAPP in CD14+ monocytic cells, whereas no IPP/DMAPP was detected in the CD14-ve fraction, or in CD3+ve T lymphocytes. To investigate whether the selective accumulation of IPP/DMAPP in monocytes is due to more efficient drug uptake by this highly endocytic cell type, an amino-derivative of zoledronate conjugated to alexafluor-680 (AF680-BP) was used to detect uptake of BP by human PBMCs using flow cytometry. Treatment of PBMCs with AF680-BP in vitro resulted in relatively high levels of uptake by CD14+ve monocytes, whereas other cell types, including lymphocytes, showed very little uptake of AF680-BP (30–60 fold increase in mean fluorescence of CD14+ve cells and 1.5–2 fold increase in mean fluorescence of CD14-ve cells following treatment with 20 μM AF680-BP for 24 hours). Dead or dying cells were excluded from the analysis based on annexin V labelling. In conclusion, we demonstrate that N-BPs induce Vgamma9Vdelta2 T cell activation through accumulation of mevalonate pathway intermediates, such as IPP/DMAPP, selectively in

CD14+ve monocytes. The accumulation of the metabolites selectively in this cell type is probably due to highly efficient drug uptake by endocytosis compared to other cell types.

Conflict of Interest: M.J. Rogers, Novartis, Grant Research Support
M.J. Rogers, Procter and Gamble, Grant Research Support
M.J. Rogers, Roche, Grant Research Support
J. Monkkonen, Novartis, Grant Research Support

Tu-P485

VITAMIN D REPLETION AND TREATMENT RESPONSE TO ANTI-RESORPTIVE AGENTS IN POST-MENOPAUSAL OSTEOPOROSIS

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The aim of the study was to determine the skeletal response to anti-resorptive agents in relationship with calcium and vitamin D intake in the routine practice. For obvious ethical reasons the study had to be observational, retrospective and uncontrolled.

The TOP (Treatment of Osteoporosis in clinical Practice) study population includes 1515 women recruited from 56 out-patient clinics for osteoporosis management distributed all over Italy. The two only inclusion criteria were: (1) Patients with osteoporosis defined by the presence of a T-score for spine or hip BMD or for heel quantitative ultrasound < -2.5 or by the presence of a previous fragility vertebral or hip fracture; (2) Patients who initiated a treatment with either raloxifene (60 mg/day), alendronate (70 mg/once week) or risedronate (35 mg/once week) 11 to 18 months earlier with a compliance $> 75\%$. Patients with secondary forms of osteoporosis, malignancies or renal failure or on treatment with corticosteroids or any other drug known to affect bone metabolism were excluded. The two groups of patients, vitamin D deficient or vitamin D repleted, significantly differed for both annualized percent changes in BMD and incidence of clinical fractures. In order to eliminate the confounding factors the BMD changes were adjusted for all parameters somewhat associated with a p value < 0.2. The % BMD changes remained statistically significantly different between the two groups at the spine and total hip (0,35 vs. 1,97, 0,58 vs. 1,60, respectively). The relative risk of incident fractures during the one year treatment adjusted for type of treatment, age, previous clinical fractures, duration of follow-up, calcium intake, in vitamin D deficient as compared to vitamin D repleted women was 1,54 (1,07 – 2,20 95% CI; p = 0,019).

In conclusion the present study has highlighted an important clinical issue in the management of post-menopausal osteoporosis: an optimal vitamin D repletion is a pre-requisite for maximizing the response to anti-resorptive agents in terms of both BMD changes and anti-fracture efficacy.

Conflict of Interest: None declared

Tu-P486

AN OBSERVATIONAL STUDY OF THE SIDE EFFECT PROFILE OF IV ZOLEDRONATE WITH SUCCESSIVE TREATMENTS

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Intravenous Zoledronate has been shown to have powerful benefits on fracture reduction in postmenopausal osteoporosis. The adverse effect profile of this mode of therapy is one factor that will determine its place in clinical practice. This study examined the side effect profile of successive doses of annual iv Zoledronate in an observational clinical setting. Zoledronate was given to a cohort of 95 patients mostly with postmenopausal osteoporosis but also a few with hyperparathyroidism, steroid induced bone loss or primary male osteoporosis. 4 mg was given iv over ½ hour with paracetamol 500 mg 6 hourly given over the next 2 days. Patients were reviewed at 3 months post treatment in the clinic unless required to attend for other reasons and asked whether or not they had experienced any adverse effects if so their nature. 95 patients have had one therapy, 35 patients 2 therapies and 9 patients 3 therapies. Following the first treatment 33 patients reported side effects, 26 of whom had aches and pains or a flu like illness lasting no more than 4 days. 2 patients had iritis within 3 days of dosing requiring an emergency attendance at Opthalmic A&E and intensive oral corticosteroids; they did not receive further therapy. One patient had prolonged aches and pains for 6 months and did not wish for further treatment. No patients apart from these 3 did not wish to be retreated. Following the second therapy only 5 patients reported side effects of whom 3 had also reported symptoms after the first treatment. In each case the clinical features were minor. Following the third therapy only 1 patient had side effects, again minor. There were no known cases of atrial fibrillation. Side effects are common following first Zoledronate treatment but rarely serious or prolonged With subsequent therapies side effects are rare and no serious consequences have been reported.

Conflict of Interest: None declared

Tu-P487

FOUR YEARS EXPERIENCE WITH THE CLINICAL USE OF BALLOON KYPHOPLASTY (BKP)

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The most frequently occurring vertebral fractures are osteoporotic and mainly occurring in older patients. Conventional management of vertebral compression fractures (VCF), consisting of pain medication and immobilization represents a serious socio-economic burden. BKP is a minimally invasive technique for the treatment of VCF, allowing stabili-zation of the vertebral body but also partly correcting the loss of vertebral height and the kyphotic angle. Inflation of the balloon tamps in the vertebral body will create a void. This allows the use of viscous cement and low pressure positioning of the cement, adding to the safety of the technique. It has been documented in the literature that the patient will experience immediate pain relief and the majority of patients can be mobilized the same day of the intervention. BKP can be performed under short general anesthesia, which makes it also suitable for older patients. In our clinic we selected BKP for the manage-ment of VCF and treated since May 2004 over 200 patients. The majority suffered osteoporotic fractures between T6 and L5, though also younger patients suffering trau-matic fractures A1 or A3.1 and patients with benign and malign infiltration were treated. When treating VCF in patients younger than 50 years we used the bioresorbable cal-ciumtriphosphate cement. In a selected case of a higher degree traumatic fracture (B1.2) we have combined the BKP with the minimally invasive instrumentation of a fixateur in-terne. We noted a significant pain reduction, measured with a visual analogue scale (VAS), in all patients. The height of the compressed vertebral body was in many cases considerably restored. In the patient group already evaluated at 4 year follow-up, a main-tained pain reduction was noted which resulted in a fast return to normal daily activities. X-ray control showed an insignificant loss of vertebral body

height during follow-up after BKP. In only one case subsequent VCF were detected on X-ray. During the presentation we will provide information on the pathophysiology of osteoporotic VCF, demonstrate the BKP procedure and provide suggestions for patient selection. We will provide statistically analyzed outcome data on our patient population regarding pain reduction, mobilization, vertebral reconstruction, intra operative complications and follow-up results. Selected cases will be used to illustrate the obtained results.

Conflict of Interest: No declared

Tu-P488

RELATIONSHIP BETWEEN LEPTIN AND ADIPONECTIN AND BONE METABOLISM IN POSTMENOPAUSAL OSTEOPOROSIS PRE AND POST ANTICATABOLIC TREATMENT

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Introduction: Adiponectin and leptin have been described as potential contributors to bone metabolism, however in vitro and in vivo studies show controversial results. Besides, the effect of anticatabolic drugs on these adipokines and their relationship with bone metabolism have not been clearly clarified. AIMS: Evaluate adiponectin and leptin levels in osteoporosic postmenopausal women and their relationship with bone mineral density (BMD), bone turnover biochemical markers and osteoclastogenesis markers. Analyze changes on adiponectin and leptin levels after treatment with raloxifene or alendronate. PATIENTS AND Methods: We selected 53 untreated women (63 $\pm$ 7 years) with postmenopausal osteoporosis (T-score < -2.5 DS) divided into two groups: women treated with raloxifene (60 mg/day; $n = 20$) or alendronate (70 mg/week; $n = 33$) during one year. All of them received calcium and vitamin D supplements. We determined at baseline and after 12 months of treatment: anthropometric data, OPG, E2, IGF-I), adiponectin, leptin, 25-hydroxyvitamin D, iPTH, osteocalcin, BALP, ALP, TRAP and BMD in lumbar spine (LS), femoral neck (FN) and total hip (TH). Results: At baseline, leptin and adiponectin serum levels were 1371,4 $\pm$ 822,4 pM/ml and 42,24 $\pm$ 26,1 μg/ml, respectively. Adiponectin was significantly correlated with BAP (r:$-$0.413;p:0.003), OPG (r:0.51;$p < 0.001$), years since menopause (r:0.295;p:0.039), but was not with BMD in any site. Leptin was significantly related to weight (r:0.41; $p < 0.01$), BMI (r:0.47; $p < 0.01$) and waist (r:0.38, p:0.01), osteocalcin (r:0.285;p:0.038) and iPTH (r:0.33;p:0.016). Leptin was correlated with LS Tscore (r:$-$0.301;p:0.04) and BMD LS (r:$-$0.266;p:0.05) after adjustment for age and weight. After 12 months, no changes were observed in leptin (p:0,46) and adiponectin (p:0,55) in alendronate group; however, a significant increase in leptin levels (973,47 $\pm$ 637,37 pM/ml vs 1305,7 $\pm$ 793,4 pM/ml; p:0.031) was detected in the raloxifene group, whereas adiponectin levels showed no significant changes (p:0,46). Moreover, the percentage changes of adiponectin levels did not differ between the two groups (p:0,79); while the percentage changes in leptin levels were near significance, between the two groups (p:0,07). Conclusions: Adiponectin and leptin levels contribute at least in part to BMD in patients with postmenopausal osteoporosis. Changes in leptin levels after raloxifene treatment could be indirectly implicated in raloxifene bone effects.

Conflict of Interest: Non declared

Tu-P489

RELATIONSHIP BETWEEN ANNUAL CUMULATIVE EXPOSURE TO IBANDRONATE, BONE MINERAL DENSITY AND CLINICAL FRACTURE REDUCTION

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Background: Greater bone mineral density (BMD) increases were seen with the approved doses of ibandronate (IBN; 150 mg monthly oral and 3 mg quarterly intravenous [IV]) compared with 2.5 mg daily IBN in women with postmenopausal osteoporosis at 2 years (MOBILE and DIVA studies].[1,2] Analyses of the relationship between changes in BMD and fracture reduction with other bisphosphonates have produced varying results, but only a limited range of doses has been examined. Data from phase III clinical trials of IBN were analysed to examine increases in BMD with different dose groups and the relationship with fracture reduction.

Methods: Data from the intent-to-treat (ITT) populations from 4 pivotal studies, (2 oral, 2 i.v.) were pooled. BONE and the low-dose i.v. fracture study were 3-year, placebo-controlled fracture trials; MOBILE and DIVA were 2-year BMD studies that captured fractures as secondary endpoints. Oral doses were: 2.5 mg daily, 20 mg intermittent, 100 mg monthly, 150 mg monthly. I.v. doses were: 0.5 mg quarterly, 1 mg quarterly, 2 mg every 2 months, and 3 mg quarterly.

Doses were grouped by annual cumulative exposure (ACE; dose [mg] x number of annual doses x absorption factor [0.6% oral, 100% i.v]). BMD increases at the lumbar spine (LS) and total hip (TH) and rates of all clinical fractures over 2 years were assessed and plots constructed to show potential associations between fractures and dose. Linear regression models, weighted by sample sizes of trials, were constructed to examine clinical fracture rate as a function of increase in LS BMD.

Results: The analysis included 8,710 patients. Plots constructed to visualise potential associations between fractures and dose group showed a trend towards decreased clinical fractures with increasing ACE. Plots of change in LS and TH BMD vs ACE showed an increase in BMD with increasing ACE. A statistically significant inverse linear relationship was seen between clinical fracture rates and gains in LS BMD at 2 years ($\beta = -0.397$, $p = 0.0046$; $R^2 = 0.65$).

Conclusion: Increasing gains in LS BMD correlated with a decreasing rate of clinical fractures. Higher IBN doses were associated with the highest BMD gains and the lowest rate of clinical fracture compared with lower doses.

1. Reginster JY, et al. Ann Rheum Dis 2006;65:654–61
2. Eisman J, et al. J Rheumatol 2007;In press

Conflict of Interest: Sebba, Research Support, speaking honoraria, Roche, Merck, Novartis; Consultant, Roche, Merck, Amgen and Novartis

Barr, Roche Employee

Harris, Sponsored presentations: Lilly, GSK, Merck, Novartis, Procter & Gamble, Roche, sanofi-aventis, Wyeth; Consultant: Amgen, Lilly, GSK, Merck, Novartis, Procter & Gamble, Roche, sanofi-aventis, Wyeth

Tu-P490

BONE TURNOVER AFTER ALENDRONATE DOSE REDUCTION FOLLOWING PROLONGED STANDARD FULL DOSE TREATMENT IN POSTMENOPAUSAL OSTEOPOROSIS PATIENTS

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Effect of bisphosphonate treatment on fracture risk reduction is mainly due to decrease in bone turnover. There are growing, though yet unsubstantiated, concerns about possible risks of life long bisphosphonates administration. On the other hand discontinuation of alendronate for 12 months led to increase of bone turnover. The effect of treatment with decreased bisphosphonates dose on BT is unknown. The study aim of was to evaluate bone turnover in patients treated with monthly or biweekly dose of 70 mg of alendronate and compare it to the standard 70 mg/wk dose. Methods: Postmenopausal patients with stable BMD and absence of fractures during standard weekly treatment (ST) for 4–5 years were switched to biweekly or monthly dose, following personal preference of each patient. Bone turnover was assessed by serum total procollagen type1amino-terminal peptide (P1NP) and serum collagen beta cross-laps (CTX) in patients on ST regimen and at 12 and 24 months of biweekly (B) or monthly (M) therapy. Results: 146 postmenopausal women were enrolled in the study: 71 on ST, 15 on B for one year, 7-on B for 2 years (BTY), 7 on M for one year, 26 on monthly for two year (MTY). Fifteen patients were tested while untreated (NT), 8-were on calcium and vitamin D supplementation (CD). Mean age was 66.8 ± 9.14.P1NP levels were 23 ± 13.5; 24.2 ± 8.8; 20.9 ± 6.0; 20.6 ± 8.2; 23 ± 8.2; 23.9 ± 9.4 in ST, B, BTY, M and MTY, respectively. CTX - 0.2 ± 0.10; 0.23 ± 0.14; 0.19 ± 0.11; 0.17 ± 0.12; 0.19 ± 0.11 in ST, B, BTY, M and MTY, respectively. P1NP in CD and NT was 35.3 ± 10.3; 43.5 ± 19.7; CTX- 0.36 ± 0.18; 0.41 ± 0.21, respectively. Normal ranges: P1NP 15.13–58.59 ng/ml; CTX 0.299 ± 014 ng/ml. There was no significant difference in BT between NT and CD patients. P1NP and CTX didn't differ between treatment groups, but were significantly lower in all treatment groups, compared to CD, $p < 0.001$ for ST, 0.026 for B,BTY, M, MTY, and compared to NT $p = 0.04$ for ST, $p = 0.02$ for B,BTY,M,MTY. Conclusion: Biweekly and monthly regimens of alendronate treatment after a prolonged standard weekly treatment keeps bone turnover stable in the premenopausal range. It could be a cost effective and possibly safe option for prolonged treatment of postmenopausal osteoporosis patients.

Conflict of Interest: E. Segal, None declared Z. Shen-Orr, None declared B. Raz, None declared S. Ish-Shalom, Merck, Grant, Research Support S. Ish-Shalom, Lilly, Grant,Research Support S. Ish-Shalom, Novartis, Grant, Research Support

Tu-P491

LONG-TERM DENOSUMAB ADMINISTRATION HAD NO OBSERVED EFFECTS ON WBC COUNTS, IMMUNE PARAMETERS, OR T-CELL-DEPENDENT IMMUNE RESPONSE IN NON-HUMAN PRIMATES

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RANKL inhibition for up to 12 months with denosumab or OPG reduced bone resorption and increased bone mineral density (BMD) and strength parameters in non-human primates. Shorter-term RANKL inhibition also preserved bone in rodent models of inflammatory bone loss without significantly altering inflammation. However, the effects of long-term RANKL inhibition on bone versus immune parameters have not been previously evaluated in non-human primates. Adult ovariectomized (OVX) cynomolgus monkeys received vehicle (OVX-Veh) or denosumab (25 or 50 mg/kg) SC Q4wk for 64 weeks (n = 14–20/group) starting 1 month after surgery. These doses were > 8-fold higher than maximal doses used in clinical trials. Sham controls received vehicle (n = 17). Blood was collected at baseline (BL) and weeks 24, 36, and 48 for white blood cell (WBC) differential counts. Immune phenotyping of lymphocyte subsets in blood was performed at BL and weeks 32 and 64. Immune responses to the T-cell dependent antigen KLH were assessed 2 weeks before the final (week 60) dose of denosumab. Blood was drawn before KLH challenge and on days 5, 6, 7, 10, 14 and 21 post-KLH to quantify primary anti-KLH IgM and/or IgG responses. Denosumab significantly reduced the bone resorption marker serum CTx and significantly increased areal BMD of the lumbar spine and the hip ($p < 0.05$ vs OVX-Veh and Sham). WBC differential analysis revealed that neutrophils, lymphocytes, monocytes, basophils, and eosinophils were present in normal proportions across all groups and time points. There were no statistically or biologically significant denosumab-related differences in numbers of T (total, T-helper or T-cytotoxic), B, or NK cells at any time point. After KLH challenge, anti-KLH-specific IgM was elevated on days 5–10, and anti-KLH-specific IgG was elevated on days 10–21, with no significant differences between denosumab- and vehicle-treated animals. Thus denosumab suppressed bone resorption and increased BMD in adult OVX cynomolgus monkeys without apparent effects on basal immune parameters or the generation of a T-cell dependent humoral immune response.

Conflict of Interest: M Stolina, Amgen, full time employee and share holder
MS Ominsky, Amgen, full time employee and share holder
J Schroeder, Amgen, full time employee and share holder
JE Atkinson, Amgen, full time employee and share holder
SY Smith, Amgen, paid contractor
L LeSauteur, Amgen, paid contractor
S Corneu, Amgen, paid contractor
PJ Kostenuik, Amgen, full time employee and share holder

Tu-P492

THE CHANGE OF BMD, BONE TURNOVER MARKER,FIBRINOGEN LEVEL BY ADMINISTRATION OF FERAMIN-Q® FOR 1 YEAR IN KOREAN POSTMENOPAUSAL WOMEN

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Background: Feramin-Q® is a compositeness of 'Black Cohosh' and 'St. John's Wort'. There have not been studies about the influence of Feramin-Q® on bone mineral density, bone turn over markers, and plasma fibrinogen in Korea. For this study, the influence of Feramin-Q® on each factors were examined.

Method: 100 participants who visited the clinique in a hospital were involved. The criteria were as follows: a woman who menopaused for at least one year and had not been treated with HRT for more than 3 months, All participants took 2–4 tablets of Feramin-Q® which contains black cohosh 0.0364 ml and hypericium 84 mg per one tablet, for one year. Pre and post numerical values on each factors were compared.

Result: Statistically, a significant increase was shown in the urine deoxypyridinoline($P = 0.000$), the serum FSH($P = 0.000$) and the total cholesterol($P = 0.025$) after taking Feramin-Q® for one year. Significantly, there were statiscal decreases in the serum estradiol($P = 0.000$) and plasma fibrinogen($P = 0.021$) after taking Feramin-Q® for one year. And there were no statiscal changes in BMD and other factors.

Conclusion: In this study, after taking Feramin-Q® for one year, there were increments of urine deoxypyridinoline, and no change in BMD. What we would like to carefully suggest is that Feramin-Q®

dose not affectively control the suppression of the born absorption in menopaused women. Moreover, there is a significant decrease of the plasma fibrinogen. Many large-scale prospective studies would be needed.
Conflict of Interest: None declared

Tu-P493

EVALUATION OF THE REDUCTION OF THE PAIN AND NEW FRACTURES IN PATIENT WITH SEVERE OSTEOPOROSIS TREATED WITH ANTIRIASSORBITIVI AND TERIPARATIDE

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Abstract: The purpose of this job is to appraise the reduction of the pain and new fractures, in patient with severe osteoporosis treated with different medicines.

Materials and Method: 250 women have been selected with an inclusive age between 50 and 80 years. Those considered fit for the study should : have been in menopause since at least 4 years have at least a vertebral fracture from brittleness

All the enlisted women have been sent to our observation by the doctors of family, with the following examinations of laboratory: ves, calcemia, got, gpt, calciuria, bony alkaline fosfatasi, idrossiprolinuria,emocromo,fosforemia, creatinemia, divided protidemia, pth. Rx in ap and ll of the line lumbar back of the vertebral column. Dexa

All the patients were submitted to an evaluation of the pain with a staircase of Visual-Analogic Autoevaluation.(VAS) After an accurate anamnesis, the vision of the prescribed examinations and considered a T-score $> -2{,}5$ the women with a vertebral collapse (group 1) they were treated with Bifosfonati, those with two or more collapses, treated previously with antiriassorbitivi, (group 2) they were treated with Teriparatide 1–34. To all them a supplement of Ca + Vit.D. was given.

The evaluation of the pain was repeated 6 - 12 - 18 months. Results Improvement of the quality of the life of patients, with a suitable clinic answer (group 2), according to the parameters of the questionnaire QUALEFFO - 41 (12) Inhibition of the Interleuchines 1 and 6 (IL 1–6) from the Teriparatide 1–34, what probable mechanism of the reduction of the pain, matter that we will treat in way deepened in a following clinical evaluation.
Conflict of Interest: None declared

Tu-P494

POSITIVE EFFECTS OF ADMINISTRATION WITH ALPHA-KETOGLUTARATE (AKG) COMBINED WITH CALCIUM SALT OF BETA-HYDROXY-BETA-METHYLBUTYRATE (CAHMB) ON SKELETAL SYSTEM PROPERTIES IN PIGS WITH DEVELOPING OSTEOPENIA

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Development of osteopenia is one of the most significant consequences of fundectomy (surgical removal of fundic part of stomach) influencing skeletal system properties in humans and animals. The aim of this study was to investigate the effects of long-term administration with alpha-ketoglutarate (AKG) combined with calcium salt of beta-hydroxy-beta-methylbutyrate (CaHMB) to fundectomized pigs on bone mineral density, geometrical and mechanical properties of tibia. Forty days old animals were divided into five groups ($n = 6$ per group). Four groups of animals were fundectomized and orally administered with placebo (FX group), AKG (AKG group), CaHMB (HMB group) or AKG and CaHMB (AH group), respectively, while the fifth group underwent sham operation (SHO group). Placebo ($CaCO_3$) and CaHMB were administered at the dosage of 0.05 g/kg of BW/day while the dosage of AKG was set at 0.4 g/kg of BW/day. Animals were sacrificed at the age of 8 months to obtain tibia for analyses. Fundectomy in pigs significantly decreased values of weight and length of tibia, volumetric bone mineral density of the trabecular and cortical bone, cross-sectional area, second moment of inertia, mean relative wall thickness, cortical index, maximum elastic strength and ultimate strength; when compared to the values obtained in the sham operated animals ($P < 0.01$). Treatment with AKG and CaHMB significantly increased all the investigated parameters in the fundectomized pigs, when compared to the placebo-treated controls ($P < 0.05$). No significant differences of the investigated parameters of tibia were found comparing the values obtained in the AKG, HMB and AH groups. In conclusion, oral administration with AKG and CaHMB was effective in diminishing osteopenic effects of fundectomy in pigs; however, additive effects of these substances on bone tissue were not observed.

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Conflict of Interest: None declared

Tu-P495

TERIPARATIDE IN CUSHING'S SYNDROME

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Background: Glucocorticoid induced osteoporosis (GIO) is the most frequent type of secondary osteoporosis but the treatments have been poorly investigated in patients with endogenous cortisol excess (a rare pathology). Although patients with Cushing's syndrome (CS) are at high risk for fractures, no recommendation has been given for the management of bone complications. Bisposphonate therapy is the current standard care in GIO but teriparatide has been recently shown to be more effective in patients on chronic glucocorticoid therapy.

Methods: In an 18-month pilot study, were evaluated effects of teriparatide on bone turnover markers and BMD in women with severe osteoporosis due to active CS. Ten patients received 20 mcg of Teriparatide once daily plus 1000 mg of calcium and 800 UI of Vitamin D3 (group 1). Other 10 women were treated with calcium and vitamin D supplements only (group 2). BMD was determined by DEXA technique at the lumbar spine (L1–L4) and femoral neck. Vertebral fractures were investigated at standard spine radiographs (Th4–L4) by a semiquantitative scoring method by Genant et al.

Results: At study entry, the groups were similar in terms of CS features, BMD values and fracture prevelence (fracture index 42 vs. 43). In group 1, spine and femoral BMD rose by $8.1 \pm 3\%$ ($p < 0.01$) and

4.3 ± 2.0% ($p < 0.05$), respectively. Bone alkaline phosphatase, a marker of bone formation, significantly increased already after 3 mos of treatment (48 ± 32%). The mean increase after 18 months was 68 ± 42% for bone ALP and 21 ± 18% for CTX($p < 0.05$, both).

In group 2, spine and femoral BMD increased by 1.6 ± 1.3% and 1.2 ± 0.9%, respectively. The mild improvements observed in BMD and turnover markers in the untreated group were not significant. Fracture index did not change in group 1 but worsen in group 2 (42 vs. 46). After 18 months, differences between the two groups were significant for all evaluated parameters.

Conclusion: In women with CS, who are at high risk for fractures, teriparatide increased significantly formation markers, lumbar and femoral BMD and stabilized fracture index.

Conflict of Interest: None declared

Tu-P496

BONE RESORPTION IS STILL DECREASED 3 YEARS AFTER THE LAST OF 3 INFUSIONS OF ZOLEDRONIC ACID IN POSTMENOPAUSAL OSTEOPOROTIC WOMEN

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Once-yearly infusion of zoledronic acid 5 mg during a 3-year period has been recently shown to significantly decrease the risk of vertebral, hip and non-vertebral fractures in women with postmenopausal osteoporosis (HORIZON Pivotal Fracture Trial). To evaluate the long term remaining effect of this treatment, we measured biochemical markers of bone resorption (serum C-telopeptide, Crosslaps, normal premenopausal value < 0.57 µg/L, or urinary d-pyridinoline, normal premenopausal value: 8–18 nmol/mmolcreatinine) in 3 former placebo (PBO) arm (79 ± 7.6 yrs) and in 4 zoledronic acid arm (77.2 ± 2.5 yrs) between 2.5 and 3 years after the last infusion. During this follow-up, all patients received calcium and vitamin D at the same dose as during the trial. Two in the PBO group and 1 in the treated-group were on raloxifen since, at least, the beginning of the trial. No treatment which could influence bone metabolism, such as other bisphosphonates, teriparatide, HRT, calcitonin, or strontium ranelate was introduced during the follow-up. Bone resorption values were at the upper limit of the normal premenopausal range, in all the former PBO patients. In contrast, in the previously zoledronic acid-treated patients, urinary deoxypyridinoline was 7.1 ± 1.5 nmol/mmol, thus at the lower limit of the normal range.

These results suggest that three yearly infusion of zoledronic acid are associated with a long-term inhibition of bone resorption lasting at least 3 years after the last administration.

Conflict of Interest: None declared

Tu-P497

TURNING INJECTION PEN STEREOTYPES UPSIDE DOWN BY INCLUSIVE DESIGN

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This study focused on the design of a novel injection pen suitable for once-daily administration of PTH (1−84) in osteoporotic patients. Commissioning a new injection pen for patients with osteoporosis involves considering the needs of the majority of osteoporosis patients, mainly postmenopausal women. Many patients with rheumatological disorders are diagnosed with osteoporosis and this can lead to problems associated with weak hands, pain and poor dexterity. The objective was to develop a pen that is comfortable and easy for osteoporotic patients to use including patients with impaired dexterity. The goal was a user-friendly and intuitive injection system that patients could use comfortably, with a friendly look avoiding negative perceptions associated with self injection devices. An inclusive design approach included critical end-user analysis. A handling study evaluated five design concepts that explored various ways of using the patients' strength in their hands and fingers, focusing especially on trigger function, its placement and size. The study comprised twelve osteoporosis patients, six with normal hand/arm function and six with reduced function due to rheumatoid arthritis. Simulated injections to the abdomen and thigh evaluated trigger force and ease of use. The results showed that a long side activator with room for many fingers was highly appreciated; 75% of the subjects thought it was 'easy' or 'very easy' to use. Surprisingly 42% of the subjects could not use the 'traditional' top trigger injection pen. Embracing inclusive design allowed more users to administer medication and resulted in a final pen design where a side activator serves as a grip and is comfortable for the user's hand. It is designed to be activated with either one or many fingers. The design incorporates an integrated, flower-shaped, needle guard that shields the needle from sight, stabilises the injection process and presents a friendly, reassuring pen appearance. Traditional top triggers excluded a large proportion of potential users. By adding the flexible grip and reducing the strength needed to operate the pen it is possible for more patients to manage their daily injections. For PTH (1–84) requiring once daily injection, the pen's favourable and inclusive design features assist in patient management, making treatment compliance easy, and enhancing patient freedom and flexibility.

Conflict of Interest: None declared

Tu-P498

THE EFFECT OF LACTULOSE ON BMD IN OSTEOPENIC POSTMENOPAUSAL WOMEN, A PILOT STUDY

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Background: Previous clinical studies have shown that prebiotic non digestible oligosaccharides (NDO) increase intestinal calcium absorption in postmenopausal (PM) women and young healthy volunteers and enhance bone mineralization during pubertal growth. The purpose of the present study is to assess whether lactulose (a NDO largely used as laxative) contributes to maintain the bone mineral density (BMD) in osteopenic PM women.

Methods: Osteopenic (baseline T-score from −1 to −2.5 SD) PM women were randomized to: Lactulose group (LG): lactulose 10 g/d + 500 mg Ca/d + vitamin D 400 IU/d, or Placebo group (PG): placebo 10 g/d + 1,000 mg Ca/d + vitamin D 400 IU/d, according to a double-blind, double dummy, parallel groups, multicenter clinical trial, to evaluate the efficacy of one year treatment with lactulose 10 g daily in the preservation of BMD and the effect on levels of: calcium, phosphorus, bone alkaline phosphatase, calciuria, PTH and 25-hydroxyvitamin D (25(OH)D), CTx and NTx. Lumbar spine (L2–L4), femoral neck and total hip BMD were determined after 6 and 12 months.

Results: 41 women were included, 19 were randomized to LG and 22 to PG. The average age of women was 57.6 y and 59.4 y in LG and in PG respectively. The baseline calcium intake (g/d) was 1.1 in LG and 0.9 in the PG. Lumbar, femoral neck and total hip BMD evolution showed no statistically significant differences between groups, neither

in PP nor in the ITT populations, (See Table 1). No significant differences between groups were observed either in the laboratory parameters studied.

Conclusions: Lactulose may contribute to the bone mass preservation. The similar treatment effects of lactulose plus vitamin D and half dose of calcium compared to the regular replacement treatment for osteopenia suggest the improvement of intestinal calcium absorption when taking lactulose. The safety profile showed that the combination of lactulose plus calcium and vitamin D is safe and well tolerated as compared to the combination of placebo plus calcium and vitamin D.

Table 1 Lumbar spine BMD (g/cm2) evolution, PP population

	Base BMD	SD	1 year BMD	SD	LG-PG (*)	Lower 95% CI	Upper 95% CI
LG	0.904	0.058	0.893	0.08	−0.012	−0.031	+0.006
PG	0.920	0.082	0.922	0.092			

(*) $p = 0.2244$ (ANCOVA model, F test)

Conflict of Interest: Study funded by Grupo Solvay Pharma.

Tu-P499

TERIPARATIDE VERSUS ALENDRONATE IN GLUCOCORTICOID-INDUCED OSTEOPOROSIS: RESULTS OF A SUBGROUP ANALYSIS IN MEN, PRE- AND POSTMENOPAUSAL WOMEN

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The purpose of these post-hoc analyses was to describe areal bone mineral density (BMD) changes, fracture incidence, and safety in men, and pre- and postmenopausal women with glucocorticoid-induced osteoporosis (GIOP) (Saag 2007, N Engl J Med). Patients taking at least 5 mg/d prednisone equivalent for 3 or more months were randomized to teriparatide 20 mcg/d (TPTD, $N = 214$) or alendronate 10 mg/d (ALN, $N = 214$) for 18 months (83 [19%] men; 67 [16%] premenopausal women). BMD was analyzed using mixed model repeated measures. Radiographic vertebral fractures were centrally assessed and analyzed with Fisher's exact test. At baseline, for ALN vs. TPTD, respectively, the median glucocorticoid dose was 7.8 vs. 7.5 mg/d, spine T-scores were (mean ± SE) -2.5 ± 0.1 vs. -2.4 ± 0.1, and 25% vs. 30% of patients had a prevalent vertebral fracture. There was no statistically significant interaction effects between treatment groups and subgroups in BMD and fracture analyses ($p > 0.658$). The mean (±SE) percent changes in LS BMD were greater with TPTD than with ALN in postmenopausal women (7.8 ± 0.7 vs. 3.7 ± 0.7, $p < 0.001$), premenopausal women (7.0 ± 1.4 vs. 0.7 ± 1.7, $p < 0.001$), and men (7.3 ± 1.5 vs. 3.7 ± 1.6, $p = 0.027$). A significant difference in hip BMD was found only in premenopausal women (TPTD 4.8 ± 1.3 vs. ALN 1.8 ± 1.5, $p = 0.006$). More new vertebral fractures occurred in the ALN than in the TPTD group in men (4 [2.4%] vs. 0, $p = 0.113$) and postmenopausal women (6 [3.6%] vs. 1 [0.6%], $p = 0.120$); 0 in premenopausal women. Overall, new vertebral fractures occurred in more patients in the ALN (10; 6.1%) vs. TPTD group (1; 0.6%) ($p = 0.005$), with no significant difference in the number of patients with new nonvertebral fractures (ALN 8 [3.7%] vs. TPTD 12 [5.6%], $p = 0.493$). Adverse events in the TPTD and ALN groups were consistent among the subgroups. The results of this active comparator trial indicate that teriparatide offers clinical benefit to men and pre- and postmenopausal women with GIOP.

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Tu-P500

EFFICACY OF CONTINUED ALENDRONATE FOR FRACTURES IN WOMEN WITHOUT PREVALENT VERTEBRAL FRACTURE: THE FLEX TRIAL

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The Fracture Intervention Trial (FIT) found that 4 years of alendronate (ALN) in women without prevalent vertebral fracture reduced the risk of non-vertebral fractures (NVF) in those with femoral neck (FN) T-score < -2.5 but not in those with higher BMD. In the main results of the FIT Long Term Extension trial (FLEX), 10 years of ALN did not significantly reduce the risk of NVFs, compared with 5 years of ALN. Continuing ALN reduced the risk of clinical vertebral fractures but not the risk of X-ray defined vertebral fractures. We tested whether the long term effect of ALN on fracture among women without a prevalent vertebral fracture differs by FLEX baseline FN T-score. In FLEX, 1099 women randomized to ALN in FIT (mean previous duration 5 years) were re-randomized to placebo (40%) or ALN 5 (30%) or 10 (30%) mg/d for an additional 5 years. The ALN groups were combined in these analyses. At FLEX baseline, 723 women (66%) did not have a prevalent vertebral fracture. We analyzed fracture results in this group, excluding 3 women without baseline hip BMD. Interaction models used continuous FN T-score. Among women without vertebral fracture at FLEX baseline, we found significant interactions between FLEX baseline FN T-score and treatment for NVF. Continuation of ALN reduced NVF in women with FLEX baseline FN T-score < -2.5 but not in women with T-score > -2. Results for clinical vertebral or morphometric vertebral fracture did not differ significantly by FN T-score at baseline. This post hoc analysis suggests that continuing alendronate for 10 years instead of stopping after 5 years reduces the risk of non-vertebral fracture in women without prevalent vertebral fracture whose FN T-score, achieved after 5 years of ALN, is < -2.5, but does not reduce risk of NVF in women whose T-score is > -2. These results are similar to those previously reported for 4 years of ALN use.

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Tu-P501

SERUM 25(OH)D LEVELS AND FALLS, FRAILTY, AND FRACTURES AMONG POSTMENOPAUSAL WOMEN IN THE HAWAII OSTEOPOROSIS STUDY

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We investigated the relationship of serum 25-hydroxyvitamin D (25OHD) levels to measures of physical performance and muscle strength and to subsequent falls and fractures in the Hawaii Osteoporosis Study (HOS). Using liquid chromatography tandem mass spectrometry, 25OHD levels (D3 and total) were measured in serum stored at −70 degrees from 495 postmenopausal women during the 8 th examination of the Hawaii Osteoporosis Study 1992–94 cohort. In the primary analyses, the relationship of total 25OHD to performance-based measurements (walking speed, timed get-up-and-go, chair stand, hand & foot reaction time, functional reach) and muscle strength (grip, triceps, and quadriceps) was explored using multivariate regression models adjusted for age, height, and weight. Logistic regression analyses adjusted for age, height, and weight were also performed to evaluate the relationship of total 25OHD to falls and to the incidence of vertebral and nonvertebral fractures during a mean 2.7 year follow-up. Secondary analyses included: 1) using Vit D3 as the predictor variable, 2) using Vit D (D3 and total) with adjustment only for age, 3) including quadriceps strength as an additional covariate in models of falling. The mean serum 25OHD level was 79.9 (SD = 23.7 nmol/L). After adjustment for age, height, and weight, only quadriceps strength had a significant association ($p = 0.0002$) with 25OHD. No significant association of 25OHD was found with either vertebral or non-vertebral fractures, or with the incidence of 1 or more falls. There was a borderline significant ($p = 0.05$) association of total 25OHD with 2 or more falls after adjustment for age alone, but there was no association in the model adjusted for age, height, weight, and quadriceps strength, suggesting that an effect on falls may be partly mediated by quadriceps strength. Study limitations: Analyses were of cross-sectional design and vitamin D levels were measured at a single point in time. Although prospective data were available for falls and fractures, the numbers of fracture events were relatively small. Also, interpretation of tests for statistical significance are less straightforward due to the large number of endpoints studied. The lack of association with fractures and falls, or with most physical performance measures, might be related to the fact that very low levels of 25OHD are less common in this population than in other studies.

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Tu-P502

SERUM VITAMIN D INCREASES DURING TREATMENT WITH A ONCE-WEEKLY TABLET CONTAINING ALENDRONATE AND VITAMIN D

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Results from a trial comparing a once-weekly tablet containing alendronate 70 mg (ALN) and cholecalciferol 2800 IU (D) to ALN alone in osteoporotic patients were reported earlier; we now report changes in serum 25-hydroxyvitamin D (25OHD) during a 24 week extension of ALN + D treatment plus additional vitamin D_3, 2800 IU once-weekly.

The base study was a 15-week, randomized, double-blind, multicenter, controlled study in winter/early spring. Postmenopausal women and men with osteoporosis (BMD T ≤ -2.5) were randomized to once-weekly ALN + D ($n = 357$) or ALN alone ($n = 351$). Patients with baseline serum 25OHD < 9 ng/mL were excluded. Sunlight and supplements containing vitamin D were restricted. The 24-week extension was conducted in summer/fall; all patients received ALN + D plus either additional vitamin D_3 2800 IU (ALN + D5600) or matching placebo once weekly (ALN + D2800) in blinded, randomized fashion. Vitamin D supplements up to 1000 IU/day (in addition to study therapy) were permitted and sunlight was not restricted. Serum 25OHD was the primary endpoint in the base study and secondary in the extension; the primary focus of the extension was the development or worsening of hypercalciuria (24-hour urine calcium increase > 25% from baseline, and to > 300 mg in women or > 350 mg in men).

Among the 652 patients who entered the extension (week 15), the mean 25OHD was 21 ng/mL. At study conclusion (week 39), the mean 25OHD was 27.9 and 25.6 ng/mL in the ALN+5600 and ALN+2800 groups respectively. Moreover, while 21.0% in the ALN+5600 and 17.6% in the ALN+2800 groups had 25OHD < 15 ng/mL at week 15, this was reduced to 3.1% and 5.6%, respectively, by week 39. The highest serum 25OHD concentration observed at week 39 was 59 ng/mL; this is well within the range observed in US adults exposed to summer sun (20 to 84 ng/mL; Barger-Lux, et al, JCEM 2002), although assay and laboratory differences may limit the value of this comparison. As reported earlier, at week 39, 4.2% in the ALN + D5600 and 2.8% in the ALN + D2800 groups had new or worsening hypercalciuria [RR, 1.48 (95% CI: 0.46–3.40)], similar to the ~4% at the end of the base study (week 15). Hypercalcemia was not observed. In summary, mean serum 25OHD increased and the proportion of patients with low 25OHD decreased during the 24-week extension study in both ALN + D2800 and ALN + D5600 groups. The safety and tolerability profile of ALN + D was maintained with 5600 IU weekly even when sunlight and additional vitamin D were permitted.

Conflict of Interest: NB, Merck and Co., Research Grant and Consulting; ML, JAW, AS, Merck and Co., Employees

Tu-P503

BIOAVAILABILITY OF ALENDRONATE AND VITAMIN D3 IN AN ALENDRONATE/VITAMIN D3 COMBINATION TABLET

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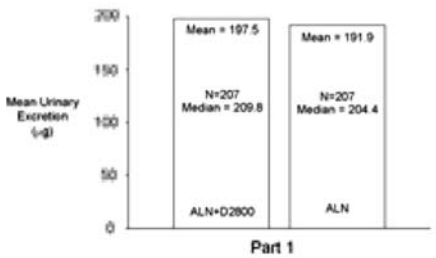
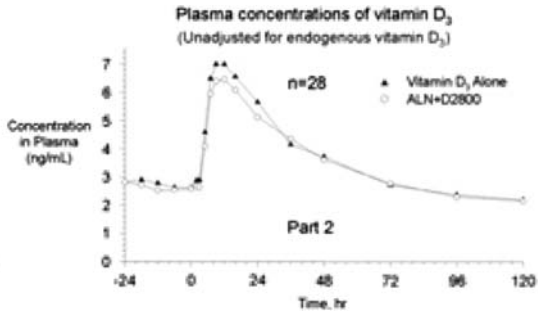
The aims of the study were to compare the urinary excretion of alendronate (ALN) after administration of an ALN 70 mg/2800 IU vitamin D_3 (ALN + D2800) combination tablet (FOSAMAX PLUS D™) versus an ALN 70 mg tablet (FOSAMAX®), and to compare the serum pharmacokinetics ($AUC_{0-120\ hr}$) of vitamin D_3 after administration of ALN + D2800 versus a vitamin D_3 2800 IU tablet without

ALN. This was an open-label, randomized, 2-part, 2-period, crossover study. Washout periods were ≥ 12 days. In Part 1, 214 participants were randomized to receive either a single tablet of ALN + D2800 or ALN 70 mg following an overnight fast and 2 hours prior to a defined meal. Urine was collected −2 hours to 36 hours relative to dose. In Part 2, 28 participants were randomized to receive a single tablet of ALN + D2800 or a 2800 IU vitamin D_3 tablet. Blood samples were collected from −24 hours to 120 hours relative to dose and processed using a mass-spectrometric based vitamin D_3 assay. All 244 subjects who participated, were included in the safety analysis. No serious adverse experiences were reported. Results from Part 1 showed that the geometric mean ratio (GMR) (ALN + D2800/ ALN 70 mg) for total urinary excretion of ALN was 1.03 (90% CI: 0.91, 1.17). Results from Part 2 showed that the serum concentration-time profiles of 2800 IU vitamin D_3 with and without ALN 70 mg (unadjusted for endogenous vitamin D_3) were similar. The serum vitamin D_3 GMRs for $AUC_{0-120\ hr}$ and C_{max} (ALN + D2800/ D_3 2800 IU; 90% CI) were 0.88 (0.81, 0.95) and 0.89 (0.84, 0.95). The ALN + D2800 combination tablet is bioequivalent to the ALN tablet with respect to alendronate bioavailability. The bioavailability of vitamin D_3 is similar in the ALN+2800 combination tablet and in a tablet containing 2800 IU vitamin D_3 without alendronate. Single doses of the ALN + D2800 combination tablet are generally well tolerated.

Conflict of Interest: NL, AP, MC, EW, LM, JW, KG, AD, Merck and Co., Employees





Tu-P504

VITAMIN D STATUS IN POSTMENOPAUSAL JAPANESE-AMERICAN WOMEN LIVING IN HAWAII: A POPULATION-BASED STUDY

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Vitamin D (vitD)has been shown to have a role in neuromuscular function and fall prevention. Fall and hip fracture rates in native Japanese and Japanese-American females in Hawaii are reported to be 1/2 that of Caucasians living in Hawaii and other parts of the US. Differences in the vitD status and the seasonal variation in serum vitD levels among these populations may contribute to this finding. Contributors to vitD status include cutaneous synthesis and dietary vitD intake. We examined serum 25OHD levels and their seasonal variation in 495 women of Japanese ancestry living in Hawaii who participated in the 8th examination of the Hawaii Osteoporosis Study (Jan. 1992 to Sep. 1994). Serum 25OHDassays were performed at the Nichols Institute using liquid chromatography tandem mass spectrometry. Mean age of subjects was 74 ± 5 years. Mean serum (± SD) total 25OHD 79.9 ± 23.7 nmol/L(31.9 ± 9.5 ng/mL); 216 women had values < 75 nmol/L(30 ng/mL), 41 had < 50 nmol/L(20 ng/mL), 3 had < 30 nmol/L(12 ng/mL), and none had < 25 nmol/L(10 ng/mL). There was little evidence of seasonal variation, with mean serum 25OHDlevels of ~28 ng/mL during Jan. and Feb., and 31 to 34 ng/mL during most other months. Study Limitations: Analyses were cross-sectional in design, and vitD levels were measured at a single point in time. Compared to published data for ambulatory Caucasians in the US and Europe, as well as other populations, low serum 25OHD level in our cohort was less prevalent. (the prevalence of 25OHD < 20 ng/mL in one US study of women with osteoporosis was 18%, compared to 8% in our study. In contrast to studies conducted at more northerly latitudes, there was little evidence of seasonal serum 25OHDvariation in Hawaii. Although the prevalence of very low vitD was lower in Hawaii than in other parts of the world, a substantial proportion (43%) had serum 25OHD values < the 75 nmol/L(30 ng/mL) considered optimal by some experts, despite the abundant sunshine in this tropical latitude.

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Tu-P505

PATIENT AND PHYSICIAN ATTITUDES TOWARD VITAMIN D IN OSTEOPOROSIS TREATMENT

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Background: Vitamin D is essential for calcium absorption and bone health, and most osteoporosis treatment guidelines recommend vitamin D supplementation. This study explored the knowledge and attitudes of physicians and patients towards supplement use in osteoporosis treatment.

Methods: Randomly selected Physicians from Malaysia, Taiwan, Philippines, Korea, and Singapore and their postmenopausal women patients with osteoporosis were surveyed. Physicians rated the importance of vitamin D and calcium in osteoporosis management on a scale of 1(not important) to 10 (extremely important) and estimated supplement use by their patients. Patients reported their use of vitamin D and calcium and their perceptions regarding these supplements.

Results: 237 physicians (37 from Malaysia, and 50 each from Taiwan, Philippines, Korea, and Singapore), and 1463 patients (251, 218, 194, 400, and 400 from Malaysia, Taiwan, Philippines, Korea, and Singapore respectively) completed the survey. 84% of patients in Malaysia, 46% in Taiwan, 16% in the Philippines, and 55% each in Singapore and Korea reported never having discussed Vitamin D

supplementation with their Physician. Physicians and patients in all countries reported that calcium was discussed more frequently than Vitamin D. Physicians reported that their patients have little knowledge of the relationship between vitamin D and calcium, and this was confirmed by patient responses.

Conclusion: Most osteoporosis patients recognize the importance of calcium, but have less awareness of that of Vitamin D. Lack of understanding about the role of Vitamin D and numerous concomitant medications can reduce compliance with Vitamin D supplementation.

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Tu-P506

ANALYSIS OF MINERAL BINDING ACTIVITIES OF BISPHOSPHONATES BY USING HYDROXYAPATITE CHROMATOGRAPHY AND ADSORPTION ISOTHERMS TOGETHER WITH DETECTION BY LIGHT ABSORPTION, FLUORESCENCE DERIVATISATION AND TANDEM MASS SPECTROMETRY

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Clinically-relevant bisphosphonates (BPs) characteristically target and reduce osteoclastic bone resorption mainly because of their strong binding to hydroxyapatite bone mineral combined with their selective inhibition of specific enzyme activities. The mineral-binding affinities differ among these pharmaceuticals and this feature may result in different biological potencies, bone tissue distribution and duration of pharmacological action. Our recent investigations have used Langmuir adsorption isotherm analysis together with novel methods of ceramic hydroxyapatite-column chromatography to assess the relative mineral-binding affinities of the BPs. The present study extends previous research in this area by comparing ceramic hydroxyapatite-binding affinities of several clinically-relevant BPs and a number of related analogue derivatives. For the quantitation of the different BPs, a number of analytical methods were used. These included: (a) fluorescence derivatisation with o-phthaldialdehyde (OPA) to detect the primary amine side chains of the alkyl-amino BPs (neridronate, pamidronate and alendronate); (b) UV absorbance at 260 nm, 282 nm and 220 nm (risedronate, minodronate and zoledronate respectively); (c) high performance liquid chromatography with tandem mass spectrometry detection (HPLC/MS/MS) to measure the BPs not detectable by either UV absorbance or OPA derivatisation (etidronate and clodronate, and N-substituted alkyl amino BPs such as ibandronate). Quantitative analysis of BPs determined by HPLC/MS/MS confirmed and extended the UV and fluorescence derivatisation methods of analysis. Significant differences were seen in mineral binding among the clinically-relevant BPs. In addition, some differences were seen in the rank order of BP binding between the hydroxyapatite chromatography and adsorption isotherm methods. It is concluded that comparisons of BP structure-binding characteristics as described in the present study facilitate a better understanding of the mechanisms of mineral binding and their clinical relevance.

Conflict of Interest: None declared

Tu-P507

PERIPROSTHETIC FRACTURE AND TERIPARATIDE

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A 90 years old lady was admitted due to left hip periprosthetic fracture, who undertook bipolar hemiarthroplasty 5 years before this episode.

The X-ray showed the fracture line near the lesser trochanter and no implant loosening sign.

Due to her advanced age and poor heart function,bed rest and non-ambulatory exercise was suggested. However, the follow-up X-ray at two weeks revealed subsidence of implant and fracture displacement accompany groin discomfort.

Her family chose non-surgery treatment and daily Teriparatide injection was used to treat her osteoporosis.

Callus formation with significant pain relief was noted at one-month follow-up. There was no further implant migration at series follow up at the end of one year usage of Teriparatide. To our knowledge, this is the first article dealing with enhanced callus formation in periprosthetic fracture using Teriparatide.

Conflict of Interest: None declared

Tu-P508

1,25 - DIHYDROXY-VITAMIN D INCREASES BONE MINERAL DENSITY IN OSTEOPENIC POST-MENOPAUSAL WOMEN. A THREE-YEAR PROSPECTIVE STUDY

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Introduction: The bone protective effect of cholecalciferol is well known. Clinical aplication of 1,25(OH)2D3 in the treatment of osteoporosis is considered possible, however, the clinical trials in this field brought about conflicting results.

Aim of the study was to evaluated the long-term effect of usually recommended pharmacological dose of 1,25(OH)2D3 on bone mineral density (BMD) in unsubstituted post-menopausal women.

Methods: The study group comprised 52 post-menopausal women with low normal or osteopenic values of BMD. Thirty-two of them were treated with 1,25(OH)2D3 (0.35−0.50 ug/day according to urine calcium) for 3 years. In parallel, another group of 20 women was treated with cholecalciferol (700 U/day). Both these subgroups received calcium (500 mg/day). Baseline vitamin D adequacy and compliance of the treatment with 1,25(OH)2D3 were checked by means of serum PTH levels, which were assessed at the start and thereafter three times in the course of treatment.

Results: Increase in BMD at the spine at the end of the 1st , 2nd and 3rd years of treatment with 1,25(OH)2D3 (expressed as a percentage of the value before treatment) was higher, but did not significantly differ from the effect of cholecalciferol. Significant increase in BMD at the hip at the end of the 3rd (but not the 1st and 2nd) year of treatment with 1,25(OH)2D3 was shown ($p < 0.05$ compared to the effect of cholecalciferol). The protective effect of cholecalciferol was found only on BMD at the spine, but not on BMD at the hip.

Conclusion: The study shows that long-term administration of 1,25(OH)2D3 is an effective treatment of low bone mass at the hip in post-menopausal women.

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