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IS01

VITAMIN D DEFICIENCY PANDEMIC: THE HEALTHFUL BENEFITS OF THE D-LIGHTFUL VITAMIN D

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The major source for vitamin D for humans is from exposure to sunlight. The solar ultraviolet B photons that are absorbed into the epidermis convert 7-dehydrocholesterol to previtamin D3. Previtamin D3 rapidly is transformed in the skin to vitamin D3 which then enters the circulation and is sequentially metabolized in the liver and kidney on carbons 25 and 1 to form 1,25-dihydroxyvitamin D. 1,25(OH)2D is a biologically active form of vitamin D responsible for regulating calcium and phosphorus metabolism for maintaining skeletal health throughout life. Very few foods either naturally contain vitamin D or are fortified with vitamin D. Inadequate sun exposure and dietary intake of vitamin D has resulted in a vitamin D pandemic world-wide. It's estimated that 30–50% of children and adults in the United States and Europe are at risk of vitamin D deficiency. It is now recognized in India, New Zealand, and Australia that vitamin D deficiency is becoming a major health problem. Vitamin D deficiency not only causes rickets in children and osteomalacia and osteoporosis in adults, but also has other dire health consequences including increased risk of common autoimmune diseases including type I diabetes and multiple sclerosis, common cancers including cancers of the breast, colon, prostate, etc., increased risk of cardiovascular heart disease and increased risk of serious infectious diseases including tuberculosis. Sensible sun exposure along with increasing intakes of vitamin D to 1,000 IU of vitamin D3/d are simple ways of preventing this vitamin deficiency. The campaign that recommends avoidance of all sun exposure has placed the world's population at increased risk of vitamin D deficiency. There is little evidence that sensible sun exposure typically no more than 5–10 minutes of arms and legs 2 to 3 times a week in the spring, summer and fall will significantly increase a person's risk of nonmelanoma skin cancer. It should also be noted that most melanomas, the most serious form of skin cancer, occur on the least sun exposed areas and are associated with the number of sun burning experiences as a child and young adult. Because of the short and long term consequences of vitamin D deficiency on health, there needs to be a reevaluation of the recommendations for sensible sun exposure and to increase the fortification of additional foods with vitamin D.

Conflict of Interest: none declared

IS02

BONE GROWTH IN LENGTH AND WIDTH: THE YIN AND YANG OF BONE STABILITY

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Bone growth in length is primarily achieved through the action of chondrocytes in the proliferative and hypertrophic zones of the growth plate. Longitudinal growth is controlled by systemic, local paracrine and local mechanical factors. With regard to the latter, a feedback mechanism must exist which ensures that bone growth proceeds in the direction of the predominant mechanical forces. How this works is unknown at present. Bone growth in length is detrimental to bone stability, but this effect is counteracted by concomitant bone growth in width. This occurs through periosteal apposition, which is the responsibility of periosteal osteoblasts. The action of these cells is mainly controlled by local factors, with modulation by systemic agents. According to mechanostat theory, periosteal apposition is regulated by mechanical requirements. An alternative model, called sizostat hypothesis, maintains that a master gene or set of genes regulate bone growth in width to reach a preprogrammed size, independent of mechanical requirements. The virtues of these two hypotheses have been the subject of much discussion, but experimental data are scarce. In the past, research in this field has focused on bone remodeling on trabecular surfaces. Future research will have to address the question how periosteal bone cells manage to integrate mechanical, hormonal and other input to shape bones that are as strong as they need to be.

Conflict of Interest: none declared

IS03

TOWARD AN EXPANDED UNDERSTANDING OF THE ROLE OF THE PERIOSTEUM IN SKELETAL HEALTH

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Bone structure is a critical component of bone strength, and overall bone size is particularly important for resistance to fracture. Cellular events at the periosteal surface determine bone size. Thus it is critical to understand the regulation of periosteal events. Although interest in this area has recently increased, there is still a dearth of information concerning periosteal physiology and disorders of periosteal function.

During growth and skeletal development periosteal modeling is dramatically active. Clearly radial bone growth occurs during development, as a result of active bone formation. But to produce the modeling drifts that result in the transformation from infant to adult proportions there is also active resorption. Disorders in these processes are well-described, including inadequate radial growth with immobilization or inborn errors and changes in bone size with the use of antiresorptive drugs (e.g. bisphosphonate therapy in children with osteogenesis imperfecta). It is highly likely that periosteal development determines adult bone size, and hence the propensity for fracture later in life. The molecular and cellular pathways that underlie these events are poorly understood.

In adults the degree to which the periosteum is active is controversial. Several histomorphometric studies in humans indicate that bone formation and resorption are present, but there are still few data. At most skeletal sites there appears to be a gradual increase in bone size with aging, suggesting active bone formation. Men may have more age-related periosteal expansion than women, but that remains uncertain. The femoral neck is of particular interest. Until recently, it was assumed that no periosteum existed at the neck, but now there is good evidence that bone formation is active at that site in adults, and that femoral neck dimensions increase with age. A new study in primates suggests that bone resorption is present at that site as well. If bone formation and resorption are both present in the periosteum, the concept of bone balance is relevant when attempting to determine the nature and regulation of bone size change in adults. Some bone and mineral disorders affect bone size via these mechanisms (e.g. hyperparathyroidism, acromegaly). To what extent alterations in periosteal bone balance contribute to bone fragility with aging or in osteoporosis is unclear.

Pharmacological therapies for increasing bone strength may act at the periosteum. For instance, several antiresorptive drugs have been reported to increase bone size, particularly in animal models, potentially by reducing resorption. Parathyroid hormone also may stimulate net increases in bone size, and androgenic compounds appear to stimulate periosteal bone formation. An understanding of periosteal responses to drugs could expand the ability to reduce fractures.

IS04

EPIDEMIOLOGY OF ASEPTIC LOOSENING

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Background: Joint replacement is increasing in the western society due to better implants, technology and an elderly population. Due to this a growing numbers of patients are at risk for revision surgery. As the techniques and implants evolve and time elapse different reasons for revisions dominate over time. To study the different reasons for revision in hip replacement we have used the data from the ongoing prospective study in the Norwegian Arthroplasty Register on joint replacement from 1987 to today.

Methods: Results of hip arthroplasty surgery in Norway will be described. An overview on reasons for failures defined as revision surgery will be presented related to time after surgery. The result of hip replacement surgery was studied from 1987 to 2005. The time periods 1987–91, 92–96, 97–01, 02–2005 were studied. Survival analysis using Kaplan Meier analysis and Cox regression analysis will be presented for different endpoints including aseptic loosening of components.

Results: In the year 2005, 29% (32 % for the hole period) of revisions were due to aseptic loosening of the acetabulum, 23 % (35) due to femoral loosening, 11% (7) due to dislocations, 9% (5) due to infections, 4% (3) due to periprosthetic fractures, 8% (6) due to pain, 4% (1) due to osteolysis in the acetabulum, 4% (2) due to osteolysis in the femur, 4% (3) due to wear of the polyethylene and 4% (4) other causes. There was 37 % less risk of revision in primary hip replacement surgery in the time period 97–01 compared to 87–91 ($RR = 0.63$: 95% CI 0.57–0.69, $p < 0.001$). From 1997 there has been an improvement of results of cemented prostheses due to fewer revisions due to aseptic loosening of the femoral stem ($RR = 0.50$: 95 CI 0.44–0.58, $p < 0.001$). There was more luxation in the later periods for cemented prostheses ($RR = 2$ (1.6–2.4)). In uncemented prostheses there was a 64 % reduced risk (RR 0.36 (0.29–0.45)) for revision, and 71 % (RR 0.29 (0.21–0.35)) and 77 % (RR 0.23 (0.15–0.33)) reduction for acetabular and femoral loosening respectively.

Conclusion: There has been an improvement in revision rate of hip replacement over the years. Aseptic loosening of the acetabulum and femoral components is decreasing but is still the dominant cause of revision in cemented implants. In uncemented implants the dominant cause of revision has been wear and osteolysis and aseptic loosening of the cup.

Conflict of Interest: none declared

IS05

THE PATHOGENESIS OF OSTEOLYSIS AND IMPLANT LOOSENING

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The pathogenesis of periprosthetic osteolysis and subsequent aseptic loosening of the implant involves complex interactions between mechanical and biological factors. It is currently thought that osteolysis develops as a result of macrophage and fibroblast-mediated osteoclast activation following a local inflammatory response to particulate wear debris generated by the prosthesis materials. We have found a clear dose–response relationship between articular bearing-surface wear and development of osteolysis. However, it remains unclear whether it is the particles themselves, or organic compounds adsorbed on their surface, that drive the inflammatory response. Other factors such as non-bearing surface wear, implant micromotion, and intra-medullary cyclic hydrostatic pressure change may also contribute to the inflammatory response.

Examination of osteolytic tissues taken at revision surgery, in-vitro and in-vivo models of osteolysis have suggested that cytokines including TNF, the IL-1 family, IL-6, RANK, RANKL, and OPG, are implicated in the osteolytic process. The genes encoding these cytokines contain polymorphic regions that are associated with inter-individual variability in bone-losing diseases such as osteoporosis, periodontitis, and inflammatory arthritis. We have examined the role of these, and other, candidate genes in affecting an individual's risk of developing osteolysis after total hip arthroplasty.

Our studies suggest that the minor allelic variants within the TNF gene promoter (TNF-238A), the IL-1 receptor antagonist (IL-1RN +2018T), and the gene encoding secreted frizzled-related protein 3 (FRZB +200Trp), together with complex haplotypes involving the RANKL, and OPG genes are associated with a differential incidence of osteolysis. We have also shown using a luciferase reporter gene assay that promoter constructs containing the TNF –238A allele have higher basal and stimulated levels of transcriptional activation versus the common –238G allele in response to polyethylene particle or bacterial toxin stimulation, providing functional evidence to support the role of this polymorphism in the development of osteolysis.

Identification of genes that are responsible for variation in the risk of osteolysis helps further our understanding of its pathogenesis. This may allow the application of emerging biologic therapies, such as those recently introduced in rheumatoid arthritis, to arrest developing osteolytic lesions before mechanical loosening occurs.

Conflict of Interest: none declared

IS06

BIOLOGIC TREATMENT STRATEGIES

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Guided bone regeneration, bone grafts, bone growth factors, and bone substitute materials have been used to restore inadequate bone tissue structures to make them proper for implant placement. We have been working with a variety of these stimulating factors e.g. gene therapy, polypeptide coatings, ceramic coatings, polymer coatings, bone substitutes / bone allograft, growth factors and drugs like bisphosphonate and PTH. We have also been working with improvements of surgical techniques in order to govern the bone to regenerate faster and more reliably, particularly in revision settings with poor ability for bone regeneration. Preclinical studies with these molecules have provided better understanding of the doses, formulation, and delivery mechanism necessary for effective bone formation in our experimental models. We have also conducted clinical randomized trials based on results from our animal experiments. The presentation will be a review of the most important basic science, preclinical studies as well as clinical studies involving these stimulating factors.

IS07

QUANTITATIVE PROTEOMICS FOR CELL DIFFERENTIATION IN BONE DEVELOPMENT

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The process of cell differentiation is controlled by variety of different signals and stimuli and it is dependent on the microenvironment. The signaling events that are initiated by Receptor Tyrosine Kinases (RTKs) often lead to different cellular outcomes such as proliferation and differentiation. Although RTKs have been a main focus of signaling research for decades, little is known about the mechanisms controlling differential growth factor effects and their impact on the process of differentiation. We found that the differentiation of human mesenchymal stem cells (hMSC) into osteoblasts is enhanced by epidermal growth factor (EGF) but not platelet-derived growth factor (PDGF), despite the ability of both to induce tyrosine phosphorylation cascades in the cells. Using quantitative mass spectrometry-based proteomic approach with triple-encoding SILAC, we determined, and quantitatively compared, the EGF and PDGF tyrosine phosphoproteomes. Three cell populations of hMSC were grown in medium containing distinct forms of arginine – either the normal Arg0 or Arg6, or Arg10 isotopic variants. Arg0 cells were left untreated and served as a control; Arg6 cells were exposed to EGF and Arg10 cells to PDGF. The combined cellular lysates were immunoprecipitated using anti-phosphotyrosine antibodies, the precipitated complexes were proteolytically digested and the resulting peptide mixture analyzed by LC-MS/MS. We identified 113 proteins that were specifically utilized by at least one of the growth factors. More than 90 percent of these signaling proteins were found both in EGF and PDGF induced signaling networks, whereas the phosphatidylinositol 3-kinase (PI3K) pathway was exclusively activated by PDGF, implicating it as a possible control point. Indeed, chemical inhibition of PI3K in PDGF-stimulated cells removed the differential effect of the two growth factors, bestowing full differentiation effect onto PDGF. Thus, quantitative proteomics can be used to directly compare entire signaling networks and discover critical differences capable of influencing the differentiation of hMSC.

Conflict of Interest: none declared

IS08

PROTEOMICS TECHNOLOGY

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Proteomics has grown explosively in recent years but the technology is daunting to the non-specialist. Here I will introduce recent trends in mass spectrometry (MS) based proteomics and report on novel applications [1–3].

MS technology: proteomics is now mainly based on mass spectrometry. Complex protein mixtures – such as organelles or whole cell lysates – are digested with an enzyme and the resulting peptides are separated by nanoscale liquid chromatography. As the peptides elute their masses are recorded and they are fragmented and identified in the mass spectrometer. Very high performance mass spectrometers with high resolution and extremely high mass accuracy have become available, tremendously increasing the confidence in the results of protein identifications. Several peptides are sequenced per second and hundreds of proteins can be identified in a single experiment.

Quantitative proteomics: Quantitation in MS-based proteomics is usually done with stable isotope labeling. Various schemes have been proposed and it is now realistic to quantify hundreds and perhaps soon thousands of proteins in mixtures [4].

Interaction proteomics: Finding interaction partners is one of the most useful applications of proteomics. Quantitative proteomics makes it possible to distinguish false positive interactions from true interactions [5].

Large-scale mapping of phosphorylation sites: Post-translational modifications are eminently suited to detection by MS. In a recent example from our laboratory, more than 6000 phosphorylation sites were quantified in response to growth factor stimulation [6]. This type of analysis now allows a direct view on signal processing in the cell.

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Conflict of Interest: none declared

IS09

BIOMARKER DISCOVERY

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A key consequence of chronic obstructive pulmonary disease (COPD) is the progressive loss of lung tissue including components of the alveolar walls and the pulmonary microvasculature. Increased levels of elastin degradation products (elastin peptides and desmosine) have been reported in the bronchoalveolar lavage fluid and blood of smokers and COPD patients. Amongst the metabolic products of the synthetic and catabolic processes of protein expression during COPD are peptides secreted into body fluids such as blood and urine. These markers are likely associated with a combination of biological processes occurring in COPD patients during cycles of tissue destruction and repair associated with chronic inflammation.

Peptide profiles from urine were compared between COPD patients, asymptomatic smokers, and non-smokers. The peptide profiling analyses were based on the initial removal of larger proteins by column chromatography using restricted access material followed by the separation of the smaller peptides by cation exchange liquid chromatography combined with mass spectrometry. Selected peptides were sequenced using tandem mass spectrometry. Total peptide profiles from each subject were used for group comparisons by univariate and multivariate statistical analyses.

We have identified panels consisting of approximately 200 individual peptide identities which are differentially expressed in COPD patients compared to never smoking controls. The patterns of relative peptide expression were correlated with measurements of lung structure by HRCT.

This exploratory biomarker discovery study has identified a number of peptide biomarker candidates in urine from COPD patients vs. controls. The biomarker candidates fall into a limited number of annotation classes, some of which may be related to known pathological processes in COPD such as lung matrix biology and others where no obvious link to COPD is known.

Conflict of Interest: C. Lindberg, AstraZeneca, employee G. Marko-Varga, AstraZeneca, employee J. Griffin, AstraZeneca, employee S. Kjellström, AstraZeneca, employee T. E. Fehniger, AstraZeneca, employee P. Broberg, AstraZeneca, employee J. Mo, AstraZeneca, employee K. Forsman-Semb, AstraZeneca, employee H. Vissing, AstraZeneca, employee I. Groth Clausen, AstraZeneca, em- ployee M. Dahlbäck, AstraZeneca, employee A. Dirksen, AstraZeneca, grant/research support S. Shaker, AstraZeneca, grant/research support

IS10

DEVELOPMENTAL ORIGINS OF OSTEOPOROTIC FRACTURE: THE ROLE OF MATERNAL VITAMIN D INSUFFICIENCY

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Osteoporosis is a skeletal disease characterised by low bone mass and micro-architectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture. The cumulative incidence of fracture from age 50 years is estimated at around 50% among white women and 20% among white men. Preventive strategies against osteoporotic fracture can be targeted throughout the life course. Thus, modification of physical activity and dietary calcium/vitamin D nutrition in the elderly and during midlife should complement high risk approaches entailing appropriate measurement of bone mineral density and targeting of antiresorptive and formation stimulating drugs. Prevention of osteoporotic fracture can also be directed earlier in the life course. Environmental influences during early life interact with the genome in establishing the functional level of a variety of metabolic processes which are involved in the pathogenesis of osteoporotic fracture. The evidence that osteoporosis risk might be programmed in this way stems from 4 groups of studies: (1) Epidemiological studies which confirm that subjects who are born light and whose growth falters in the first year of postnatal life, have significantly lower bone size and mineral content at age 60–75 years; (2) Epidemiological cohort studies have demonstrated that subsequent lower trajectories of childhood growth are associated with an increased risk of hip fracture among such men and women; (3) Detailed physiological studies of candidate endocrine systems which might be programmed have shown that birthweight and growth in infancy alter the functional settings of the GH/IGF-1 and hypothalamic pituitary adrenal axes; (4) Studies characterising the nutrition, body build and lifestyle of pregnant women which relate these to the bone mass of their newborn offspring have identified a number of important determinants of reduced fetal mineral accrual (maternal smoking, low maternal fat stores and maternal vitamin D deficiency, intense levels of weight-bearing physical activity in late pregnancy). These data suggest that undernutrition and other adverse influences arising in fetal life or immediately after birth have a permanent effect on body structure, physiology and metabolism, which might independently influence the later risk of cardiovascular disease and osteoporotic fracture.

Conflict of Interest: none declared

IS11

READING THE DNA METHYLATION SIGNAL

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The DNA of vertebrate animals is covalently modified by methylation of the cytosine base in the dinucleotide sequence 5′CG3′. In mammals, DNA methylation patterns are established during embryonic development and maintained by a copying mechanism when cells divide. The heritability of DNA methylation patterns allows epigenetic marking of the genome to be stable through multiple cell divisions and therefore constitutes a form of cellular memory. The existence of DNA methylation patterns raises two important questions: (1) How are the patterns formed? (2) How are they read to generate biological outcomes? We have focussed on the second question and have identified a set of proteins that recognise and bind to methylated sites in the genome. Most of these proteins are transcriptional repressors that recruit corepressor complexes, which modify chromatin structure to insure gene silencing. To understand the biology of these proteins and their role in human disease, we have created mouse gene knockouts for methyl-CpG binding domain (MBD) proteins and identified cases of gene mis-regulation that can be attributed to the absence of one of these proteins. For example, efficient repression of the Xist gene on the active X chromosome and of exocrine pancreatic enzyme genes in the mouse colon requires MBD2. Interestingly, despite their common DNA binding sites, these proteins apparently do not substitute for one another. Thus, it seems that the bona fide target genes regulated by each methyl-CpG binding protein are distinct. Our work has begun to reveal the molecular basis for this specificity. MECP2 is of particular interest as mutations affecting this MBD protein are the primary cause of Rett Syndrome, which is the most common inherited form of mental retardation affecting human females. Delayed onset symptoms include developmental delay, loss of purposeful limb use and breathing abnormalities. As there is no obvious neurodegeneration in post-mortem brains of RTT patients, the question of reversibility arises and is of obvious relevance for therapeutic approaches to RTT. We earlier created a mouse model for RTT that lacks an intact Mecp2 gene and mimicks several features of the disorder including late inset. Using a mouse with an Mecp2 allele that can be conditionally activated, we asked whether neuronal defects can be rectified if MeCP2 is provided after abnormal neuronal morphology and symptoms have arisen. The results suggest that most or all symptoms are in fact reversible.

IS12

OSTEOCYTES

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Imagine being intrigued and absolutely fascinated by an observation, hypothesizing the significance of the observation, but being unable to prove or disprove the hypothesis. This is essentially what has occurred in past decades with regards to determining the function of osteocytes. After a flourish of interest in the late sixties, early seventies, only a few investigators continued to carry on with the study of osteocytes in a time when many described the cells as 'passive', 'inactive', and 'placeholders'. Recently, many of the intriguing hypotheses formulated by early osteocyte pioneers have been validated, while several novel functions have been discovered. These discoveries have dramatically increased as evidenced by citations, mainly due to new, advanced technology that our predecessors did not have access to thirty to forty years ago.

Whereas the concept of osteocytes as mechanosensors and transducers of messages of bone formation and/or resorption has always had a following, this area of research is dramatically expanding. Osteocytes appear to be the earliest cell in bone to respond to loading, potentially to shear stress generated by bone fluid flowing in the lacuno-canalicular system along the cell's dendritic processes, cell body, and/or primary cilia. These cells appear to be able to extend and retract their dendritic processes potentially affecting both magnitude of shear stress and communication between cells. Not only do the cells respond with small molecule signaling through release of ATP, nitric oxide, and prostaglandin, but also send signals between cells through gap junctions. Functional hemichannels have been discovered on osteocytes that play a role in osteocyte viability and as portals for the release of prostaglandin. Markers for early osteocytes, E11/gp38 and for late osteocytes, Sost, have been discovered. Other molecules highly expressed include Phex, Dmp1, MEPE, and potentially FGF23, therefore these cells may act as an endocrine organ to regulate phosphate metabolism. The perilacunar matrix surrounding the osteocyte is hypomineralized compared to the surrounding bone matrix and can be modified by the osteocyte especially in response to insult such as high dose glucocorticoids. Therefore the osteocyte can function as a mechanosensory cell, a mechanotransduction cell, as a regulator of local and systemic mineralization and systemic mineral metabolism.

Conflict of Interest: Proctor and Gamble-Graduate Student grant

IS13

OSTEOCYTE FUNCTION AND CLINICAL BONE DISEASE: PATHOGENESIS AND TREATMENT

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Three decades ago, interest in osteocytes centred on their buffering role in calcium homeostasis. Then Evans and colleagues found that osteocyte death was prevalent in the elderly femoral neck. Noble and Tomkinson showed that osteocyte apoptosis was enhanced by estrogen suppression in adults and during skull growth in children, in the latter case in the zone programmed for early resorption. As with apoptosis in other tissues, it seemed that the dying osteocyte signals to osteoclasts, targetting tissue resorption at the microscopic level. The accumulating empty lacunae of Evans, confirmed by Power et al, could thus result from inefficient targetting of osteoclasts. The work of Noble, Verborgt and their colleagues showed that osteocytes were responsive to mechanical loading, with both under- and overloading associated with apoptosis. In sequence, local tissue resorption followed. In the case of overloading, osteocyte apoptosis could be massive, but only if the tissue damage was sufficient to result in microdamage. Teriparatide and bisphosphonates may preserve osteocytes from apoptosis, partly explaining their effects in osteoporosis. The recent demonstration that the SOST gene product sclerostin is almost exclusively an osteocyte product that suppresses LRP5-wnt signaling led Poole, Bezooijen and colleagues to investigate the role of sclerostin in vivo. The majority of osteocytes stain for sclerostin, but young osteocytes surrounded by matrix undergoing primary mineralisation do not. Thus the developing basic multicellular unit (BMU) forming bone is exposed to little sclerostin unlike the lining cells that cover completed BMUs. In recent work, Kneissel and colleagues have shown that teriparatide as daily injections suppresses sclerostin expression suggesting that the lining cell is a sclerostin-suppressed osteoblast. In vivo Robling found that mechanical loading suppresses sclerostin in proportion to strain. In vivo also, Ominsky found that neutralizing antibodies to sclerostin increased bone formation. Osteocytes now appear to be the key regulators of skeletal structure and strength, responding to endocrine, paracrine and loading signals by activating osteoclasts and osteoclasts as appropriate within their own BMU. There are important clinical implications of our new understanding of osteocyte function, both in rehabilitation and therapy. These will be expanded as we increase our understanding of the regulation of osteocyte signalling.

Conflict of Interest: J Reeve P&G Research support, consultant J Reeve Eli Lilly, consultant

IS14

CALCIMIMETICS AND HYPERPARATHYROIDISM

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Secondary hyperparathyroidism is an inevitable consequence of untreated chronic uraemia. It can be viewed as an adaptive response to sustained phosphate retention, failure of calcitriol synthesis and hypocalcaemia. For many years the therapeutic approach to these abnormalities has been relatively constant comprising, respectively, dietary phosphate restriction with oral phosphate binders, replacement of the deficient active vitamin D ligand, and calcium supplementation by the oral and/or transdialytic route. The powerful calcaemic effects of the traditional 1 alpha hydroxylated vitamin D compounds are often problematic and have prompted a search for new compounds that might inhibit the parathyroids more selectively, without exhibiting unwanted effects on intestine and perhaps bone as well. A number have shown promise at early stages of development but, while clearly highly effective in the setting of placebo controlled studies, they have shown little if any advantage when compared properly with existing standard therapy using calcitriol or alfacalcidol. To call these new agents 'non calcaemic' is clearly misleading at the moment.

Cinacalcet, the first marketed calcimimetic agent, is now widely used in the treatment of hyperparathyroid states. Predictably, and in the knowledge that its principle action at the level of the parathyroid is to shift the sigmoidal calcium–PTH curve to the left, treatment of hyperparathyroid patients with cinacalcet is associated with simultaneous lowering of both PTH and calcium. In the setting of chronic kidney disease patients with persisting hyperparathyroidism despite receiving standard therapy with vitamin D metabolites and phosphate binders, the addition of cinacalcet resulted in substantially improved biochemical profiles, even in patients with severe disease. Calcimimetic treated patients also experienced marked reduction of the rates of parathyroidectomy, fracture and cardiovascular hospitalisation. Further outcome studies are now in progress.

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IS15

THE BONE–VASCULAR INTERFACE

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Tissue calcification is a complex and coordinated process in bone, teeth as well as in extraosseous sites. However, the most threatening localization of unwanted calcification is at vascular sites, where it manifests as both medial and intimal calcification of arteries. Atherosclerotic plaque calcification is associated with cardiovascular events such as myocardial infarction and stroke. Medial calcification causes arterial stiffness, increased pulse pressure and increased pulse wave velocity, and contributes to left ventricular dysfunction. Vascular calcifications are usually progressive, while their severity is highest in patients with chronic kidney disease (CKD) and associated with increased mortality in this population. Age, duration of dialysis, inflammation and diabetes mellitus are major predictors of progressive calcification. Bone turnover is also a major player in this context, since a healthy bone is the key buffer of excess calcium and phosphate ions, while high-turnover as well as adynamic bone disease are both incapable to sufficiently incorporate circulating calcium and phosphate into the bone matrix. The resulting hyperphosphatemia and hypercalcemia contribute to extraosseous calcifications not only by passive precipitation, but by active induction of cellular events. Both trigger osteogenic differentiation of vascular smooth muscle cell (VSMC) into osteoblast-like cells, while especially high phosphate may be the master switch towards unwanted calcification processes. Newly discovered calcium(Ca)-regulatory factors with calcification-inhibitory properties include fetuin-A, matrix Gla protein (MGP), osteoprotegerin (OPG) and pyrophosphates (PP). In cohorts of dialysis patients, low serum fetuin-A and high OPG levels were found to be associated with increased extraosseous calcification as well as mortality, and further clinical data currently emerge for additional Ca-regulatory factors. These novel insights may help to develop new therapeutic targets to counteract progressive soft-tissue and cardiovascular calcifications including anti-inflammatory strategies (fetuin-A upregulation), vitamin K supplementation (MGP activation), humanized antibodies (OPG replacement) and use of bisphosphonates (PP replacement).

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IS16

VITAMIN D (DEFICIENCY/SUFFICIENCY) AND THE NEW ACTIVE COMPOUNDS

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Vitamin D is 25-hydroxylated in the liver and 1-alpha-hydroxylated in the kidney. As such patients with reduced kidney function have reduced ability to perform the 1-alpha-hydroxylation activity, resulting in low levels of 1,25(OH)2D. This leads to a reduced suppression of PTH synthesis resulting in secondary hyperparathyroidism. At the same time the low levels of 1,25OH2D result in reduced intestinal calcium absorption, which also leads to decreased synthesis and secretion of PTH and to the development of parathyroid hyperplasia.1,25(OH)2D deficiency further leads to skeletal complications (renal osteodystrophy). The hormone, 1,25(OH)2D, has a number of non-renal and non-skeletal and non-parathyroid related effects, that all might be affected in the condition of vitamin D deficiency.

Therefore, patients with reduced renal function are frequently treated with vitamin D analogs. A major problem is that this treatment often results in the development of hypercalcemia. A number of different vitamin D analogs have therefore been created, which may have different effects on intestinal calcium absorption, cell differentiation, etc.

The actual knowledge on the effects and present status of some of the 'non-calcemic vitamin D analogs' will be presented.

Conflict of Interest: none declared

IS17

G PROTEIN-COUPLED RECEPTOR SIGNALLING MECHANISMS

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The G protein-coupled receptor (GPCR) family represents the largest and most versatile group of cell surface receptors that recognise a wide variety of chemical signals and transduce the ligand-binding event into intracellular responses (Hill, 2006). Drugs that are active at GPCRs have therapeutic actions across a wide range of human diseases. In this presentation, I will review the general characteristics of ligand–receptor interactions (agonists, antagonists and

inverse agonists) and discuss the potential for different ligands to recognise different conformations/sites of the same receptor to stimulate cell signalling cascades. A good example of this is the ability of the classic beta-adrenoceptor antagonist propranolol to act as an inverse agonist of cyclic AMP accumulation in CHO cells expressing the human beta-2 adrenoceptor whilst acting as an agonist of MAP kinase activation in the same cells (Baker et al., 2003).

GPCRs are now recognised to exist as dimers and higher oligomers and it is becoming increasing apparent that coupling to other signalling proteins and their cellular location may play an important role in determining the final signalling outcome of receptor stimulation (Hill, 2006). This means that the ligand-binding properties of GPCRS may vary depending on their cellular location in microdomains within a given cell. I will also review how new imaging approaches can be used to investigate these interactions in microdomains of single living cells.

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Conflict of Interest: Founder and Director of University of Nottingham spin-out company CellAura Technologies Ltd

IS18

CANNABINOIDS AND BONE

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Mammalian tissues express at least two types of cannabinoid receptor, CB1 and CB2, both G protein coupled. Endogenous ligands for these receptors also exist. These 'endocannabinoids' are all eicosanoids, prominent examples including arachidonoyl ethanolamide and 2-arachidonoyl glycerol, both of which are synthesised on demand, removed from their sites of action by tissue uptake processes and metabolised by intracellular enzymes. A clear role for the endocannabinoid system has been demonstrated in a variety of physiological processes including appetite control, cardiovascular regulation and pain processing.

Recent reports have demonstrated the involvement of the cannabinoid receptors and ligands in bone physiology. Mice lacking either the CB1 or CB2 receptor have abnormal bone phenotypes. Furthermore, cannabinoid receptor agonists and antagonists have been shown to reduce bone loss following ovariectomy and have direct effects on osteoclasts and osteoblasts in vitro (Idris et al, 2006; Ofek et al, 2006; Tam et al, 2006). In addition, a significant association between specific CB2 receptor genotypes and osteoporosis has been reported (Karsak et al, 2005).

Despite the striking effects of synthetic cannabinoids on the skeleton in vivo, the role of endocannabinoids on bone metabolism is unknown. Since the CB1 receptor is highly expressed in the hypothalamus, it is possible that endocannabinoids may affect bone remodelling via sympathetic projections; it is also possible that endocannabinoids may act locally in the bone microenvironment.

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Conflict of Interest: none declared

IS19

G PROTEIN COUPLED RECEPTORS FOR FSH AND TSH: NOVEL ACTIONS IN SKELETAL PHYSIOLOGY AND PATHOPHYSIOLOGY

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We have evaluated the direct actions of FSH and TSH on bone and bone cells (Abe et al., 2003; Sun et al., 2006). We suggest that elevated FSH and lowered TSH levels contribute to the bone loss in hypogonadal and thyrotoxic states. We find that haploinsufficiency of FSH and the TSH receptor in heterozygotic mice, which have conserved ovarian and thyroid function, display high and low bone mass, respectively. This suggests that the actions of FSH and TSH on the skeleton are independent of the hormones they release from the ovary and thyroid gland. We have identified G protein-coupled FSH and TSH receptors on osteoclasts. Activation of the TSH receptor causes a dramatic reduction in osteoclast formation, bone resorption and osteoclast survival through the inactivation of MAP kinase and NF-kappaB signaling. The reverse occurs with FSH in both mouse and human osteoclasts. Interestingly the osteoclast FSH receptor couples to a Gi2alpha, the genetic deletion of which in Gi2alpha-/- mice abolishes FSH-induced osteoclast formation, as well as MAP kinase, Akt and NF-kappaB signaling. Additionally FSH stimulates and TSH inhibits the secretion of the osteoclastogenic cytokine TNFalpha from macrophages (Hase et al., 2006; Iqbal et al., 2006). Genetic deletion of the TNFalpha gene thus abrogates the osteoporosis in TSH receptor deficient mice. Epidemiologic studies also reveal a close correlation between fracture risk and serum TSH levels in hyperthyroid patients. Furthermore, patients with TSH receptor mutations have bone loss despite being rendered euthyroid. Recombinant TSH inhibits bone turnover in post-menopausal women, and ovariectomy-induced bone loss in mice. Likewise, clinical data implicating elevated FSH in the genesis of post-menopausal osteoporosis are just beginning to evolve. Strong correlations exist between bone mass and FSH rather than estrogen levels across the menopausal transition. Furthermore, estrogen-deficient women with high, but not low/normal FSH levels experience bone loss. Together, these studies implicate anterior pituitary hormones in the direct control of bone mass in health and disease.

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Conflict of Interest: Procter and Gamble – research support Servier – research support Genzyme – research support Speaking and adhoc advisory boards of Procter and Gamble, Aventis, GSK, Rocke and Merck

IS20

BONE, FAT AND AGING: PPAR-GAMMA AS KEY COMPONENT OF BONE LOSS DUE TO AGING AND ANTI-DIABETIC GLITAZONES

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Osteoporosis, obesity and diabetes are at the center of interest due to their prevalence in our increasingly sedentary and aging society. Recent evidence clearly indicates that a single protein, peroxisome proliferator-activated receptor gamma (PPAR-gamma), controls bone mass, energy expenditure and glucose metabolism. Unsaturated fatty acids, prostaglandin J2, and certain phospholipids are natural activators of PPAR-gamma, whereas anti-diabetic drugs thiazolidinediones (TZDs) are artificial high affinity PPAR-gamma agonists. Besides its role in energy metabolism and adipocyte development and function, PPAR-gamma plays an important role in bone marrow mesenchymal stroma/stem cells (MSC) lineage allocation. Adipocyte-specific isoform PPAR-gamma2 acts in MSC as a positive regulator of adipocyte and a dominant-negative regulator of osteoblast differentiation. PPAR-gamma2 expression increases in MSC with aging. Animal models of either bone accrual or bone loss, which depend on the status of PPAR-gamma activity, indicate that PPAR-gamma is a key regulator of bone homeostasis. Thus, PPAR-gamma insufficiency leads to higher bone mass and protects against bone loss with aging, whereas PPAR-gamma activation with TZD rosiglitazone results in a significant bone loss, which resembles age-related bone loss and is associated with structure/function changes in bone marrow. Similarly, anti-diabetic TZD therapy in human leads to the bone loss in older diabetic women. In order to determine molecular mechanisms by which PPAR-gamma2 suppresses osteoblastic and promotes adipocytic differentiation of MSC, we have performed high-throughput analysis of gene expression using a microarray of 38,000 murine transcripts. This analysis revealed that PPAR-gamma2 controls activity of multiple regulatory pathways essential for MSC growth and differentiation, including Wnt, IGF-1 and TGF-beta/BMP signaling. Results from these experiments provide a vast array of information about hierarchical interactions between different regulatory pathways and may ultimately lead to the identification of a master regulatory mechanism by which PPAR-gamma2 controls osteoblast differentiation.

In conclusion, therapeutic modulation of PPAR-gamma activity with anti-diabetic TZDs may cause adverse effects on bone. Thus, understanding PPAR-gamma bone-specific activities may lead to the development of anti-osteoporotic therapies, which will selectively target anti-osteoblastic activity of this nuclear receptor.

Conflict of Interest: none declared

IS21

PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA: LESSONS FROM HUMAN GENETIC STUDIES

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At a time when the twin epidemics of obesity and type 2 diabetes threaten to engulf even the most well-resourced Western healthcare systems, the nuclear receptor peroxisome proliferator-activated receptor ((PPARγ) has emerged as a bona fide therapeutic target for treating human metabolic disease. The novel insulin-sensitising antidiabetic thiazolidinediones (TZDs e.g. rosiglitazone, pioglitazone), which are licensed for use in the treatment of type 2 diabetes, are high affinity PPARγ ligands, whose beneficial effects extend beyond improvement in glycaemic control to include amelioration of dyslipidaemia, lowering of blood pressure and favourable modulation of macrophage lipid handling and inflammatory responses. However, a major drawback to the clinical use of existing TZDs is weight gain, reflecting both enhanced adipogenesis and fluid retention, neither of which is desirable in a population who are already overweight and prone to cardiovascular disease. Accordingly, the 'search is on' to identify the next generation of PPARγ modulators that will promote maximal clinical benefit by targeting specific facets of the metabolic syndrome (glucose intolerance/diabetes, dyslipidaemia and hypertension), while simultaneously avoiding undesirable side effects of PPARγ activation (e.g. weight gain). This article outlines the important clinical and laboratory observations made in human subjects harbouring genetic variations in PPARγ that support such a therapeutic strategy.

Conflict of Interest: GlaxoSmithKline, Speakers Bureau

IS22

PRIMARY HYPERPARATHYROIDISM: WHAT TO DO AND WHEN TO ACT

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The clinical presentation of primary hyperparathyroidism (pHPT) has changed as a result of the increasing accessibility to biochemical analyses. The introduction of biochemical auto-analysers was followed by a rise and later a fall in the incidence of pHPT. Today the diagnosis in the majority of cases is made accidentally in patients with mildly increased serum calcium and few or no specific symptoms. Whereas operative treatment in patients with higher calcium levels or organ specific symptoms is well established, the value of surgical treatment of mild or borderline pHPT in asymptomatic patients has been a matter of discussion. So far, two Consensus Development Conferences have been performed in order to provide guidelines and perspectives for treatment in relation to the change in the clinical presentation of the disease. In the absence of randomized controlled trials, these recommendations were based on clinical experience and data derived from epidemiological studies or non-randomized, prospective cohort studies.

On a population basis, increased serum calcium levels have been reported to be associated with an increased mortality related largely to cardiovascular disease, especially in younger men. Increased mortality, primarily from cardiovascular disease has also been found in epidemiological studies on pHPT, but these studies do not specifically address the situation in patients with slightly elevated calcium levels, or the value of operative treatment. Other studies have found unchanged or even reduced mortality among patients with mild pHPT although the calcium level by it self has been found to be an independent predictor of death.

It appears that most patients with asymptomatic, mild pHPT can be followed without treatment. Observed for a decade, calcium and PTH levels seem to be stable and bone mass do not deteriorate. However, age seems to be an important risk factor for progressive disease, with increased risk among younger patients. In general, it is recommended that patients, who do not meet surgical criteria, are monitored closely for development of indications for operation, especially increasing calcium levels and deterioration of renal function and bone mass.

Conflict of Interest: none declared

IS23

PAGET'S DISEASE

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Paget's disease of bone (PDB) is a common condition characterized by focal abnormalities of increased bone turnover leading to complications such as bone pain, deformity, fractures, and deafness. Environmental and genetic factors both appear to contribute to the pathogenesis of PDB. The most widely studied environmental trigger is paramyxovirus virus infection. Several studies have been conducted to try and determine whether or not paramyxoviruses are present in PDB but the results have been conflicting. Other potential triggers for the disease include low dietary calcium intake in childhood and repetitive mechanical loading of affected bones, although the mechanisms by which these factors cause PDB is unclear. Paget's disease has a strong genetic component and in many cases is inherited in a simple autosomal dominant fashion. Several susceptibility loci for the disease have been identified by genome wide scans and mutations have now been identified in four genes that predispose to PDB and related disorders. These are The TNFRSF11A gene, which encodes RANK, the TNFRSF11B gene, which encodes OPG and SQSTM1, which encodes a scaffold protein in the NFkappaB signaling pathway. The rare syndrome of hereditary inclusion body myopathy, PDB and fronto-temporal dementia (IBMPFD) is caused by mutations in the VCP gene which acts as a chaperone for IkappaB amongst many other functions. Management of PDB is generally based on giving antiresorptive drugs such as bisphosphonates and/or giving analgesics or NSAID's. With modern fourth generation bisphosphonates such as Risedronate and Zoledronate, it is now possible to restore bone turnover to normal in the vast majority of patients but the long term effects of bisphosphonate therapy on the complications of PDB are unclear. Recent insights into the effects of bisphosphonate therapy on clinical outcomes in PDB have been provide by the PRISM study which involved 1324 patients who were randomised to receive symptomatic treatment or intensive bisphosphonate therapy. Several important clinical endpoints such as fracture, joint replacement, progression of deafness and quality of life were evaluated in PRISM and the initial results will be presented at the meeting.

Conflict of Interest: Consultancy for Novartis, Proctor & Gamble, Sanofi/Aventis

IS24

NEW MODELS FOR BONE AND CARTILAGE RELATED DISORDERS AND NEW WAYS OF MOUSE MODELS CHARACTERIZATION

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Mouse model production and their systemic characterization are bottlenecks in the process of proper understanding of molecular mechanism in diseases. We have undertaken in the Munich ENU-Mouse Mutagenesis Screen a large-scale and genome wide production of mouse models for inherited human diseases. The new main focus is on bone and cartilage diseases. Bone densitometry, blood screens for bone parameters and turnover markers have been implemented in the F1 screen. We have isolated 16 new mouse models in different fields of bone and cartilage disorders.

To appreciate the fact that many genes show pleiotropic effect we established a unique mouse phenotyping center (German Mouse Clinic, GMC). In the GMC, experts from various fields of mouse physiology and pathology in close cooperation with clinicians work side by side at one location. The examinations comprise the following areas: bone and cartilage, allergy, behavior, clinical chemistry, cardiovascular analyses, energy metabolism, eye development and vision, host–pathogen interactions, immunology, lung function, molecular phenotyping, neurology, nociception, steroid metabolism and pathology. The systemic characterization of the new mouse models in the GMC has revealed the syndromic character of many bone related disorders.

Data will be presented from the analysis of mouse models for osteogenesis imperfecta, and osteoarthritis. Our data contributed in these mutant lines either to the identification of the mouse lines as a model system for these diseases, or we discovered further similarities of the mutant line with the human syndromes.

We are currently implementing new challenge experiments for genotype–environment interactions in the analysis of the mutant line. 'Environmental platforms' are tested with different standardized challenge experiments. This will help us to identify genetic predispositions as susceptibility factors for environmental influences.

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IS25

HEDGEHOG AND BONE MORPHOGENETIC PROTEIN SIGNALING DURING DERMAL BONE REGENERATION OF THE ZEBRAFISH FIN RAYS

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Teleosts, including zebrafish, can regenerate many organs and tissues following amputation during adulthood. The relative simplicity and accessibility of the caudal fin, and of its skeletal elements, make the fin an excellent system to investigate the molecular pathways involved in bone regeneration. The skeletal elements of the ray are acellular and form, as intramembranous bone,

by the direct mineralization of a bone matrix. We showed that the Hedgehog and Bmp signaling pathways are both involved in the regeneration of the dermal bones of the rays and in the regenerate growth. Indeed, ectopic expression of Shh or Bmp2b induces an ectopic formation of bone matrix; the Hedgehog action being mediated by the activation of the Bmp pathway. On the other hand, blocking the Hedgehog pathway using cyclopamine inhibits the growth of the regenerate and formation of new scleroblasts secreting the bone matrix. Similarly, misexpression of Chordin, an antagonist of Bmps, leads to a transient inhibition of regenerate growth and to a reduced bone matrix deposition due to a defect in scleroblasts maturation and function. This is accompanied by the downregulation of *runx2a/b*, their target, *col10a1* and of *sox9a* and *col2a1*. This analysis revealed the surprising finding of the presence in the fin regenerate of several factors that are normally expressed in chondrogenic elements during endochondral bone formation (*col10a1, col2a1, sox9* and *ihha*) although fin rays form without cartilage intermediate. These results suggest that the dermal bones of the rays possess intermediate characteristics of cartilage and bone.

We are now examining the respective roles of Shh and Ihha. *Ihha* is expressed in the differentiating scleroblasts adjacent to *shh*-expressing cells located in the basal epidermal cells. This expression suggests that Ihha may have a more direct role than Shh in the scleroblast differentiation. In contrast, the dynamic expression patterns of shh suggest that it may rather be involved in patterning the dermal bone, notably the formation of the ray branches. We are using a zebrafish transgenic line in which EGFP expression recapitulates shh expression to investigate the effect of laser ablation of *shh*-expressing cells. Preliminary data indicate that ablation of these cells significantly delays ray branch formation supporting the hypothesis of Shh role in patterning ray branching and more generally, in patterning the bony rays. (supported by CIHR).

Conflict of Interest: none declared

IS26

SPEEDING UP FUNCTIONAL EVALUATION FOR THE MOUSE

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Due to the wonderful properties of ES cells, it is now possible to plan a systematic assault upon the mouse genome via gene targeting. This task requires the optimization of all steps involved in the production of targeted cells and mice. Of these steps, we are focused on high throughput generation of targeting constructs, as well as functional and proteomic studies with ES cells.

Recombineering is the use of homologous recombination for DNA engineering in E. coli. Because illegitimate recombination in E. coli is almost nonexistent, it is possible to recombineer through multiple steps in liquid culture without cloning. This permits the establishment of parallel processing protocols, termed 'recombineering pipelines', for high throughput generation of DNA constructs. We have built pipelines for several tasks, including (i) targeting constructs for conditional alleles, (ii) targeting constructs for knocking-in protein tags, and (iii) production of BAC transgenes. It is now straightforward to generate gene libraries for functional and proteomic applications.

Although systematic genome wide mutagenesis of the mouse is achievable, genome-wide phenotyping remains a huge task. We think that two experimental approaches based on ES cells and standardized ES cell differentiation routines will reduce the scale of task. First, conditional mutagenesis for microarray and functional studies, which requires a strategy for double targeting. Second, protein tagging via knock-ins, for proteomic and regulomic analyses, which requires an optimized tag for affinity chromatography and mass spectrometry. Ideally, the tag also presents options for in situ immunofluorescence and/or chromatin immunoprecipitation. As dramatically illustrated in yeast, transcriptome and proteome data sets are strongly complementary and lead to a simplification of functional enquiry. The methodology to generate these data sets in ES cells is now available.

Conflict of Interest: A.F. Stewart, Gene Bridges GmbH, Consultant A.F. Stewart, Artemis Pharmaceuticals GmbH, consultant A.F. Stewart, Cibasa, consultant

IS27

BIOLOGICAL FUNCTIONS OF CONNEXINS DEDUCED FROM TRANSGENIC MOUSE MUTANTS

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After complete genomic sequencing it became clear that the mouse and human genomes contain 20 and 21 connexin genes, respectively (1). Almost all of the different connexin coding DNAs have been deleted in the mouse genome. These studies allowed to assign gross functional properties to the different connexin isoforms. In contrast, connexin mutations in the human genome frequently result in the exchange of single amino acid residues, leading to an abnormal protein conformation. This could dominantly affect the same nonmutated isoform or a different connexin isoform in the same cell in a transdominant manner. Thus, the investigation of human connexin mutations can yield functional information on the interaction of connexin isoforms and can thus lead to new insights into the biological function of the connexin channel network.

Recent examples from our laboratory regarding ablation of connexins in the conductive and working myocardium of transgenic mice will be summarized. This allowed us to correlate the expression pattern of different 'cardiac' connexin isoforms with the conduction properties of the corresponding connexin channels. (2). In addition, the expression of human connexin mutations in the orthologous mouse connexin genes can yield similar phenotypic abnormalities as observed in humans and thus allow mechanistic studies which cannot be carried out with tissues from human patients. We have inserted single base mutations in connexin43 (from a patient suffering from oculodentodigital dysplasia) or in connexin31 (from a patient suffering from the genodermatosis Erythrokeratodermia variabilis) into the corresponding mouse connexin genes, and results from the characterization of these transgenic mice will be presented.

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IS28

CONNEXINS IN SKELETAL DEVELOPMENT AND MAINTENANCE

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Bone is a dynamic tissue that is constantly remodeled in response to a large number of stimuli, and control of bone remodeling requires a tightly orchestrated interplay among, osteoblasts, osteocytes and osteoclasts. Gap junctional communication allows for direct diffusion of signaling molecules and metabolites, thus equalizing hormonal and local signals among the osteoblast–osteocyte network. Osteoblasts and osteocytes are functionally coupled by gap junctions formed primarily by connexin43 (Cx43) and connexin45. Mouse genetics studies have demonstrated the importance of Cx43 in postnatal bone growth and maintenance. To overcome the lethality of the germline null mutation, we have used a Cre/loxP gene replacement approach to ablate the Cx43 gene (Gja1) at different stages of osteoblast differentiation. Conditional Gja1 ablation using a 2.3kb fragment of the α1(I) collagen promoter results in reduced peak bone mass, osteopenia, osteoblast mineralization defects, and dramatically attenuated response to PTH, the consequence of an inability of Cx43 deficient osteoblasts to fully activate in response to the hormonal anabolic stimulus. Instead, deletion of Gja1 using osteocalcin promoters only minimally affects bone mass and osteoblast differentiation. We have also used the Dermo-1 (Twist2) promoter, which is embryonically expressed in cells that give rise to chondrocytes and osteoblasts. Deletion of Gja1 at this early stage of chondro-osteogenesis results in mice of normal size at birth, but with age these animals remain substantially smaller than their littermates up to 1 year. They also exhibit shortened, tubulated limb bones, and small, narrow skull and long nasal bones, resulting in a pointed snout. Similar craniofacial abnormalities occur in germline Gja1 null mice, and are remindful of malformations reported in mice expressing a mutant form of Gja1 (G60S), which partially phenocopies the human disease oculodentodigital dysplasia. Therefore, Gja1 ablation in osteochondroprogenitor cells results in a more severe skeletal phenotype than that observed when Gja1 is deleted later in osteoblast differentiation. While Cx43 controls gene expression and the function of differentiated osteoblasts, the emerging in vivo data reveal a more prominent role of this gap junction protein at earlier steps of osteogenesis, perhaps in the control of osteoblast commitment from stromal cell precursors.

Conflict of Interest: none declared

IS29

RECENT DEVELOPMENTS IN OSTEOPOROSIS TREATMENT

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The objective of treating patients with osteoporosis is to reduce current fracture risk. Current treatment options, especially bisphosphonates, teriparatide and strontium ranelate, are quite effective in decreasing the risk of vertebral and nonvertebral fracture and, in general, are well tolerated. However, success of treatment is limited by contraindications or intolerance, inconvenient dosing regimens and, especially, poor adherence to therapy. As old clinical questions are answered, new questions and challenges are identified, resulting in a constant refining of our treatment strategies.

The importance of adequate intake of vitamin D as a component of our treatments has re-emerged, including the appreciation that higher doses than previously believed are required to achieve optimal benefits.

The availability of intravenous bisphosphonate regimens that have been documented to improve BMD and reduce fracture risk and that are registered for the treatment of osteoporosis provides attractive alternatives to patients with contraindications or intolerance to oral dosing or who have complex medical problems that preclude the stringent logistics of oral dosing. Alternate forms of parathyroid hormone-like proteins are under development.

Recent discoveries of new pathways regulating or affecting skeletal metabolism provide new molecular targets for therapy. Strategies currently under investigation include anti-resorptive agents that inhibit cathepsin K or RANK ligand. The recently discovered and incompletely understood Wnt/β-catenin signaling pathway, including the LRP5 receptor system, provides attractive targets to stimulate bone formation. Of these approaches, inhibiting the activity of sclerostin, an osteocyte-derived inhibitor of the Wnt-signaling pathway in osteoblasts, has been explored most productively in preclinical models.

Having new antiresorptive agents would provide options with different tolerability profiles, safety issues, and dosing regimens. Whether these agents will be more effective inhibitors of fracture risk is uncertain, and it is uncertain whether these new agents will be more effective than current antiresorptive drugs, such as bisphosphonates. New anabolic agents may provide the opportunity to restore damaged skeleton and possibly to 'cure' osteoporosis.

Perhaps the most important development is the anticipated availability of the WHO absolute fracture risk algorithm that will allow more accurate and appropriate identification of patients who will most benefit from our current and new treatment options.

IS30

TREATMENT OF OSTEOPOROSIS

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Both antiresorptives and anabolic therapy have been shown to be effective for reducing fracture risk in clinical trials ranging from less than 2 years to over 4 years. However, for each type of treatment, there are questions about optimal duration of treatment, although the nature of these questions is quite different.

For antiresorptives, which are often prescribed for periods as long as 10 years, there have been concerns that long-term reduction of bone resorption might lead to eventual decreases in bone strength with a consequent increase in fracture risk. The only long term data with a primary fracture outcome has been the WHI which showed reductions in hip and clinical fracture risk for as long as 9 years of estrogen versus placebo. For bisphosphonates, there are no long-term placebo-controlled trials but there had been several small extension studies (without placebo) for alendronate and risedronate with BMD and bone marker endpoints. More recently, the largest such study of long-term alendronate, the FLEX (FIT Long term Extension) study, compared five years of alendronate use followed by 5 more years to five years of alendronate followed by placebo. This study found that there was greater bone loss in those who stopped but the loss was only moderate (2–3% over 5 years) compared to continuing. Bone markers increased but only gradually in those who stopped. While numbers were small, incidence of non-vertebral fractures and morphometric vertebral fractures were similar, although clinical vertebral fractures while rare, were reduced by 50% in those who continued. The study also included a small bone biopsy substudy which was consistent with no decrease in bone quality with 10 years of alendronate treatment. The study concluded that long-term alendronate was safe in terms of bone quality but that many women can perhaps take a drug holiday for up to 5 years without a substantial increase in fracture risk. Whether BMD and bone markers can help identify who should continue and who can take a holiday will be discussed.

For anabolic therapy, the teriperatide fracture trial, which had to stop after about 21 months, showed an intriguing pattern of increasing fracture risk reduction over that time suggesting a very dramatic reduction might have been seen had the study been able to continue until its planned 3 year endpoint. On the other hand, several small BMD experiments with differing patterns of PTH use (one year followed by bisphosphonate, 3 month cycles, once per week) have suggested that several shorter periods of treatment might yield similar effects to longer term daily use, at much lower cost.

OC001

HIGH BONE MASS LRP5 MUTATION LEADS TO INCREASED BONE FORMATION AND INHIBITION OF ADIPOGENESIS IN HUMAN MESENCHYMAL STEM CELLS

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Background: Mutations in the Wnt coreceptor, LRP5, have been linked to alterations in bone mass leading to either osteoporosis or high bone mass phenotype. However, the cellular mechanisms underlying these changes are still controversial. Here, we examined the effects of LRP5 mutations on mesenchymal stem cell (MSC) proliferation and differentiation. Method: We have established three different human MSC (hMSC) lines stably expressing either LRP5WT (hMSC-LRP5WT), LRP5T244 (hMSC-LRP5T244, inactivation mutation leading to osteoporosis) or LRP5T253 (hMSC-LRP5T253, activation mutation leading to high bone mass) using retrovirus mediated gene transduction of our telomerized hMSC (hMSC-TERT). Real-time PCR, dual luciferase assay for Wnt signaling activation, and in vivo bone formation assays were employed. Results: hMSC-TERT cells express Wnt ligands, Fizzled receptors and other Wnt signaling components. The functional activity of the transgenes were verified by dual luciferase assay that demonstrated marked stimulation of Wnt signaling in both hMSC-LRP5WT and hMSC-LRP5T253 compared to hMSC-LRP5T244 upon treatment with Wnt3a and such stimulation was inhibited by Wnt antagonist DKK1. Impaired Wnt signaling in hMSC-LRP5T244 was due to decreased trafficking of LRP5T244 compared to LRP5T253 mutated proteins as assessed by Western blotting. Wnt3a inhibited cell proliferation in hMSC-LRP5WT and hMSC-LRP5T253 through down regulation of DKK1. Both hMSC-LRP5WT and hMSC-LRP5T253 showed enhanced in vitro osteoblast differentiation in response to Wnt3a and this effect was abolished in hMSC-LRP5T244. Similarly, hMSC-LRP5WT and hMSC-LRP5T253 but not hMSC-LRP5T244 formed significant amount of ectopic bone when implanted subcutaneously with Hydroxyapatite/tricalcium phosphate (HA/TCP) in SCID/NOD mice. In contrast, hMSC-LRP5T244 exhibited enhanced adipocyte differentiation capacity compared with hMSC-LRP5WT and hMSC-LRP5T253. Conclusion: LRP5 mutations and the level of Wnt signalling determine differentiation fate of hMSC into osteoblasts or adipocytes and provide supportive evidence for the possible targeting of hMSC as method for increasing bone formation.

Conflict of Interest: None declared

OC002

N-CADHERIN INTERACTION WITH LRP5 ANTAGONIZES WNT SIGNALING AND DELAYS OSTEOBLAST DIFFERENTIATION AND BONE MASS ACQUISITION

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Bone formation is an important physiological process that controls bone mass acquisition. This process involves the progressive differentiation of osteoprogenitor cells into mature osteoblasts. In this study, we show that the cell–cell adhesion molecule N-cadherin acts as a negative regulator of osteoblast differentiation in vitro and in vivo. A moderate (2-fold) overexpression of N-cadherin (N-Cadh) in murine MC3T3-E1 osteoblasts reduced the expression of the osteoblast differentiation markers Runx2, Osterix and alkaline phosphatase (ALP) and delayed osteogenic capacity. Immunoprecipitation analysis showed that N-Cadh interacts with LRP5. We showed that N-Cadh/LRP5 interaction in osteoblasts reduced basal and Wnt-dependent GSK3 recruitment to LRP5, leading to permanent GSK3 activation. This in turn resulted in increased beta-catenin degradation, decreased beta-catenin nuclear translocation, reduced TCF/LEF transcription and delayed osteoblast differentiation in response to Wnt3a. These effects were reversed after transfection with constitutive active beta-catenin. These results indicate that N-Cadh/LRP5 interaction has a negative impact on osteoblast differentiation by increasing beta-catenin degradation. To assess the functional role of N-Cadh-LRP5 interaction in vivo, we generated transgenic mice that overexpressed N-cadh under the control of an osteoblast-specific Col1a1 promoter fragment. Histomorphometric analysis revealed that N-Cadh transgenic mice showed decreased bone formation activity. The decreased osteoblast activity in N-Cadh transgenic mice resulted in a dose-dependent decrease in trabecular thickness and delayed peak bone mass acquisition, as shown by bone mineral density and microCT analyses. Overall, these data point to a novel function for N-cadherin as a negative regulator of osteoblast differentiation, bone formation and bone mass by antagonizing Wnt signaling through functional interaction with LRP5.

Conflict of Interest: None declared

OC003

MICE LACKING SCLEROSTIN HAVE INCREASED BONE FORMATION AND BONE STRENGTH

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We previously reported a complete lack of sclerostin resulted in increased bone mineral density (BMD) and cortical area at the proximal tibia of SOST knockout (KO) mice. However, it had not been determined whether the increase in bone mass was associated with increased bone formation, or whether lack of sclerostin altered bone strength. Therefore we analyzed femurs from 5 to 6.5-month old SOST KO mice (17 male and 16 female) and wild type (WT) mice (11 male and 11 female) using micro-CT, mechanical testing, and bone histomorphometry (n = 10–12 per group). Micro-CT analysis of the distal femur revealed that SOST KO mice had significant increases in trabecular bone volume fraction (2.5 fold in male and 3.8 fold in female), volumetric BMD (2.5 fold in male and 3.2 fold in female), trabecular number, trabecular thickness and decreased trabecular spacing compared with gender-matched WT mice. Lack of sclerostin was also associated with a shift from rod-like to plate-like trabeculae (decreased structural model index) and increased connectivity density at the distal femur ($p < 0.05$ vs WT). SOST KO mice had normal lamellar structure. Female and male SOST KO mice had 3 and 11 fold increase, respectively, in osteoblast surface, while osteoclast surface remain unchanged when compared with WT mice. SOST KO mice had significantly increased mineralizing surface (MS/BS, 12 fold in male and 9 fold in female) and bone formation rate (BFR/BS, 12 fold in male and 10 fold in female) compared with gender-matched WT mice. Micro-CT analysis of the femur diaphysis revealed that SOST KO mice had significantly greater cortical area (2 fold) associated with both a significant increase in periosteal perimeter and a decrease in endocortical perimeter compared with WT mice ($p < 0.05$). These geometry changes were associated with 2–3 fold increases in maximum load, stiffness, and energy to failure at the femur midshaft ($p < 0.05$ vs. WT). SOST KO mice had significant increases in MS/BS and BFR/BS at endocortical surface compared with the gender-matched WT mice. SOST KO mice also had increases in MS/BS and BFR/BS at the periosteal surface compared with WT mice. The results indicated that the lack of sclerostin in KO mice increased cortical bone strength and bone formation at both the periosteal and endocortical surfaces. In conclusion, lack of sclerostin increased bone formation, improved trabecular architecture and increased cortical bone strength regardless of gender in SOST KO mice.

Conflict of Interest: Employees of Amgen Inc.

OC004

SKELETAL GROWTH DEFICIT AS RESULT OF ANTAGONISM BETWEEN HOXA2 EXPRESSION AND CHONDROCYTE DIFFERENTIATION: MORPHOLOGICAL AND DENSITOMETRIC STUDY

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Background: Hox proteins provide patterning cues to determine embryonic axial structures like the neural tube, neural crest derivatives or the somites. In contrast, Hox gene expression fades out once differentiation programmes are initiated. By generating an inducible transgene allowing Cre-mediated ectopic and heterochronic Hoxa2 expression in mouse, we recently showed that Hoxa2 induction at the precise onset of chondrocyte differentiation impairs overall chondrogenesis and gives rise to chondrodysplasic phenotype as well as delayed cartilage hypertrophy, mineralization and ossification. Aim: The present study assessed the overall bone growth defect and the vertebral bone mineral density of transgenic mice for ectopic and heterochronic induction of Hoxa2 in collagen IIa1 expressing cells. Materials and methods: 20 double transgenic mice, 10 wildtype littermates and 10 single transgenics were under study. Vertex–sacrum distance was measured in all mice from birth to death. Under anaesthesia, neonate mice were scanned in the sagittal plane with peripheral Quantitative Computed Tomography (pQCT) in order to measure density and height of vertebral bodies. They were scanned again after death. Statistical analysis of the data was performed with two-tailed t tests and differences were considered significant at $p < 0.05$. Results: Growth delay of the double transgenic mice, as measured by vertex–sacrum distance, appeared maximal from the 5th postnatal day and was accounted for by the reduced height of their vertebral bodies, as compared with the control littermates. Bone mineral density of the vertebral bodies was not significantly different between the transgenic and control littermates. Conclusions: Skeletal growth delay induced by ectopic and heterochronic expression of Hoxa2 is mainly due to chondrogenic lineage impairment, leading to delayed but qualitatively normal ossification. Further studies are performed in order to identify the molecular pathways involved in the negative interaction between Hoxa2 and chondrocyte differentiation.

Conflict of Interest: None declared

OC005

DLX5 REGULATES POSITIVELY OSTEOGENESIS DURING EMBRYONIC DEVELOPMENT

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Dlx5 is an homeodomain transcriptional factor expressed during skeletal development. Targeted inactivation of Dlx5 in the mouse results in reduced bone formation at birth suggesting an important role in the control of osteogenesis. However, so far, it has been difficult to elucidate the role of Dlx5 in bone formation as Dlx5-null mice exhibit perinatal lethality. In this study we examine the developmental defects in long bone formation of Dlx5-null embryos and we provide cellular and molecular evidence supporting a role of Dlx5 in regulating osteoblast differentiation and bone-related gene transcription.

Femora of 18.5 dpc embryos were used for histomorphometric analysis. The study of osteoblastic differentiation and molecular analyses were performed on calvaria-derived primary osteoblasts culture of the same embryos.

β-gal staining of sagittal sections of femurs confirmed that Dlx5 is expressed in proliferating and pre-hypertrophic chondrocytes of the growth plate and at higher level in periosteal and trabecular osteoblasts. Histomorphometry revealed a significant reduction of the total bone volume measured in between the two chondro-osseous junctions of femurs, of Dlx5-null mice compared to wild-type. To standardise our measurements for the quantification of other bone parameters, a 0.06 mm^2 area situated at 100 μm from the growth plate was analysed. Comparing Dlx5-null to wild-type mice, a significant decrease in the trabecular bone volume BV/TV and trabecular number (Tb.N) associated with a significant increase in the trabecular separation (Tb.Sp) was noted. In primary cultures, no major change in Dlx5 expression was observed during osteoblastic differentiation at day 7 and 14. Analysis of embryonic osteoblasts revealed a significant reduction in proliferation, alkaline phosphatase activity and mineralized nodules formation in the absence of Dlx5. Real-time PCR quantification showed a significant decrease in the expression of alkaline phosphatase, osteocalcin, bone sialoprotein and Runx2 in cultures of Dlx5-null osteoblasts.

This work is the first detailed description of the bone phenotype of Dlx5-null mice and presents evidence suggesting a role for Dlx5 in positively regulating osteoblast proliferation and differentiation. Finally, we demonstrate that Dlx5 is essential for the activation of expression of bone-specific genes including the key transcription factor Runx2 and thus is indispensable in the regulatory system that mediates bone formation.

Conflict of Interest: None declared

OC006

GENETIC VARIATION IN APOLIPOPROTEIN E IS RELATED TO HIP FRACTURE RISK. 25 YEARS FOLLOW-UP OF 9181 ADULTS FROM THE GENERAL POPULATION

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Background: Apolipoprotein E (ApoE) is a protein involved in lipoprotein metabolism. In target cells such as osteoblasts it induces uptake of the lipid soluble vitamin K, which is important for carboxylation of osteocalcin. Two single nucleotide polymorphisms (SNPs) at codon 112 and 158 give rise to three classic ApoE alleles (e2, e3, e4) and six genotypes producing combinations of three isoforms of the circulating protein (APOE-2, -3 and -4). In some, but not all previous studies, presence of the e4 allele has been associated with increased fracture risk. Additional variation in the gene may contribute to difference in protein function, which has been demonstrated for three 5′ promoter SNPs. We aimed to investigate if genetic variation in the ApoE gene is a marker for risk of hip fracture. Methods: We performed a prospective study of participants in the Copenhagen City Heart Study with 25 years follow-up comprising 9181 Caucasian men and women selected at random to represent the Danish general population. Main outcome measure was first ever hip fracture (n = 265). ApoE genotyping for the classical e-alleles and three additional 5′ SNPs was performed using standard PCR-RFLP analysis and risk was estimated by Cox multivariate regression models. Results: According to genotype, women with ApoE43 (25%) had increased hip fracture risk (RR 1.57, P = 0.009). Analyzing according to presence of the e4 risk allele rendered a similar risk estimate for e4 carriers, as this group mainly consist of ApoE43 women. ApoE44 women did not have increased risk. In men, no association was found. In additional analyses of 5′ SNPs, the −219G > T was associated with hip fracture risk in men only, the T

allele carriers had increased risk of fracture (RR 1.90, P = 0.019). Conclusion: In conclusion, the contribution of genetic variation in the ApoE gene to hip fracture risk varied between genders. The classic e4 allele was a risk marker for hip fracture in women, while in men, a 5′ promoter SNP (-219G > T) was a more useful marker.
Conflict of Interest: None declared

OC007

A RARE HAPLOTYPE IN THE 5′ FLANK OF THE COLIA1 GENE IS ENRICHED IN HIP FRACTURE PATIENTS AND REDUCES BONE STRENGTH INDEPENDENT OF BMD

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Background: Osteoporosis is a common disease with a strong genetic component and the COLIA1 gene is an important candidate for susceptibility. Three single nucleotide polymorphisms (SNP) have been identified in the 5′ flank of the COLIA1 gene (-1997G/T; -1663IndelT and +1245G/T) which have been associated with BMD in various populations. The mechanisms by which these SNP predispose to osteoporosis are unclear however. Methods: We analysed the effects of individual SNP and haplotypes on bone strength by biomechanical testing of bone cores obtained from femoral heads of undergoing surgery for hip fracture. We also analysed the effects of the SNP on gene expression using a combination of promoter–reporter assays and electrophoretic mobility shift assays (EMSA). Haplotypes were inferred from genotype data using the PHASE program.

Results: Biomechanical testing showed that yield strength was strongly associated with all three polymorphisms independently of BMD such that samples obtained from carriers of the -1997 T allele, the -1663del T allele and +1245 T allele all had a 25% reduction in bone strength when compared with non-carriers ($p < 0.001$). Two common haplotypes accounted for 90% of alleles in the samples studied; −1997G / −1663insT / +1245G (G-InsT-G; 76%) and −1997T / −1663delT / +1245T (T-delT-T; 12.2%). Interestingly the T-delT-T haplotype (T-delT-T) was 401 times more frequent in the hip fracture samples analysed than in the general population from the same region (12.2% vs 0.03%; $p < 0.0001$). The T-delT-T haplotype was associated with a 27.5% reduction in bone strength compared with the common haplotype ($p < 0.001$) and this was independent of BMD ($p < 0.001$). Gel shift assays showed that DNA binding to Sp1 was significantly increased in the −1997T, and the +1245T alleles and binding to NMP4 was increased in the −1663delT allele. Reporter assays showed significantly increased rates of transcription in association with the −1663delT allele and the +1245T allele ($p = 0.024$). Conclusion: We conclude that a rare haplotype in the 5′ flank of the COLIA1 gene is markedly increased in hip fracture patients and is associated with a substantial reduction in bone strength independent of BMD. The rare alleles increase DNA–protein binding and increase collagen transcription and presumably adversely influence bone strength by disrupting the normal ratio of type 1 to type 2 collagen in bone.
Conflict of Interest: None declared

OC008

HIGHER PERIMENOPAUSAL BONE MASS AND REDUCED POSTMENOPAUSAL BONE LOSS IN WOMEN CARRYING THE C161T SNP IN THE PPAR GAMMA GENE. THE DANISH OSTEPOROSIS PREVENTION STUDY

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Background: Differentiation of mesenchymal precursor cells involves activation of peroxisome proliferator-activated receptor gamma (PPARgamma) when switching from osteoblastic lineage to adipocytic lineage. PPARgamma is expressed in adipocytes and primary osteoblasts. In some settings, agonists (glitazones) reduce bone formation. By contrast, PPARgamma is inhibited by estradiol, directing the precursor cells towards osteoblastogenesis. Polymorphisms in the PPARgamma gene may be associated with development of osteoporosis by affecting bone formation. A previous Japanese study has suggested association between bone mineral density (BMD) and a C161T silent single nucleotide polymorphism (SNP) in exon 6 of the PPARgamma gene. Methods: In this study, we genotyped 1484 Danish postmenopausal women (mean age (SD) 50.1 (2.9) years) for the C161T SNP. The women participated in a trial investigating the effect of hormone replacement therapy (HRT) on development of osteoporosis. We investigated association between genotype and BMD at baseline and change in BMD during 10 years in untreated women. BMD was measured by dual energy X-ray absorptiometry at the lumbar spine, femoral neck, and total hip using Hologic scanners. Genotyping of the C161T SNP was performed by RFLP analysis. Results: Genotype frequencies were CC 71.1% CT 27.1% and TT1.8%. Body mass index (BMI), fat mass, and lean body mass did not differ by genotype. At baseline spine BMD (age- and BMI-adjusted) was higher in TT women (1.064 g/cm^2 versus 1.028 and 1.011 in CC and CT women, $P = 0.03$ ANCOVA). This contrasts with previous findings in Japanese women, where the T allele was associated with low BMD. BMD at hip sites did not differ by genotype. After 5 and 10 years 765 and 684 HRT-free women remained in the study. After 10 years bone loss at the spine was lower in TT women (0.4 % per year versus 0.7 and 0.9 in CC and CT women, $P = 0.006$). Conclusion: These results could indicate that the PPARgamma C161T polymorphism is a marker for peak bone mass and long-term postmenopausal bone loss.
Conflict of Interest: None declared

OC009

ASSOCIATION BETWEEN BONE TURNOVER MARKERS AND BONE LOSS OVER 5 YEARS IN RANDOMLY SELECTED ELDERLY WOMEN: THE MALMÖ OPRA STUDY

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Background: Bone turnover markers (BTM) can be used to identify individuals with high bone turnover. High turnover may increase the risk for future bone loss and fracture. The aim of this study was to evaluate if measurement of BTMs longitudinally and at multiple occasions could improve the prediction of bone loss. Methods: The Malmö Osteoporosis Prospective Risk Assessment (OPRA) study is a randomly selected population based cohort of elderly women, all 75 years of age at inclusion (n = 1040). Areal BMD (aBMD) at total body and total hip was measured by dual-energy x-ray absorptiometry at baseline and after five years. Seven bone turnover markers (S-CTX, S-TRACP5b, S-OC[1–49], S-TotalOC, S-cOC, S-boneALP, urinary osteocalcin) were measured at baseline and after 1, 3 and 5 years. Women who attended the entire 5-year follow-up and did not take any bone-active medication were included in the analysis (n = 573). Results: The baseline levels of BTMs were weakly correlated to 5-year change in BMD at total body (beta/sd from −0.07 to −0.10, p = 0.02–0.09). The correlation was more pronounced and statistically significant for all BTMs when we used the average of two measurements of each marker (beta/sd from −0.12 to −0.23, $p < 0.01$). Adding a third and a fourth BTM measurement further strengthened the correlation (beta/sd from −0.15 to −0.30, $p < 0.001$). The results were similar for all BTMs except for S-boneALP. Longitudinal changes in BTMs did not correlate to bone loss as strongly as did the average value of multiple measurements of each marker. Women who had constantly high S-CTX values (highest tertile) during the 5-year follow-up period lost significantly more bone at the total body (−2.6%) than women who had constantly low S-CTX (lowest tertile) (–0.2%, $p < 0.0001$). Women with constantly high S-CTX also had a greater bone loss at the hip (−8.3%) than women with constantly low S-CTX (-5.1%, $p = 0.010$). Conclusions: Bone turnover as estimated by BTMs correlates to future decrease in aBMD in randomly selected elderly women without bone-active medication. Multiple serial measurements improve the precision and strengthen the correlation. Women with constantly high bone turnover loose significantly more bone over five years than women with constantly low bone turnover.
Conflict of Interest: None declared

OC010

USE OF DXA-BASED STRUCTURAL ENGINEERING MODELS OF THE PROXIMAL FEMUR TO PREDICT HIP FRACTURE

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Several DXA-based structural engineering models of the proximal femur [1–3] have been developed to estimate stress in the bone due to sideway falls. Their usefulness in predicting hip fracture has not yet been established and we therefore evaluated these models as diagnostic tools for hip fracture.

We re-analysed the hip DXA scans of 204 postmenopausal women using a special version of Hologic's software that produces a pixel-by-pixel BMC map for each scan. Fifty-one of the women (age 58–81 yrs) had sustained a low-trauma hip fracture prior to a DXA scan of the un-fractured hip. For each case, 3 age-, height- and weight-matched controls were identified. Curved-beam (CB) [1], curved composite beam (CCB) [2] and finite element (FE) [3] models were generated from each map and the stress calculated at each pixel. The elastic modulus and yield stress of the models were determined according to Keyak [4]. Index of fracture risk (IFR) was defined at each pixel as the stress divided by the yield stress. The maximum and mean IFR in the femoral neck (FN), narrowest

FN (NFN), middle intertrochanter (MI), intertrochanter and total hip (TH) were calculated. The usual hip structure analysis (HSA) parameters were also calculated for the NFN and MI.

We performed forward logistic regression followed by ROC analysis, expressed as area under the curve (AUC), to identify the best discriminators between cases and controls. Three HSA and one IFR parameters were identified and they were TH BMD, MI cortical thickness (CTH), FN axial length (AL) and mean IFR (avgIFR) over MI from the FE models. Specificity for hip fracture was consistently high (93–94%). Sensitivity and AUC were 33% and 0.778 respectively when using TH BMD alone as predictor. Following the sequential addition of MI CTH, FN AL and MI avgIFR, sensitivity increased to 45%, 57% and 61%, whereas the AUC increased to 0.848, 0.877 and 0.885. The increases in AUC were significant at $p < 0.005$ when compared with TH BMD alone.

In conclusion, this study suggests that HSA and FE models, derived from BMC maps, can enhance hip fracture discrimination over TH BMD alone.

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Conflict of Interest: None declared

OC011

DELETION OF THE GHRELIN RECEPTOR GHSR CORRECTS THE TRABECULAR, BUT NOT THE CORTICAL BONE CHANGES IN THE FEMORAL HEAD OF OB/OB MICE

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Background: There exists an intriguing and complex relationship between fat and bone cells with respect to aging and osteoporosis, which is mediated in part by leptin. Genetically obese mice (*ob*/*ob*), that lack leptin, have a heterogeneous bone phenotype, with differential effects on cortical and trabecular compartments. Besides its role in bone metabolism, leptin is most well known for its anorexigenic properties. Opposed in action to leptin is ghrelin, a potent orexigenic peptide hormone derived from the stomach. Ghrelin and leptin also act as each other's antagonists in gonadal and immune system function.

Objective: To determine if ghrelin opposes leptin action on bone metabolism.

Methods: Characterization of femoral micro-architecture in 6 months old male wild type, *ob*/*ob*, ghrelin receptor knockout ($Ghsr^{-/-}$), and *ob*/*ob*.$Ghsr^{-/-}$ mice using micro-computed tomography.

Results: Deletion of *Ghsr* alone did not significantly alter bone micro-architecture in wild type mice. Deletion of leptin reduced cortical volume (Ct.V: 3.03 vs. 3.95 mm^3, $p < 0.001$) and thickness (Ct.Th: 148.05 vs. 210.49 mum, $p < 0.001$) in the femoral head of wild type mice, while it increased endocortical volume (Ec.V: 5.18 vs. 4.42 mm^3, $p < 0.05$). Tissue volume (Ct.V + Ec.V) remained unaffected (TV: 8.21 vs. 8.37 mm^3, NS). Conversely, deletion of leptin increased trabecular bone volume (Tb.V: 0.94 vs. 0.73 mm^3, $p < 0.01$), trabecular number (Tb.N: 0.52 vs. 0.36 /mm, $p < 0.001$) and connectivity (628 vs. 411, $p = 0.08$) in wild type mice. Additional deletion of *Ghsr* in *ob*/*ob* mice restored the changes to wild type levels in trabecular bone (Tb.V: 0.71 mm^3, $p < 0.001$; Tb.N: 0.41 /mm, $p < 0.01$; connectivity: 345, $p < 0.01$), but not in cortical bone (all not significant).

Conclusion: We found that leptin deficiency has a negative effect on cortical and a positive effect on trabecular bone micro-architecture, confirming the heterogeneous skeletal effects observed by others in *ob*/*ob* mice. Knocking out ghrelin signaling compensates for the effect of leptin deficiency on trabecular bone. These observations demonstrate the positive activity of ghrelin signaling in bone, and suggest that ghrelin and leptin have opposing actions on bone metabolism.

Conflict of Interest: None declared

OC012

THE SEROTONINERGIC RECEPTOR 5-HT2B REGULATES OSTEOBLAST FUNCTION AND BONE MASS IN AGEING MICE

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The monoamine serotonin (5-hydroxytryptamine, 5-HT) has been mainly investigated as a neurotransmitter. However, recent studies suggest that serotonin might play a role in bone metabolism. In order to assess action of 5-HT on bone we first searched for the 5-HT receptors on osteoblast. 5-HT1A, 5-HT2A and 5-HT2B receptors were present on murine calvaria osteoblast but we observed an increase in the number of receptors across differentiation only for the 5-HT2B receptor. Therefore, because 5-HT2B receptor could be important for osteoblast function we investigated the bone phenotype of the 5 -HT2B receptor knock-out (5-HT2B−/−) mice that has been previously generated (Nebigil et al. PNAS 2000). Compared to wild type (WT) mice, the absence of 5-HT2B receptor leads to osteopenia that was significant from of the age of 4 months and increased by 12 and 18 months (femoral BMD at 18 months : 0.085 ± 0.001 g/cm^2 in WT vs 0.077 ± 0.002 g/cm^2 in 5-HT2B−/−, $p = 0.01$). Histomorphometry at the femoral metaphysis was performed in 4- and 18-month-old mice and showed, at both time points, a decreased trabecular bone volume (61% at 4 months and 55% at 18 months; $p < 0.001$) and trabecular thickness ($p < 0.01$ at 18 months). Furthermore, cortical thickness were decreased in 5-HT2B−/− compared to WT mice (4 months, $p < 0.0444$ and 18 months, $p < 0.0001$). Modifications of bone microarchitecture was related to alteration in bone formation in 5-HT2B−/− mice compared to WT : bone formation rate was 31% lower ($p < 0.002$) due to decrease in mineralizing surfaces (33%, $p < 0.002$). Moreover, compared to WT mice, both serum alkaline phosphatase (121 ± 39 vs 54 ± 25 UI/l, $p < 0.01$) and serum osteocalcin (135 ± 27 vs 98 ± 18 ng/ml, $p < 0.01$) were decreased in 5-HT2B−/− at 18 months. To further investigate this formation defect, ex vivo cultures of calvarial osteoblast was performed and showed a decrease in proliferation, differentiation and mineralization in osteoblast from 5-HT2B−/− compared to wild-type. Finally, we investigated the CFU capacity of marrow precursors from 12 months old mice. Osteoblast CFU number was decreased in 5-HT2B−/− ($p < 0.001$). In conclusion, we show that the 5HTB2 receptor is important for recruitment and proliferation of osteoblast in adult mice indicating that the 5-HT2B receptor acts as a physiologic regulator of bone remodelling. Furthermore, serotonin is an important player in the regulation of bone mass in ageing mice.

Conflict of Interest: None declared

OC013

USE OF SELECTIVE SEROTONIN REUPTAKE INHIBITORS AND RISK OF HIP/FEMUR FRACTURES: A POPULATION-BASED CASE–CONTROL STUDY

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Background: Selective serotonin reuptake inhibitors (SSRIs) have been previously associated with increased risk of falling and hip/femur fracture. Recent findings have shown that serotonin transporter inhibiting properties may decrease bone mineral density. However, cumulative SSRI exposure, serotonin transporter inhibiting properties and hip/femur fracture risk have not been studied yet.

Aim: To evaluate the association between SSRI use and hip/femur fracture risk.

Methods: We conducted a case–control study using PHARMO (n = 6,763). PHARMO RLS includes a virtually complete medication history of more than two million community-dwelling residents in the Netherlands from 1985 onwards, further linked to hospital admission records. This study used drug dispensing data and hospitalization data. Cases were defined as adult patients with a first admission for hip or femur fracture (index date) between January 1, 1991 and December 31, 2002. Up to 4 control patients were matched to each case by year of birth, gender and region and were assigned the same index date. Our analyses were adjusted for 24 general risk factors for fractures and exposure to other antidepressants. Smoothing spline plots were used to visualize the association between timing and continuous duration and risk of hip/femur fracture.

Results: Current users ($> = 1$ dispensings within 30 days before the index date) of SSRIs had a significantly increased risk of hip/femur fracture compared to never users (adjusted (adj.) OR 2.32; 95% CI, 1.91–2.81) (Figure 1). A rapid fall in fracture risk occurred after SSRI discontinuation for > 3 months: adj. OR 1.25 (95% CI 1.08–1.44). Among current users, the highest risk estimates were found in patients with a cumulative SSRI exposure of $> = 3.6$ g paroxetine equivalents. (adj. OR 2.48; 95% CI, 1.94–3.16). High degrees of serotonin transporter inhibition properties were associated with the highest increase of hip/femur fracture risk.

Conclusions: Current use of SSRIs was associated with increased risk of hip/femur fracture, particularly after intake of more than 3.6 g paroxetine equivalents. The degree of inhibition of the serotonin transporter was associated with an increased risk of hip/femur fracture. Our results support the new hypothesis that SSRIs may increase risk of hip/femur fracture by neural regulation of bone

mineral density. Hip/femur fracture risk assessment may be considered for elderly taking SSRIs.
Conflict of Interest: None declared

OC014

β-ARRESTIN2 INHIBITS PTH-STIMULATED OSTEOCLASTOGENESIS AND CORTICAL BONE REMODELING IN RESPONSE TO LOW CALCIUM DIET

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β-arrestin2 (βarr2) is a cytoplasmic molecule expressed in osteoblasts that regulates PTH-stimulated intracellular signaling. βarr2 KO mice have low bone mass and a negative mineral balance in trabecular bone in response to intermittent PTH. We hypothesized that βarr2 influences osteoclastogenesis and thereby the changes in bone microarchitecture induced by a low calcium diet. Adult βarr2 KO and WT mice were fed either a low calcium (LCa, 0.02% Ca, 0.4% Pi) or regular diet (RCa 0.6% Ca, 0.4% Pi) for 4 wks (n = 7–9/gr.). Compared to RCa, LCa significantly decreased total body BMD, trabecular and cortical bone microarchitecture, as evaluated by pDXA and micro-CT, respectively. In general, the deleterious effects of LCa on cancellous bone were similar in WT and KO mice, whereas mid-femoral cortical bone was more severely affected in KO: Bone area (KO −14%, $p < 0.05$ vs RCa, WT −8%, ns), BA/TA (KO −10%, $p < 0.01$, WT −2%, ns), CortTh (KO −13%, $p = 0.06$, WT −2%, ns). LCa also increased sTRACP5b significantly more in KO (+41% vs baseline) than WT mice (+5%, $p < 0.01$ vs KO). Thus, βarr2 restrains bone resorption and cortical bone remodeling induced by secondary hyperparathyroidism. To investigate the arrestin-mediated mechanisms of bone resorption, OPG and RANKL mRNA expression were analyzed by qRT-PCR in femurs of WT and βarr2 KO mice receiving PTH (80 μg/d) continuously (cPTH) or vehicle (VEH) for 7d (n = 3/gr). In cortical bone, RANKL/OPG ratio was higher in cPTH KO (3.4) compared to either VEH KO (2.4) or cPTH WT (2.7). In contrast, in trabecular bone, RANKL/OPG was slightly higher in cPTH KO than VEH KO, but not different from cPTH WT. In primary bone marrow cultures from WT and KO treated 7d with PTH (1–100nM) the RANKL/OPG protein ratio was increased nearly 6-fold in the conditioned medium from KO compared to WT mice. Moreover, the PTH dose-dependent increase in TRAcP+ multinucleated cells and resorption index (on bone slices) was significantly greater in KO than WT cultures. An excess of OPG inhibited PTH-induced osteoclastogenesis, confirming the relevance of this pathway in the βarr2 null phenotype(s). In contrast, increasing concentrations of RANKL stimulated osteoclastogenesis similarly in WT and KO cultures, suggesting that RANK signals in osteoclasts are βarr2-independent. In conclusion, βarr2 inhibits osteoclastogenesis and cortical bone remodelling in response to continuous PTH by regulating RANKL/OPG expression.
Conflict of Interest: None declared

OC015

TRANSGENIC DISRUPTION OF GLUCOCORTICOID SIGNALLING IN MATURE OSTEOBLASTS ATTENUATES K-RN SERUM-INDUCED ARTHRITIS IN VIVO

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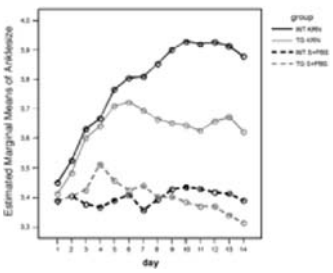
Background/Aims: Transgenic overexpression of 11beta-hydroxysteroid dehydrogenase type 2 (HSD2), a glucocorticoid (GC) inactivating enzyme under the control of a 2.3Kb collagen type I promoter (Col2.3-HSD2), abrogates intracellular GC signalling exclusively in mature osteoblasts. Since GC are important immune modulators, we investigated the impact of osteoblast-targeted disruption of GC signalling on joint inflammation and bone catabolism using the KRN serum transfer model of autoimmune arthritis.

Methods: KRN arthritis was induced in 5-week-old male Col2.3-HSD2-transgenic (tg) mice (n = 28) and their wild-type (WT) littermates (n = 27). Twelve tg and 13 WT mice served as controls receiving either normal serum or PBS. Body weight and paw swelling (ankle size, clinical arthritis scored 0–6)were assessed daily from day 0 (induction) to day 14. Serum cytokine levels (IL-1beta, IL-2, IL-4, IL-6, IL-10, IL-12, TNF-alpha, IFN-gamma, G-CSF, and M-CSF) were determined using a multiplex ELISA system (BioRad) at days 7 and 14.

Results: Both tg and WT animals developed acute arthritis. However, the inflammatory response was significantly blunted in tg mice from day 7 onwards (fig. 1). Clinical score and changes in body weight gain were closely correlated with changes in ankle size. In both tg and WT mice, mean serum TNF-alpha, M-CSF IL-6, IL-12 and G-CSF levels were significantly lower on day 14 when compared to day 7 (IL-1beta, IL-2, IL-4, IL-10 and IFN-gamma were not detectable by ELISA in any of the animals). However, there was no difference in cytokine levels between tg and WT mice at any time point, suggesting that the less severe inflammatory arthritis in tg mice may be mediated by local regulation.

Conclusions: Mature osteoblasts appear to modulate inflammatory response via a glucocorticoid dependent pathway.



Conflict of Interest: None declared

OC016

THE BINDING BETWEEN SCLEROSTIN AND LRP5 IS DRASTICALLY IMPAIRED BOTH IN HIGH-BONE-MASS LRP5 MUTANTS AND IN THE PRESENCE OF DKK1

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The low density lipoprotein receptor-related protein 5 (LRP5) acts as a co-receptor in canonical Wnt signaling, an important signaling pathway involved in a wide range of cellular and physiological processes. This pathway has been shown to play a role in bone metabolism as loss-of-function and gain-of-function mutations in LRP5 result in respectively autosomal recessive osteoporosis pseudoglioma syndrome and autosomal dominant high-bone-mass (HBM) phenotypes. Previous studies demonstrated that activating mutations associated with HBM phenotypes cause reduced binding affinity for DKK1, consequently leading to impaired inhibition of canonical Wnt signaling.

The aim of our study was to further delineate the mechanisms by which these HBM LRP5 mutations alter Wnt signaling. For this, we studied the effect of six HBM-LRP5 missense variants on sclerostin binding and antagonism of canonical Wnt signaling. Previously, it has been shown that sclerostin binds to the first and/or second β-propeller domains of LRP5, and interestingly, all HBM-associated LRP5 mutations are located in the first β-propeller domain. Furthermore, we evaluated whether the presence of DKK1 affects sclerostin binding to LRP5.

Our data showed that all HBM-LRP5 mutants were able to transduce Wnt1 signals at equal or slightly increased levels compared to wild type LRP5. Conversely, our data suggest that modulation of canonical Wnt signaling is impaired. We were able to confirm that a decreased inhibition by DKK1 contributes to the molecular mechanisms of the LRP5 mutations. We additionally observed an impaired physical binding of sclerostin to all HBM-LRP5 mutants and subsequently a reduced inhibition of canonical Wnt signaling by sclerostin. Furthermore, we provided evidence that sclerostin and DKK1 do not physically interact. Interestingly, our results also indicate that DKK1 prevents sclerostin to act as an inhibitor in LRP5-mediated Wnt signaling.

To conclude, our data suggest that impaired antagonism of both sclerostin and DKK1 contributes to the aetiology of the craniotubular hyperostoses. Understanding the molecular mechanisms by which HBM-LRP5 variants potentiate canonical Wnt signaling and increase osteoblastic bone formation will provide further insights into the control of bone mass regulation and may result in novel treatment targets for osteoporosis.
Conflict of Interest: None declared

OC017

IDENTIFICATION OF NOVEL PHOSPHOPROTEINS ON PLASMA MEMBRANES OF HUMAN MESENCHYMAL STEM CELLS USING TITANIUM DIOXIDE CHROMATOGRAPHY AND MASS SPECTROMETRY

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The molecular mechanism controlling differentiation of human mesenchymal stem cells (hMSC) are not known in details. Phosphorylation of plasma membrane (PM) proteins is often the initiating step in signal transduction processes. Thus, we characterized the phosphoproteins in plasma membranes of human mesenchymal stem cells (hMSC) in undifferentiated and differentiated state in order to understand how the cells coordinate responses to signals initiated by extracellular microenvironment to determine a lineage-specific differentiation.

We developed a highly selective mass spectrometry based strategy for the identification of phosphoproteins from highly complex biological samples using titanium dioxide (TiO2) micro-columns and Liquid chromatography Electrospray Ionization tandem Mass Spectrometry (LC-ESI-MS/MS). By combining this enrichment strategy with an efficient plasma membrane purification protocol we were able to identify > 750 phosphopeptides in 376 unique phosphoproteins from approximately 100 μg purified hMSC plasma membranes. This is to our knowledge the highest number of identified phosphopeptides from such low amount of starting material, illustrating the strength of our strategy. A significant number of the identified phosphorylation sites have never been described in the literature before. Bioinformatic analysis employing the Protein Center software (Proxeon Bioinformatics, Odense, Denmark) showed that more than 60% of the identified proteins were membrane proteins and ~75% were related to the membrane.

Many stem cell markers were identified in addition to other proteins of highly biological significance. Six phosphorylation sites were identified in the human Epidermal growth factor receptor, whereas 14 phosphorylation sites were found in the human Ephrin type-A receptor 2 (Tyrosine-protein kinase receptor ECK) of which only seven were reported in the UniProt Database.

Different phosphatase inhibitors were tested for the efficiency of preserving the phosphorylations present at the time for harvesting the cells. The use of these inhibitors led to improved phosphopeptide recovery compared to the control and a specific tyrosine phosphatase inhibitor (Na-Pervanadate) proved to be necessary for the identification of tyrosine phosphopeptides. More than 100 tyrosine phosphorylation sites were identified from the plasma membrane fractions after Na-pervanadate treatment, whereas only 10 sites were identified in the control sample.

Conflict of Interest: None declared

OC018

BIPHENYLCARBOXYLATES INHIBIT BONE RESORPTION IN VITRO AND OVARIECTOMY INDUCED BONE LOSS IN VIVO WITHOUT IMPAIRING PTH INDUCED BONE FORMATION

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Many diseases of the skeleton such as osteoporosis and Paget's disease, are characterised by excessive bone loss due to increased osteoclastic bone resorption. Whilst several inhibitors of osteoclastic bone resorption are available clinically, the most successful anti-resorptive agents, Bisphosphonates (BP) also inhibit bone formation and blunt the anabolic effect of PTH. Here we describe a novel series of orally active biphenylcarboxylate derivatives which have potent anti-resorptive effects, but do not adversely affect osteoblast function or inhibit the anabolic effects of PTH. We have recently shown that ABD56, the butanediol ester of biphenylcarboxylic acid, is a potent inhibitor of osteoclast formation *in vitro* and ovariectomy (Ovx) induced bone loss *in vivo*. However, as ABD56 is an ester, it is susceptible to rapid degradation by acid and esterases, making it unsuitable for oral delivery. We therefore developed derivatives where the ester linkage is replaced with a ketone or reduced ketone. The most potent osteoclast inhibitors identified were the halogen substituted biphenylcarboxylate ketone derivatives ABD328 and ABD350, which inhibited osteoclast formation in RANKL-stimulated bone marrow cultures with an IC50 of 0.88 μM and 2.3 μM respectively. All derivatives promoted osteoclast apoptosis and prevented RANKL-induced IκB phosphorylation. We observed no effect on osteoblast growth, alkaline phosphatase activity, collagen or osteocalcin production, or PTH-induced activation of Raf, MEK1/2 and ERK MAPK, at concentrations of up to 20 μM. In contrast to the BP Alendronate and Risedronate, ABD328 did not inhibit bone nodule formation at concentrations of up to 2 μM. ABD328 fully prevented Ovx-induced bone loss in mice when given intraperitoneally (5mg/kg/day) and orally at 20mg/kg/day. Treatment of the mice with PTH 1–34 (80 μg/kg/day) lead to an 18 ± 5% increase in bone volume and trabecular and cortical thickness as assessed by μCT. ABD350 also prevented Ovx-induced bone loss when given intraperitoneally at 5mg/kg/day and had no inhibitory effect on PTH induced bone formation at this dose. Biphenylcarboxylates inhibit osteoclast formation and promote osteoclast apoptosis, but have no significant inhibitory effects on osteoblast activity *in vitro* or PTH-induced bone formation *in vivo*. Because of their lack of effect on the anabolic properties of PTH, these agents may be of therapeutic value in combination treatment with PTH for osteoporosis.

Conflict of Interest: None declared

OC019

OSTEOCYTES SUBJECTED TO PULSATING FLUID FLOW INHIBIT OSTEOCLAST FORMATION AND BONE RESORPTION

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Osteocytes are the predominant bone cells and likely the professional mechanosensors of bone. A strain-derived fluid flow through the lacuno-canalicular porosity seems to mechanically activate them, resulting in the production of signalling molecules such as nitric oxide (NO). We investigated whether osteocytes, osteoblasts, and periosteal fibroblasts subjected to pulsating fluid flow (PFF) modulate the formation and activity of osteoclasts via soluble factors, thus affecting bone resorption. Osteocytes, osteoblasts, and periosteal fibroblasts were isolated from fetal chicken calvaria via enzymatic digestion. Separation of the periostea from calvaria occurred to obtain fibroblasts. The osteocyte specific Mab 7.3 was used to separate osteocytes from osteoblasts by immunomagnetic separation. Cells were treated for 1 h with a PFF (0.70 ± 0.30 Pa, 5 Hz) or were kept under static conditions and the conditioned medium was collected. Bone marrow cells from five-week-old male mice were cultured in the presence of RANKL and M-CSF, and conditioned medium was added (1: 1, vol: vol). After 6 days of culture, the number of TRAP-positive multinucleated cells was counted and the percentage of bone resorption was calculated. We found that the formation of osteoclasts was inhibited by conditioned medium obtained from osteocytes that were subjected to 1 h PFF. For osteoblast PFF conditioned medium such effect was, to a lesser extent, also observed, but not for periosteal fibroblast PFF conditioned medium. Furthermore, in line with the decreased number of osteoclasts, PFF conditioned medium of osteocytes, but not of osteoblasts or periosteal fibroblasts, resulted in a decreased bone resorption. The NO synthase inhibitor NG-Nitro-L-arginine methyl ester (L-NAME) attenuated the inhibitory effects of osteocyte PFF conditioned medium on osteoclast formation and resorption, suggesting that a change in NO release is at least partially responsible for the inhibitory effects of osteocyte PFF conditioned medium. We conclude that osteocytes subjected to PFF inhibit osteoclast formation and resorption via soluble factors, and the release of these factors was at least partially dependent on activation of an NO-mediated pathway in response to PFF. Thus, the osteocyte appears to be more responsive to PFF than the osteoblast or periosteal fibroblast in producing soluble factors that affect osteoclast formation and bone resorption.

Conflict of Interest: None declared

OC020

OSTEOCYTES AND OSTEOBLASTS EXHIBIT DIFFERENT GENE EXPRESSION OF PROTEINS INVOLVED IN CANONICAL AND NON-CANONICAL WNT SIGNALING PATHWAYS AFTER MECHANICAL LOADING

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Osteocytes are the professional mechanosensory cells of bone. Strain-derived flow of interstitial fluid through the lacunocanalicular network seems to mechanically stimulate osteocytes to activate cellular signaling transduction pathways that regulate bone metabolism [1, 2]. It has been shown that members of the canonical Wnt pathway, i.e. β-catenin and lipoprotein receptor-related protein 5 (LRP5), are involved in the regulation of mechano-regulated bone adaptation [3]. However, whether the non-canonical pathway also plays a role in this process is still unknown. The aim of our study was to assess if mechanical loading by pulsating fluid flow affects gene expression of proteins involved in canonical as well as non-canonical Wnt signaling pathways in cultured osteocytes and osteoblasts.

MLO-Y4 osteocytes and MC3T3-E1 osteoblasts were submitted to 1 h of pulsating fluid flow (PFF, 0.7± 0.3 Pa, 5 Hz) in a laminar flow chamber, and post-incubated without PFF for 0.5 to 3 h. Gene expression of proteins related to the Wnt canonical pathway (Wnt3, LRP5, LRP6, β-catenin, APC) and the canonical Wnt signaling target gene c-jun, and to the non-canonical pathway (Fzd6, Wnt5, Sfrp4) was studied using RT-PCR. Statistical analysis was performed using Student t-test. Differences were considered significant if $p < 0.05$.

In osteocytes 1 h PFF upregulated gene expression at 1 to 3 h after PFF treatment of Sfrp4, Wnt3, β-catenin, APC, and c-jun, and down-regulated the expression of LRP5 and Fdz6. PFF did not affect gene expression of LRP6.

Wnt 5 was detectable but not quantifiable. In osteoblasts 1 h PFF down-regulated expression of Wnt5, LRP6, β-catenin, APC, and c-jun at 0.5 to 3 h after PFF treatment. There was no effect on gene expression of SFRP4, LRP5 and Fzd6.

This is the first study showing that mechanical loading leads to differential gene expression of proteins related to different Wnt signaling pathways by osteocytes and osteoblasts. The canonical Wnt signaling pathway is likely involved in mechanosensing by osteocytes and osteoblasts, but the non-canonical pathway might also play a role in osteoblasts.

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Conflict of Interest: None declared

OC021

CLINICAL RISK FACTORS ENHANCE THE PERFORMANCE OF BMD IN THE PREDICTION OF FRACTURES

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The aim of this study was to evaluate a risk assessment tool based on clinical risk factors with and without BMD. A total of 46,340 men and women from nine population-based studies were studied in whom BMD and clinical risk factors were documented at baseline. The clinical risk factors, identified from previously published meta-analyses, comprised body mass index (as a continuous variable), a prior history of fracture, a parental history of hip fracture, use of oral glucocorticoids, rheumatoid arthritis, current smoking and alcohol intake of 3 or more units daily. Separate models using Poisson regression were developed for hip fracture and other osteoporotic fractures, with and without hip BMD. Fracture risk was expressed as gradient of risk (GR, the risk ratio/SD change in risk score). For hip fracture, clinical risk factors alone predicted fracture with a GR of 2.1/SD at the age of 50 years (see table below). This GR decreased with age; being 1.8/SD at 70 years and 1.7/SD at 90 years. The use of BMD alone provided a higher GR for hip fracture at all ages and at the age of 70 years was 2.8/SD. Hip fracture prediction was improved somewhat further with the combined use of clinical risk factors and BMD, an effect more marked at younger ages, so that GR was 4.2/SD at 50 years decreasing to 2.9/SD at 70 years . For other osteoporotic fractures, the GRs were lower than for hip fracture. For example, the GR with the use of clinical risk factors alone was 1.6/SD at the age of 70 years. Fracture prediction was similar to that provided by BMD alone (GR = 1.4/SD) and was not markedly increased by the combination (GR = 1.6/SD). The combined use of clinical risk factors and BMD improves the GR and therefore the sensitivity (i.e. detection rate) of fracture risk assessment. The models developed will provide the basis for the integrated use of validated clinical risk factors in men and women to aid in fracture risk prediction.

Table:

Age (years)	BMD only	Clinical risk factors alone	Clinical risk factors + BMD
(a) hip fracture			
50	3.7 (2.7–5.2)	2.1 (1.6–2.7)	4.2 (3.1–5.7)
70	2.8 (2.4–3.2)	1.8 (1.7–2.1)	2.9 (2.6–3.4)
90	1.7 (1.5–1.9)	1.7 (1.5–1.9)	2.0 (1.7–2.4)
(b) Other op fract			
50	1.2 (1.1–1.3)	1.4 (1.3–1.6)	1.4 (1.3–1.6)
70	1.4 (1.3–1.5)	1.6 (1.5–1.6)	1.6 (1.5–1.7)
90	1.6 (1.4–1.8)	1.8 (1.7–2.0)	1.8 (1.7–2.0)

Conflict of Interest: None declared

OC022

EFFECT OF ONCE-YEARLY INFUSION OF ZOLEDRONIC ACID 5 MG ON BIOCHEMICAL MARKERS OF BONE TURNOVER: DATA FROM HORIZON-PFT

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Intravenous zoledronic acid (ZOL), given as a single infusion, has been shown to decrease bone turnover and improve bone density. We evaluated the impact of once-yearly infusions of ZOL 5 mg on biochemical markers of bone turnover in a subset of patients in selected centers in the HORIZON-PFT trial, a multinational, 3-year, randomized, double-blind, placebo-controlled clinical trial involving over 7000 postmenopausal women with osteoporosis. In the ZOL 5 mg group (n = 257), the geometric means for β-C-terminal telopeptide of type I collagen (β-CTX), a marker of bone resorption corresponded with 74%, 61%, 57%, and 52% decreases from baseline at 6, 12, 24, and 36 months, respectively. The 61% decrease vs baseline at 12 months is between the 12-month decreases with alendronate (73.8%) and risedronate (54.7%) reported by Rosen et al. (J Bone Miner Res 2005;20: 1). In the placebo group (PBO, n = 260) the geometric mean at 36 months for β-CTX corresponded to a 6% increase from baseline. Serum β-CTX levels were significantly reduced in the ZOL group versus PBO at all postbaseline time points ($P < .0001$). Median serum β-CTX levels were within the premenopausal reference range (0.13–0.54 ng/dL) at the end of each annual dosing cycle. During year 3, serum β-CTX was measured at 9–11 days and at 1, 3, 6, and 12 months postinfusion. At 9–11 days, the median level in the ZOL group was 0.04 ng/mL, rising to 0.13 by 6 months. Markers of bone formation were significantly reduced from baseline with ZOL 5 mg compared to PBO ($P < .001$, all comparisons). For bone-specific alkaline phosphatase (ALP), geometric means at 6, 12, 24, and 36 months corresponded to decreases from baseline of 37%, 30%, 37%, and 31% in the ZOL group. In the PBO group, the geometric mean for bone ALP corresponded to a 6% decrease at month 6; there were no decreases relative to baseline at later timepoints. Similarly, serum N-terminal propeptide of type I collagen (PINP) was decreased in the ZOL group, with geometric means corresponding to decreases from baseline of 61%, 59%, and 53% at 12, 24, and 36 months, respectively. In contrast, geometric means for P1NP in the PBO group corresponded to decreases of 2%, 8%, and 2% at 12, 24, and 36 months. We conclude that once-yearly infusions of ZOL 5 mg resulted in significant reductions in biochemical markers of bone resorption and formation compared to placebo over 3 years. The decrease in β-CTX in the ZOL 5 mg group is comparable to that seen with other bisphosphonates.

Conflict of Interest: P. Delmas, Novartis, Consultant, Speaker D. Bauer, Novartis, Grant/Research support D. Black, Novartis, Grant/Research support S. Boonen, Novartis, Grant/Research support F. Cosman, Novartis, Grant/Research support R. Eastell, Novartis, Grant/Research support, Consultant, Speaker

OC023

STRONTIUM RANELATE DECREASES VERTEBRAL FRACTURE RISK WHATEVER THE LEVEL OF PRETREATMENT BONE TURNOVER MARKERS

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Objective: Pretreatment bone turnover levels may influence antifracture efficacy of anti-osteoporotic treatments. To choose the best treatment for the patient's individual profile, vertebral antifracture efficacy of strontium ranelate was studied according to pretreatment bone turnover markers (BTM) levels.

Materials and methods: Two double-blind placebo-controlled studies were performed in women with postmenopausal osteoporosis. SOTI (N = 1649) focused on vertebral fracture efficacy and TROPOS (N = 5091) on non-vertebral

efficacy, incident vertebral fracture being a prespecified secondary end-point in patients having spine Xrays (N = 3640).

The risk of new vertebral fracture over 3 years was compared between groups in 5082 women with paired spinal Xrays and baseline bALP, or sCTX values, pooled from SOTI and TROPOS and stratified into quartiles (Q) of baseline BTM.

Results: Baseline characteristics were similar between groups (age 74.0 ± 6.2 years; lumbar BMD T-score -3.0 ± 1.6; femoral neck BMD T-score -3.0 ± 0.7).

Over 3 years of treatment, the risk of new vertebral fractures was significantly lower in the strontium ranelate group than in the placebo group regardless of the class of BTM considered. New vertebral fracture risk reduction compared to placebo was 32% (p = 0.040) in the lowest quartile of bALP (bALP ≤ 9.3 ng/mL), 39% (p = 0.001) in Q2 (9.3 ≤ bALP < 11.5 ng/mL), 43% (p < 0.001) in Q3 (11.5 ≤ bALP < 14.5 ng/mL) and 40% (p < 0.001) in Q4 (≥14.5 ng/mL). For sCTX, new vertebral fracture risk reduction compared to placebo was 36% (p = 0.001) in Q1 (sCTX ≤ 2931 pmol/L), 29% (p = 0.018) in Q2 (2931 ≤ sCTX < 4003 pmol/L), 46% (p < 0.001) in Q3 (4003 ≤ sCTX < 5338 pmol/L) and 44% (p < 0.001) in Q4 (≥5338 pmol/L). Treatment effects were not different between the quartiles (interaction test: p = 0.349 for bALP and p = 0.129 for sCTX).

Results were reinforced when analysing patients with both a value of bALP and sCTX in the lowest quartile or in the highest quartile with a risk reduction of having a new fracture compared to placebo of 33% (p = 0.042) and 42% (p = 0.007), respectively.

Conclusion: The efficacy of strontium ranelate to significantly reduce incident vertebral fracture is largely independent of pretreatment bone turnover suggesting that strontium ranelate offers clinical benefits to women across a wide range of metabolic states and disease severity.

Conflict of Interest: None declared

OC024

EFFECTS OF PRIOR ANTIRESORPTIVE THERAPY ON THE BONE MINERAL DENSITY RESPONSE TO TWO YEARS OF TERIPARATIDE TREATMENT IN POSTMENOPAUSAL WOMEN WITH OSTEOPOROSIS: FINAL RESULTS FROM THE EUROFORS TRIAL

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Aim: The objective of this predefined analysis of the Eurofors trial was to investigate the effect of a prior predominant antiresorptive (AR) therapy on the BMD response at the spine and hip in women who continuously received TPTD for 2 years after predominant AR use for at least one year. Predominant AR was defined as an AR taken for > 12 months, with any other AR being taken for < 3 months.

Methods: A subgroup of 245 patients had received a predominant AR (alendronate [ALN], risedronate [RIS], etidronate [ETI], non-bisphosphonate [NBP]) before starting TPTD. The BMD response was examined in multivariate analyses including type and duration of predominant prior AR, lag time between stopping prior AR and starting TPTD, baseline BMD and P1NP values, age, time since menopause and body mass index (BMI).

Results: Mean age (SD) was 69.2 (7.0) years. Median (Q1–Q3) AR therapy duration and lag time were 27.9 (19.8–48.9) months and 28.0 (15.0–43.0) days respectively. Table lists the mean adjusted 2-year BMD changes vs baseline [absolute: g/cm^2 (SD) and group level % change from mean baseline BMD].

All end-point values were statistically different vs. baseline. No pairwise differences between groups were shown at the LS, TH and FN, with the exception of a significantly higher increase in LS BMD in the pre-ETI group (§p < 0.05 vs other groups). Multivariate analysis showed that BMD changes at all sites measured were associated with baseline BMD and PINP levels, and either with age or time since menopause. The previous type and duration of prior AR treatment, lag time between stopping AR medications and start of TPTD, and either baseline BMI or its change over time were not associated with BMD increase.

Conclusion: BMD response at the spine or hip to 2 years of TPTD treatment in prior predominant AR users is influenced by the degree of bone turnover suppression, but not on the type of AR, its previous duration, or the interval between its discontinuation and the onset of TPTD.

Table: Mean 2-year BMD changes vs baseline: g/cm^2 (SD)

Predominant AR subgroup (n)	Lumbar Spine (LS)	Total Hip (TH)	Femoral Neck (FN)
Pre-ALN	+0.062	+0.013	+0.019
(107) (%)	(0.004)	(0.003)	(0.004)
	(+9.3)	(+1.9)	(+3.4)
Pre-RIS	+0.064	+0.016	+0.024
(59) (%)	(0.006)	(0.005)	(0.005)
	(+9.6)	(+2.3)	(+4.1)
Pre-ETI	+0.089	+0.024	+0.021
(30) (%)	(0.009)§	(0.007)	(0.008)
	(+13.3)	(+3.5)	(+3.6)
Pre-NBP	+0.065	+0.013	+0.016
(49)* (%)	(0.007)	(0.006)	(0.006)
	(+9.4)	(+1.8)	(+2.7)

*Raloxifene = 22; HT = 20; Calcitonin = 6; Vit D metab = 1 case

Conflict of Interest: F. Marín, C. Barker, H. Oertel, T. Nickelsen, are full-time employees of Eli Lilly and Company.

OC025

A SOLUBLE ACTIVIN RECEPTOR TYPE IIA (ACTRIIA) ACTS AS A NOVEL BONE ANABOLIC AGENT

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Activin, a member of the TGF-β superfamily, has been described as both a positive and negative regulator of bone metabolism. Evidence exists for a role of Activin as both an inhibitor and promoter of osteoblastic cell activity, and as a co-factor in osteoclast induction. Previously, we showed 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), reversed bone loss in ovariectomized mice (Pearsall et al. J Bone Min. Res. 21(S1) 2006). The aim of this study was to determine whether RAP-011 increases bone mass under basal, non-estrogen deficient, conditions and to define mechanisms involved. To this end, 12-week old C57BL/6 female mice received intraperiotoneal injections of RAP-011 (10 mg/kg) or PBS (VEH) twice weekly and euthanized after 2, 4, 6 and 12 weeks of treatment for assessment of static and dynamic histomorphometry and μCT analysis. Histomorphometry of the distal femur showed that RAP-011 increased trabecular bone volume by 45%, 120%, 130% and 248% vs. VEH at 2, 4, 6 and 12 weeks, respectively (all, p < 0.01). The increased bone volume was secondary to an increase in bone formation, since RAP-011 treated mice exhibited a significant increase in mineralized bone surface (80 to 120 %), mineral apposition rate (14 to 25%) and bone formation rate (115% to 155%) relative to VEH. Osteoblast number/perimeter and osteoblast surface were not increased, indicating an increase in osteoblastic function in RAP-011 treated mice. After two weeks of RAP-011 treatment, osteoclast and eroded surfaces were reduced by 20% and 29% respectively. While this trend is maintained, it is not significantly different at the later time points. Confirming the histomorphometric results, ex vivo μCT of 5th lumbar vertebrae revealed that, compared to VEH, treatment with RAP-011 increased vertebral trabecular bone volume by 8%, 29%, 39% and 51% at 2, 4, 6 and 12 weeks, respectively (all, p < 0.05). The increase was mainly due to increased trabecular number (19 to 48%, p < 0.01), with a slight increase in trabecular thickness (+4 to 6%, p < 0.05). In conclusion, the soluble ActRIIa (RAP-011) has skeletal anabolic activity in intact mice, and stimulates bone formation resulting in an increase in trabecular bone mass.

Conflict of Interest: Scott Pearsall: Employee and Shareholder of Acceleron Pharma.

OC026

LYMPHOCYTIC CELLS, AS THE MYELOMA B-CELL CLONE, CONTRIBUTES TO THE FORMATION OF OSTEOCLASTS IN MYELOMA PATIENTS

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Osteolytic bone destruction is a major clinical manifestation of bone cancers. It is a dogma that osteolysis is caused solely by osteoclasts, multinucleated cells of monocytic origin, indirectly recruited and activated by factors released from the malignant cells. Here, we demonstrate that resorbing osteoclasts of myeloma patients contains a mixture of nuclei either with or without translocated chromosomes of the lymphocytic originating myeloma B-cell clone. These malignant nuclei of lymphocytic origin are transcriptionally active, appear fully intermixed with the other nuclei, and are not associated with CD138-stained plasma membrane remnants of myeloma cells, as would happen if they came from dying myeloma cells phagocytosed by the osteoclasts, as occasionally seen. The contribution of lymphocytic malignant nuclei to the osteoclast nuclei population analyzed in our study, ranged from 33% to 48%, proving that intermixed myeloid and lymphocytic originating hybrid osteoclasts is widely spread in myeloma patients. The hybrid osteoclasts contained more nuclei than normal osteoclasts, and their occurrence correlated with the proximity of myeloma cells. In vitro studies have previously shown that myeloma cells and pro-B cells can differentiate into bone resorbing osteoclast-like cells. We succeeded in generating osteoclast–myeloma clone hybrids through co-cultures. The origins of the respective nuclei were traced analyzing the content of translocations, BrdU, or the Y chromosome of male myeloma cells in female osteoclasts. In conclusion, a substantial number of osteoclasts of myeloma patients are actually osteoclast–myeloma clone hybrids. Osteoclast–myeloma clone hybrids reflect a previously unrecognized mechanism of bone destruction, allowing the direct participation of malignant cells in bone breakdown. It also proves that not only monocytes, but also cells of the lymphocytic lineage can contribute to the generation of osteoclasts. Finally, the possibility that malignant cells corrupt host cells by transfer of malignant DNA may have been underestimated so far in cancer research.

Conflict of Interest: None declared

OC027

MICE WITH A TRUNCATING MUTATION AFFECTING THE UBIQUITIN-ASSOCIATED DOMAIN OF SQSTM1 EXHIBIT FEMORAL SHAFT EXPANSION AND INCREASED OSTEOBLAST ACTIVITY IN VIVO AND ABNORMALITIES OF OSTEOBLAST AND OSTEOCLAST FUNCTION IN VITRO

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Paget's disease of bone (PDB) is a common condition characterized by focal increases in bone turnover, with excessive osteoclastic bone resorption coupled to an increase in new bone formation. Mutations affecting the ubiquitin-associated (UBA) domain of the Sequestosome 1 gene (SQSTM1) have been identified in up to 40% of patients with familial PDB and 10% of patients with sporadic PDB. Here we report upon our preliminary analysis of the skeletal phenotype of mice with a truncating mutation at codon 409 of the SQSTM1 gene which deletes most of the UBA domain. The mutant mice are fertile, and offspring of heterozygote crosses shows the expected Mendelian distribution of genotypes. Radiological screening has shown evidence of femoral expansion in heterozygote and homozygote carriers of the mutation when compared with wild type mice by 8.63 ± 4.31% ($p < 0.0001$) and this was accompanied by a small decrease in femoral length (4.62 ± 5.1%; $p < 0.05$). Initial histological observations of long bones showed highly active and numerous osteoblasts in mutant mice and large areas of osteoid indicating bone formation was not impaired. Transmission electron microscopy showed no obvious abnormalities in osteoclasts or nuclear inclusions. However, studies *in vitro* showed a 8.6 ± 14.4% increase in RANKL-induced osteoclast formation in mice which carry the mutation compared with wild type mice ($p = 0.03$). Surprisingly, studies of cultured calvarial osteoblasts showed reduced levels of alkaline phosphatase activity in cells cultured from mutant mice compared with wild type (−46.5 ± 16.9%; $p < 0.001$), although there was no difference in growth between mutant and wild type cell cultures. In conclusion, mice carrying a truncating mutation of the SQSTM1 gene exhibit femoral expansion, and increased osteoblast activity *in vivo* as well as abnormalities of osteoclast and osteoblast activity *in vitro*. These data confirm that the SQSTM1 UBA domain regulates bone cell activity *in vitro* and *in vivo*, although further studies are in progress to conduct more detailed characterisation of the skeletal phenotype and define the mechanisms by which this mutation regulates bone cell activity.

Conflict of Interest: None declared

OC028

INTERPLAY BETWEEN INTERLEUKIN-6 AND c-SRC IN THE REGULATION OF OSTEOBLAST FUNCTION

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The non-receptor tyrosine kinase c-Src plays remarkable roles in the regulation of bone cell activity. c-Src maintains osteoblasts (OBs) in a less differentiated state in which cell proliferation is active and further differentiation is inhibited. In a large-scale trascriptome study of mouse primary OBs treated with a well-known c-Src inhibitor (PP1), we noted a ~60% decrease of expression of the pro-inflammatory cytokine interleukin-6 (IL-6), also confirmed by RT-PCR and ELISA assay. Our previous work had shown that NSE/hIL-6 transgenic mice, which have high levels of circulating IL-6, display reduced OB activity, therefore we hypothesised that c-Src and IL-6 could be functionally associated in regulating OB function and that they could contribute to imbalanced bone remodelling in pathologic situations. In a time-course experiment in which we treated mouse primary OBs with PP1, we investigated IL-6 expression and found a time-dependent reduction of IL-6 mRNA in PP1- vs. vehicle-treated OBs. IL-6 mRNA decrease started after 2 hours of treatment and reached minimal levels at 48 hours, matching with similar time-dependent increase of OB differentiation markers. The same result was also obtained in OBs in which c-Src was inhibited by siRNA treatment for 48 hours. In order to explain how c-Src inhibition influenced IL-6 expression, we tested the involvement of "Signal Transducer and Activator of Transcription" (STAT) proteins, among which STAT3 is described to drive the transcription of IL-6 gene and to be a c-Src substrate. In the PP1 time-course experiment we showed that c-Src-dependent STAT3-phosphorylation was decreased during c-Src inhibition, suggesting involvement of this transcription factor in the c-Src-dependent regulation of IL-6. We then tested a possible role for IL-6 on c-Src signalling: primary OBs were treated with recombinant human IL-6 (rhIL-6), resulting in a time-dependent increase of c-Src activating tyrosine-416-phosphorylation vs. control, vehicle-treated OBs. This effect was apparent at 7 days of treatment, suggesting the induction of a long-term mechanism. Total c-Src was not changed over the treatment time, thus indicating lack of a direct transcriptional regulation of c-Src expression, but rather suggesting involvement of intermediate factors. In conclusion, we have obtained evidence of an interplay between c-Src and IL-6 in OBs. Our results indicate that the two pathways are associated possibly through the STAT3 signalling molecule.

Conflict of Interest: None declared

OC029

STATINS SENSITIZE HUMAN OSTEOSARCOMA CELLS TO CHEMOTHERAPY AND REDUCE TUMOR CELL INVASION AND MIGRATION

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Osteosarcoma is the most common primary tumor of bone in children and young adults. These tumors are characterized by a highly malignant and metastatic potential. Despite improvement in chemotherapeutic strategies, the survival rate of patients who relapse on therapy has not improved since the last decade. This highlights the need for the development of new therapeutic strategies to prevent the development of metastasis and increase survival in patients with osteosarcoma (OSA). We recently showed that lipophilic statins, which are HMG-CoA reductase inhibitors, induce apoptosis in human osteosarcoma cells through alteration of the RhoA-MAPK ERK½-Bcl2 pathway, independently of the genetic status of the OSA cells (Cell Death Diff 2006). In this study, we investigated the effects of atorvastatin in combination with chemotherapeutic drugs on cell death and tumorigenesis in four human OSA cell lines. Doxorubicin, cisplatin and atorvastatin induced additive effects on cell viability inhibition as well as additive effects on apoptotic cell death induction (up to about 40-fold increase in effector caspases activity) in the all human OSA cell lines, showing that atorvastatin sensitizes tumor cells to chemotherapy. Furthermore, we performed invasion and migration assays using modified Boyden chambers coated or not with basement membrane Matrigel, respectively. In parallel, trans-endothelial invasion through HUVEC monolayer was evaluated under the different treatments. We showed that atorvastatin alone reduced OSA cell migration and invasion, and exhibited additive effects when combined with doxorubicin or cisplatin (up to 95% inhibition of invasion, $p < 0.05$ vs untreated cells). Zymography and biochemical assays showed that atorvastatin markedly inhibited MMP-2 activity, whereas MMP-9 activity was slightly decreased. Reverse zymography indicated that TIMPs were not modified by atorvastatin treatment. Furthermore, we found that atorvastatin reduced MMP-2 activity through inhibition of RhoA activity and JNK phosphorylation. Consistently, activation of JNK signaling with anisomycin abolished the inhibitory effect of atorvastatin on MMP-2 activity. These results show that targeting RhoA-JNK-MMPs with statins re-

duces tumorigenesis in human OSA cells, which provides a potential pharmacological therapeutic strategy combining lipophilic statins with cytotoxic drugs to reduce tumor burden in human osteosarcoma.
Conflict of Interest: None declared

OC030

CANNABINOID RECEPTOR AGONISTS ARE POTENTIAL BONE ANABOLIC AGENTS WHICH STIMULATE BONE FORMATION IN VITRO AND INCREASE BONE MASS IN VIVO

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Background: The endocannabinoid system plays a key role in regulating bone metabolism. Mice lacking the cannabinoid type 1 receptor (CB1), which is predominantly expressed in the brain, have high bone mass and are protected from ovariectomy induced bone loss, whereas mice lacking the cannabinoid type 2 receptor (CB2) develop age related osteoporosis. The effects of cannabinoids on bone cell activity and bone mass are poorly understood and it is yet unknown if endocannabinoids are produced within the bone micro-environment. We investigated bone cells for expression of the enzymes involved in endocannabinoid metabolism by quantitative PCR, and studied the effects of cannabinoid receptor ligands on bone cell activity in vitro and in vivo. Results: Osteoblasts (OB) and osteoclasts (OC) express CB1 and CB2. NAPE-PLD and DAGL, the enzymes that synthesise Anandamide and 2-AG respectively, were detectable in the bone micro-environment. The enzymes involved in endocannabinoid breakdown, FAAH and MAGL, were also expressed in bone cells. The non-selective endogenous agonists Anandamide and 2AG, as well as the CB2 selective agonists HU308 and JWH133, significantly stimulated RANKL-generated OC formation and expression of several osteoclast specific genes including TRAcP, RANK, and the Calcitonin receptor, at concentrations between 10–300nM ($p < 0.001$). However, higher concentrations of these agents inhibited OC formation. The endogenous cannabinoids and CB2 selective agonists stimulated bone nodule formation significantly in long term OB cultures in a dose dependent fashion between 10–300nM ($p < 0.01$) and also enhanced BMP-2, COL1A1 and Osteocalcin mRNA expression in mature osteoblasts. To determine the role of CB2 agonists on bone metabolism *in vivo*, we studied the effects of JWH133 (0.1–1.0 mg/kg/day) in ovariectomised and sham-operated mice. JWH133 in low doses (0.1mg/kg/day) worsened ovariectomy induced bone, whereas higher doses (1mg/kg/day) were protective and increased bone mass by about 10% ($p < 0.05$) in sham operated mice. Conclusions: We have shown that the enzymes for endocannabinoid metabolism are expressed in the bone micro-environment. CB2 agonists enhance bone formation *in vitro* and have an anabolic effect *in vivo*. At low concentrations, however, CB2 agonists enhance osteoclast formation and exacerbate ovariectomy induced bone loss. CB receptor agonists could be of use as anabolic agents, although they might need to be combined with antiresorptive drugs.
Conflict of Interest: None declared

OC031

HEMATOPOIETIC STEM CELL TARGETED NEONATAL GENE THERAPY CURES OC/OC MICE FROM OSTEOPETROSIS

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Infantile malignant osteopetrosis (IMO) is a progressive, rare autosomal recessive disorder affecting osteoclast function. 50% of the affected children have a mutation in the Tcirg1 gene coding for one subunit of an osteoclast specific proton pump, OC116. The resulting dense, sclerotic bones cause symptoms including pancytopenia and progressive visual loss and ultimately death. So far, the only curative treatment is hematopoietic stem cell (HSC) transplantation. The oc/oc mouse has a mutation in the gene homologous to Tcirg1 giving rise to similar symptoms as in patients leading to death at the age of 3–4 weeks. We have previously shown that the oc/oc mouse can be treated with neonatal HSC transplantation. In this study we set out to develop HSC directed gene therapy for osteopetrosis in the oc/oc mouse model.

Fetal liver (FL) cells from oc/oc mice were depleted of Ter119+ cells and transduced with a retroviral vector expressing OC116 and GFP. In vitro transduction efficiency was 60–85%. One-day-old oc/oc mice were irradiated (400cGy) and transplanted i.p. with transduced FL cells (1–3.5 × 106). 7 out of 14 mice survived past the expected lifespan and had 8–53% GFP+ cells in peripheral blood at 3, 6 and 12 weeks. Analysis of bone structure with X-ray and histopathology showed a partial correction at 8 weeks and an almost normal structure at 18 weeks, indicating a correction of osteoclast function. In vitro culture on bovine bone chips of osteoclasts from transplanted animals showed bone resorption, albeit at lower levels than for wild type cells.

Conclusion: We have demonstrated that the osteoclast defect seen in oc/oc mice can be successfully corrected by neonatal transplantation of gene modified hematopoietic cells and that this can lead to long-term survival of treated mice. This represents a significant step towards the development of gene therapy for IMO.
Conflict of Interest: None declared

OC032

IN VIVO DIFFERENTIATION OF MURINE DENDRITIC CELLS INTO OSTEOCLASTS RESCUES THE OSTEOPETROTIC PHENOTYPE OF OC/OC MICE

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The control of osteoclast (OCL) differentiation by activated T cells has revealed the importance of the interactions between immune and bone cells. However, the role of other immune cells in this process remains unclear. Dendritic cells (DCs) are professional antigen presenting cells responsible for T cell activation and share a common precursor with OCLs. Furthermore, in vitro monocyte-derived DCs are able to generate functional OCLs in vitro.

In this study, we assessed whether mature DCs isolated from the spleen of wild type mice were able to generate OCLs both in vitro and in vivo. We first demonstrate that purified splenic DCs efficiently differentiate into fully functional OCLs in vitro, when cultured in the presence of RANK-L (receptor activator of NF-kB) and M-CSF (macrophage-colony stimulating factor), the main osteoclastogenic factors. This differentiation is inhibited by the addition of GM-CSF (granulocyte macrophage-colony stimulating factor), a growth factor necessary for the generation of DCs. To further analyze the differentiation potential of mature DCs in vivo, we have used the osteopetrotic oc/oc mice characterized by an increased osteoclastogenesis, although these OCLs are inactive for resorption due a deletion in the Tcirg1 gene. We show that mature DCs isolated from normal mice are able to rescue the osteopetrotic phenotype of oc/oc mice by differentiating in vivo into functional OCLs. This differentiation is favoured by the high level of RANK-L expression driven by CD4+ T cells, in the bone marrow of oc/oc mice. This report is the first demonstration that DCs are efficient for the treatment of a bone resorption disease in mice. Our results open new insights in the filiation between DCs and OCLs and offer new basis for analyzing the relations between bone and immune systems.
Conflict of Interest: None declared

OC033

THE BETA-GLUCURONIDASE KLOTHO DIMINISHES OSTEOCLASTOGENESIS AND SUBSEQUENT OSTEOCLASTIC BONE RESORPTION

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Klotho is a β-glucuronidase that was associated with prolonged aging. In the bones of mice lacking klotho, osteoblast and osteoclast differentiation are retarded, which leads to a low bone turnover phenotype. However, no data has appeared on the direct effect of klotho on individual bone cells. Only recently, we found that klotho improves the function of the epithelial calcium channel TRPV5 as well as its homolog TRPV6 (Chang *et al.*, 2005, Science, 310, 490–493). Besides hypercalciuria and intestinal hyperabsorption of Ca^{2+}, mice lacking TRPV5 display thinner bone structures and disturbed osteoclastic bone resorption despite that TRPV6 is still present in osteoclasts. In this study we explored the direct effects of klotho on osteoclast differentiation and activity and whether it improves osteoclast function in the TRPV5 knockout osteoclasts.

Bone marrow cultures treated with MCSF and RANKL to induce osteoclast formation were co-incubated with klotho-containing medium (produced by transfected HEK293 cells) and control medium (medium of HEK293 cells transfected with an empty vector). Compared to empty vector controls, klotho strongly inhibited osteoclast differentiation as assessed by tartrate-resistant acidic phosphatase staining. This was already evident after 3 days of culture and progressed to day 6 of culture. Following this, osteoclast activity, as assessed by analyses of resorption pit number (detected by coomassie staining), was greatly reduced by klotho compared to controls. To assess whether klotho acts on osteoclasts through its β-glucuronidase activity, we studied the effect of β-glucuronidase on osteoclast formation. Beta-glucuronidase perfectly mimicked the

klotho effect on osteoclast formation and resorption. Klotho treatment even further diminished the residual resorption by the TRPV5 knockout osteoclasts, excluding a potential improvement of osteoclast function via a positive effect on TPRV6 function. Finally, klotho mRNA was expressed in femoral bone as well as in osteoclasts generated from bone marrow cultures treated with MCSF and RANKL.

In conclusion, this is the first study demonstrating that klotho directly inhibits early osteoclastogenesis and bone resorption by its β-glucuronidase activity and that klotho can act as a para-/autocrine regulator of bone resorption. Finally, the data show that the detrimental effect of klotho on osteoclast development is dominant over its ability to improve TRPV5 and TRPV6 function.

Conflict of Interest: None declared

OC034

SMALL MOLECULE INHIBITORS OF TRAF-DEPENDENT SIGNALING AS ANTI RESORPTIVE AND ANTI-RHEUMATIC DRUGS

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We have previously shown that the butanediol ester of biphenylcarboxylic acid (ABD56) causes osteoclast apoptosis, and inhibits bone resorption in vitro and in vivo. However, the exact mechanism of action of ABD56 was not clear. Here we studied the effects of ABD56 on signal transduction in cells of the osteoclast lineage, to further elucidate its mechanism of action. Pretreatment of Osteoclasts (OC) generated in M-CSF and RANKL stimulated mouse bone marrow cultures or M-CSF dependent bone marrow macrophages (OC-Pre) with ABD56 (50 μM) for 45 minutes prior to stimulation with RANK-L (100ng/ml), completely abolished the RANKL-induced phosphorylation of IKKalpha and IkappaB, and ultimately prevented NFkappaB translocation to the nucleus. However, ABD56 not only abolished NFkappaB signaling, it also inhibited RANK-L-induced phosphorylation of Raf, MEK1/2 and ERK MAPK, and completely suppressed c-FOS activation. These results suggest that ABD56 acts at a stage in the signal transduction pathway before the NFkappaB and AP-1 pathways diverge. We therefore studied recruitment of TRAF6 to RANK, one of the earliest events in this cascade. ABD56 strongly suppressed RANKL-induced co-immuno-precipitation of RANK and TRAF6, and prevented translocation of TRAF6 to the cell membrane. Many pro-inflammatory cytokines act through TRAF-dependent receptors, and we found that ABD56 also potently inhibits TNFalpha-, IL-1-, and LPS-induced signaling. In contrast, ABD56 failed to inhibit M-CSF-, PTH- and PMA-induced signaling, pathways which do not involve TRAFs. TNFalpha, IL-1 and RANKL are important mediators of inflammation and bone loss in inflammatory diseases such as rheumatoid arthritis (RA). As ABD56 is a potent inhibitor of all three cytokines, we investigated the effects of ABD345, a more potent and metabolically stable derivative of ABD56, in the collagen induced arthritis (CIA) mouse model of RA. In this model, ABD345 strongly inhibited the development of arthritis at a dose of 20mg/kg/day. In conclusion, ABD56 and its derivatives act by inhibition of TRAF-recruitment to pro-inflammatory cytokine receptors. This suggests that, apart from their potential as anti-resorptive drugs, these compounds have great potential as treatments for inflammatory diseases such as RA. As these compounds are relatively simple small molecules, they could provide a more cost-effective alternative for current anti-TNF therapy.

Aymen I Idris is the recipient of the ECTS-Amgen Bone Biology Fellowship.

Conflict of Interest: None declared

OC035

CHONDROADHERIN IS DECREASED IN YOUNG OSTEOPOROTIC PATIENTS AND REDUCES BONE RESORPTION BY INHIBITING OSTEOCLAST FUNCTION AND DIFFERENTIATION

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Chondroadherin (CHAD) is a 38 kDa, leucine-rich protein that promotes chondrocyte attachment through binding to the integrin alpha2beta1. It is associated with the cartilage pericellular-territorial matrix, and is particularly enriched in the late proliferative-prehypertrophic zone in the growth plate. The protein is also prominent in adult cartilage of all origins and is expressed in bone where it plays a role during development. Gene profiling analysis shows that CHAD mRNA levels are about 50% ($p < 0.005$) reduced in bone biopsies of young osteoporotic females versus age-matched controls. We hypothesised that the protein plays a role in the regulation of bone cell activities and tested the effects of a cyclic peptide, representing the integrin binding sequence, in murine calvarial osteoblasts and bone marrow osteoclasts in culture. The peptide had no effect on the osteoblast functional parameters alkaline phosphatase activity, nodule mineralization, and transcription of osteoblast-specific genes, including alkaline phosphatase, runx2, collagen 1, osteocalcin and neither on osteopontin. In contrast, we noted marked reduced expression of the osteoclastogenic cytokines Rankl (−43%), IL-1beta (−34%) and IL-6 (−31%) ($n = 3$, $p < 0.05$), while no effect on opg, M-CSF, pthrp, TNFalpha, IL-12 and IL-18 mRNAs was noted. Consistently, the peptide reduced bone marrow in vitro osteoclastogenesis and bone resorption by 70% ($p < 0.05$, IC50 = 12.5 microM). It reduced to a lesser extent (50%, $p < 0.05$) osteoclastogenesis in purified bone marrow macrophage cultures treated with M-CSF and RANKL, suggesting that the peptide affects osteoclast formation both directly and through the attenuation of osteoclastogenic cytokine production by osteoblasts. The peptide reduced osteoclast adhesion to substrate (−20%, $p < 0.01$), suggesting interference with the cell-matrix interaction. We conclude that CHAD, as shown by its isolated integrin-binding domain, regulates directly osteoclast function and differentiation. CHAD also indirectly reduces osteoclast development and activity by attenuating osteoblast cytokine production. This integrin binding domain of CHAD thus appears to act as a regulator of the normal balance between bone formation and resorption. These findings provide a mechanistic correlation to the lower CHAD mRNA found in bone biopsies from women with primary osteoporosis.

Conflict of Interest: None declared

OC036

BALLOON KYPHOPLASTY AND NON-SURGICAL MANAGEMENT IN PATIENTS WITH ACUTE VERTEBRAL BODY COMPRESSION FRACTURES: A RANDOMIZED COMPARATIVE TRIAL

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BACKGROUND The international, multicenter, randomized, controlled Fracture Reduction Evaluation (FREE) trial was initiated to compare effectiveness and safety of balloon kyphoplasty (BKP) to non-surgical management (NSM) for the treatment of acute painful VCFs. We describe the primary endpoint of the ongoing 2-year study.

METHODS Patients with 1–3 non-traumatic VCFs were randomly assigned to BKP or NSM. The primary endpoint, the change in QOL as measured by the SF-36 Health Survey Physical Component Summary (PCS) at one month, and device/procedure-related safety were analyzed in the intent-to-treat population. Secondary endpoints (at one month) were analyzed in a per protocol population and included SF-36 subscales, the global health measure EQ-5D, self-reported back pain and back function using the Roland Morris Disability Questionnaire (RMDQ). All patients were referred for appropriate osteoporosis medical therapy.

RESULTS Among the BKP ($N = 149$) and NSM ($N = 151$) cohorts, mean patient age was 73 years and 77% were female. Thirty-nine BKP (26%) and 36 NSM (24%) patients had >1 VCF treated. For PCS, the mean baseline scores were 26.1 ± 1.46 for BKP and 25.7 ± 1.47 for NSM; at one month follow-up, the means scores were 33.6 ± 1.46 for BKP and 27.4 ± 1.49 for NSM. The difference in change was 5.73 points (95% confidence interval, 3.72–7.75) in favor of BKP over NSM ($p < 0.0001$). All physical component SF-36 subscales and the total EQ-5D score were significantly improved for BKP compared to NSM. Mean improvements in back pain were 3.2 ± 2.6 (7 days) and 3.3 ± 2.6 (1 month) for BKP, and 1.0 ± 1.7 (7 days) and 1.4 ± 2.2 (1 month) for NSM ($p < 0.0001$ for difference at both time points). Mean improvement in RMDQ was 5.9 ± 6.2 for BKP and 2.0 ± 4.1 for NSM ($p < 0.0001$ for difference). There was one device-related serious adverse event (a soft tissue hematoma) and one procedure-related serious adverse event (a postoperative urinary tract infection). There were no bone cement-related serious adverse events.

CONCLUSIONS Compared to non-surgical management, balloon kyphoplasty demonstrated superior short-term pain, function and quality of life outcomes with no difference in serious adverse events for the treatment of acute, painful vertebral compression fractures. Follow-up to two years will document whether these superior outcomes are sustained and whether BKP affects the natural history of osteoporotic vertebral compression fractures. (Clinical trials.gov number, NCT00211211)

Conflict of Interest: None declared

OC037

THE WHO RISK FACTOR INDEX, RISK OF VERTEBRAL FRACTURES AND REDUCTION IN FRACTURE RISK WITH ALENDRONATE: THE FRACTURE INVERVENTION TRIAL

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BACKGROUND: The WHO Risk Index predicts hip and nonspine fractures. We tested its ability to predict vertebral fractures (VF) compared with that of BMD. We also tested its ability to identify women with the greatest reduction in risk of fractures with alendronate (ALN) treatment.

METHODS: After baseline assessment of risk factors (RF) and BMD, 6,456 postmenopausal women with VF or femoral neck (FN) BMD T-score ≤ -1.6 were randomized to ALN or placebo (PBO) with radiographic assessment of VFs and validation of nonspine fractures over 3 to 4 years. We calculated the WHO Index based on RF alone (Index) and the WHO Index including RF and FN BMD (Index + BMD). We tested and compared the associations between Index, Index + BMD, FN BMD alone and L-spine BMD alone in the placebo group. We then compared fracture rates in high and lower risk women defined by the median value of the Index (25.1% 10-year risk of osteoporotic fx) or Index + BMD (27.8% 10-year risk) or by FN BMD T-scores alone (at T-score of −2.5)

RESULTS: The Index alone (RR/SD = 1.8; 95% CI = 1.6, 2.0), the Index + BMD (1.9: 1.7, 2.2), FN BMD alone (1.8; 1.6, 2.0), and spine BMD alone (1.9; 1.7, 2.3) had similar associations with risk of VF. The benefits of ALN for VF were significantly and similarly greater for women defined as high risk by the Index, Index + BMD or FN BMD (Table). The effect of ALN on nonspine fractures was greater for high risk than for lower women defined by FN BMD but not when defined by the risk factor Index alone or Index + BMD. Among women with 'osteopenia' (no VF and FN T-score −1.5, −2.5) ALN did not reduce the rate of nonspine fractures in those with a high risk (−2.5%; −6.6, 1.6) or low risk Index alone (0.7%; −2.0, 3.3).

CONCLUSION: The WHO Index predicts risk of VF and identifies women who benefit more from ALN for prevention of vertebral fractures but does not identify women that benefit more from ALN for prevention of nonspine fracture.

Table: Differences in rates: PBO-ALN (95% CI)

	VERTEBRAL FX	NONSPINE FX
FN BMD T < −2.5	5.5% (3.3, 7.8)*	3.9% (1.1, 6.7)*
FNBMD −1.6 to −2.5	1.9% (0.7, 3.1)	0.4% (−1.6, 2.4)
Hi risk Index	4.6% (2.6, 6.6)*	1.9% (−0.5, 4.4)
Lo risk Index	2.1% (0.9, 3.3)	1.7% (−0.5, 3.8)
Hi risk Index + BMD	5.2% (3.2, 7.2)*	1.5% (−1.0, 3.9)
Lo risk Index + BMD	1.4% (0.3, 2.4)	2.1% (0.0, 4.2)

*P < .05 for the difference between the risk groups.

Conflict of Interest: S. Cummings, Lilly, Amgen, Novartis, Pfizer, Zelos, Merck, Research support, consultation, honoraria

OC038

A HIGH-DENSITY SNP SCREEN IDENTIFIES ENPP1 AS A CANDIDATE LOCUS FOR FEMORAL SHAFT BONE DENSITY IN OLDER MEN: THE OSTEOPOROTIC FRACTURES IN MEN STUDY (MROS)

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In contrast to DXA, quantitative computed tomography (QCT) provides a 3-dimensional measure of volumetric bone mineral density (vBMD) that is not confounded by bone size. Little is known about the genetic determinants of vBMD in humans. To investigate the potential genetic determinants of vBMD at the femoral shaft, we systematically screened 374 physiologically defined candidate genes for bone metabolism in MrOS. Specifically, 882 Caucasian American men ≥65 with vBMD measures were selected from the Minneapolis and Pittsburgh clinics.

Genetic variation in each gene was captured by creating a reference SNP panel using Phase I HapMap that spanned 10kb downstream and 30kb upstream of each gene. Tag SNPs were then selected using a pairwise correlation method (r2 ≥0.80). Potentially functional SNPs that were either non-synonymous, predicted to alter a transcription factor binding site, or a putative exon splice enhancer were also genotyped. 4108 SNPs in 371 genes were genotyped using the Illumina platform, met stringent quality control criteria, and were analyzed for their association with vBMD. Analyses assumed a recessive and additive model of inheritance and were adjusted for age and clinic site. Principal components methods were used to account for population substructure and the most promising results (defined as $p < 0.001$) identified.

Using these criteria, 12 SNPs in 11 genes were associated with vBMD. The most significant association was with an intronic tag SNP (MAF = 11%) in ectonucleotide pyrophosphatase/phosphodiesterase 1(ENPP1), a gene whose protein product is involved in mineralization. Individuals with two copies of the minor allele had 6.9% lower vBMD compared to those with two major alleles ($p = 0.00003$). This association was driven by 45.8% lower bone mineral content ($p = 0.00003$) and 10.6% thinner cortices ($p = 0.0009$) in those with the rare genotype, but not by differences in cross-sectional area ($p = 0.37$). This tag SNP was not predicted to be functional by an in silico analysis using FastSNP. Upon closer inspection, 6 other SNPs in this gene region were associated with vBMD ($p < 0.05$). Three of these SNPs were identified as being potentially functional: two are predicted to alter intronic splice enhancers and one is a non-synonymous coding SNP within an exonic splice enhancer. These findings require confirmation, but implicate ENPP1 as a novel candidate gene influencing vBMD and bone mineral content but not size at this largely cortical skeletal site.

Conflict of Interest: None declared

OC039

A MOUSE MODEL OF EARLY ONSET PAGET'S DISEASE OF BONE CAUSED BY AN INSERTION MUTATION AFFECTING THE RANK SIGNAL PEPTIDE

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Previous studies have shown that activating mutations in the first exon of the *TNFRSF11A* gene (which encodes RANK) cause early onset familial Paget's disease of bone (PDB) and related conditions such as familial expansile osteolysis, and expansile skeletal hyperphosphatasia. In order to investigate how mutations in the *TNFRSF11A* cause early onset PDB, we have successfully generated a "knock-in" mouse line for the *84dup27* mutation of *TNFRSF11A* which mimics the mutation observed in the syndrome of early onset PDB by using a gene targeting approach in embryonic stem cells. Homozygote animals are viable and fertile but they are smaller in size compared to heterozygote and wild type littermates possibly because they develop early loosening of teeth and tooth loss by 6 weeks of age. Radiological analysis by Faxitron has shown that homozygous carriers of the mutation develop osteolytic and osteosclerotic lesions in the tibia, femur, humerus, phalanges and vertebrae at about 6 weeks of age. Heterozygote animals initially appear normal but some animals develop osteolytic lesions in the tibiae at the age of 8 months. We are currently performing further experiments to characterise the skeletal phenotype of these animals in more detail including analysis of bone histology, markers of bone turnover and micro CT. Here we present the first animal model of early onset PDB and show that homozygote animals develop many of the features which characterise the human disease including tooth loss and mixed osteolytic and osteosclerotic lesions early during life. More detailed studies of these animals are in progress in order to advance our understanding of the molecular mechanisms by which these lesions develop and why they target to specific regions in the skeleton. The mice will also be a valuable resource with which to explore the effects of therapeutic interventions

Conflict of Interest: None declared

OC040

MICRORNA REPERTOIRE OF PRIMARY HUMAN BONE DERIVED CELLS AND MG63-CELLS – POLYMORPHIC BINDING SITES IN PUTATIVE TARGET GENES

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MicroRNAs (miRNAs) are endogenous short RNA molecules (19–24nt) that inhibit translation of genes with partially complementary sequence to the miR-

NA in their 3́-UTRs. Although miRNAs have mainly been studied in tumor biology and embryonal development, they may also be involved in common phenotypic variation, either if being differentially expressed or when binding sites within target genes are polymorphic (Clop et. al., Nat Genet. 2006 Jul;38). Using a custom microarray targeting 300 human miRNAs we have examined miRNA expression in human primary bone derived cells and in the MG63 cell-line.

The microarray comprises positive and negative control probes and probes targeting 378 miRNAs. Each probe was spotted in quadruplicate to CodeeLink glass slides (Amersham Biosciences). From four individuals (two female and two male) undergoing orthopedic surgery bone samples were subdivided by scalpel and washed in PBS. Bone pieces were seeded in flasks containing alpha-MEM, with penicillin, streptomycin, L-glutamine and FCS (10 %). From cells, a fraction of small RNA (< 40 nt) was Cy3-labeled and hybridized to the microarrays. After hybridization the microarrays were washed and scanned. Fluorescence intensities were extracted with the GenePix software.

50 miRNAs were identified as expressed in primary bone cells and a similar expression profile was observed for the MG63-cells. miRNAs being ubiquitously expressed in primary bone cells included miRNAs: 183, 33, 345, 140, 34a, 450, 421, 122a, 485, 452, 153, 147, 513, 26b, and 489 as well as the let7-family of miRNAs. For miRNAs being expressed in bone cells we globally identified all binding sites either introduced or lost by genotyped SNPs present in 3́-UTRs of human genes. Several such SNPs reside in genes with known functions on bone metabolism and some are localized to regions harboring QTLs for bone traits.

We have characterized miRNA expression in bone derived cells and have globally identified polymorphic miRNA-binding sites in 3́-UTRs of human genes. Characterization of miRNA expression will further our understandings of processes regulating gene expression in the osteoblast. Further characterization of polymorphic miRNA-binding sites by association analyses and functional studies will be needed to conclude if these sites are involved in normal bone phenotypic variation.

Conflict of Interest: A. Kindmark, Astra Zeneca, Consultant

OC041

IDENTIFICATION OF NOVEL MUTATIONS IN RANKL AS A CAUSE OF OSTEOCLAST-POOR OSTEOPETROSIS

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Autosomal recessive malignant osteopetrosis (ArOP) is a genetically heterogeneous group of bone diseases characterized by defects in osteoclast formation (osteoclast-poor), or, more commonly, osteoclast function. In about 30% of cases, including all the osteoclast-poor cases, the genetic basis of the disease is as yet unknown. The remainder are caused by loss of function mutations in the proton pump subunit ATP6a3, or the chloride channel ClC-7, or the "grey-lethal" protein OSTM1. These proteins are functionally linked by the fact they are essential for ruffled border formation and proton secretion. We have previously shown that this osteoclast phenotype (i.e., normal formation, but absence of ruffled border and resorption) can be reproduced in vitro when osteoclasts are differentiated from patient monocytes in the presence of synthetic M-CSF and RANKL. To find genes mutated in osteoclast-poor ArOP we analysed a large series of patients lacking mutations in the known causative genes. Here we report on 6 patients from 4 unrelated families. Three of these patients had undergone bone marrow transplantation (BMT), but although engraftment was successful, no improvement in bone structure had resulted. This suggested a defect outside the osteoclast lineage and prompted us to analyse the genomic structure of RANKL. We found RANKL mutations in all patients: an intronic mutation causing exon skipping, a frameshift mutation and a missense mutation. Interestingly, the same missense mutation was found in patients in two unrelated families. In vitro, monocytes from the untransplanted patients were able to differentiate normally to multinucleated resorbing osteoclasts. By contrast, preliminary data suggest that osteoclasts from normal donors are not able to differentiate in the presence of synthetic RANKL carrying the mutations found in the patients. These findings demonstrate that osteoclast-poor osteopetrosis can be the result of loss of function mutations in RANKL. Therefore osteoclast-poor ArOP patients should be routinely screened for RANKL mutations as BMT will not bring any benefit. Therapeutic regimes should be developed to reconstitute RANKL in the bone environment in such patients.

Conflict of Interest: None declared

OC042

LOSS OF APC IN SKELETAL PRECURSOR CELLS INHIBITS BOTH OSTEOGENIC AND CHONDROGENIC DIFFERENTIATION

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During skeletal development, osteoblasts and chondrocytes are derived from a common precursor cell. Intracellular levels of β-catenin determine lineage commitment of this bi-potential progenitor: high levels induce osteoblast differentiation, whereas low levels lead to chondrocyte differentiation. Whether the Adenomatous polyposis coli (Apc), the key regulator of β-catenin expression, is involved in this process is currently unclear.

To address this issue we generated conditional knockout mice in which the mouse Apc was tissue-specifically ablated by crossing floxed Apc mice with transgenic mice expressing the Cre enzyme under the control of a rat Col2a promoter.

Conditional homozygous Apc mutants died perinatally. E16.5 mutant embryos were much shorter, displaying severely altered skeletogenesis. Although all axial skeletal elements were present, these elements were completely misshaped and lacked structural integrity. Microscopical analysis proved that defects in endochondral bone formation were already present at E12.5. In marked contrast with the controls, the conditional mutant skeletal elements displayed high levels of nuclear β-catenin, indicating an efficient Apc deletion. In all bone primordias, β-catenin positive cells lacked an Alcian Blue and Col2 positive extracellular matrix, and they did not express chondrogenic markers like Sox9 or Col2 at the mRNA level. This indicated that Apc loss completely blocked chondrocyte differentiation. We then checked whether these cells had redirected their differentiation program into osteoblasts. Surprisingly, the β-catenin positive cells did not express typical markers for the osteogenic lineage like RunX2, Osx, Col1, Pthr and Osc either. Therefore, we concluded that homozygous Col2Cre-mediated Apc deletion results in an uncontrolled rise of intracellular β-catenin levels. β-catenin positive cells displayed neither chondrogenic, nor osteogenic marker expression, suggesting a complete arrest in their differentiation potential, and maintenance of an undifferentiated phenotype. In vitro differentiation experiments using the murine mesenchymal progenitor KS483 cell line confirmed that Apc knock-down blocks differentiation of precursor cells into chondrocytes, osteoblasts as well as adipocytes.

In conclusion, we propose that maintenance of a skeletal precursor cell population as well as regulation of lineage commitment requires a tight regulation of intracellular β-catenin levels and that this is controlled by Apc.

Conflict of Interest: None declared

CC001

DEFICIENCY OF COMPONENTS OF THE COLLAGEN PROLYL 3-HYDROXYLATION COMPLEX CAUSES A RECESSIVE BONE DYSPLASIA RESEMBLING LETHAL/SEVERE OSTEOGENESIS IMPERFECTA

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Classical osteogenesis imperfecta (OI) is a dominant genetic disorder caused by mutations within the genes coding for type I collagen, COL1A1 and COL1A2. However, 10–15% of OI patients do not have a collagen mutation and a recessive form of OI has long been suspected. Recently, Morello et al. reported severe bone dysplasia in a KO mouse for cartilage associated protein (CRTAP) and a hypomorphic CRTAP mutation in type VII OI. CRTAP, prolyl 3-hydroxylase 1 (P3H1) and cyclophilin B form a complex which 3-hydroxylates α1(I)Pro986. Therefore, we postulated that deficiency of a member of this col-

lagen modifying complex may cause recessive OI. With real-time PCR, we screened fibroblast RNA samples from 10 lethal or severe OI patients who have no collagen mutation for deficiency of CRTAP or P3H1 mRNA. Three contain minimal CRTAP mRNA, while P3H1 mRNA is significantly reduced in the other seven patient samples. The expected null mutations in both alleles of CRTAP or P3H1, respectively, were identified by DNA sequencing. Among the 3 patients with CRTAP null mutations, two have homozygous mutations and the other is a compound heterozygote. For the 7 patients with null P3H1 mutations, three have homozygous mutation and the other four have compound heterozygous mutations. All tested patients' parents are heterozygous carriers. Five of our seven P3H1 null patients have a common mutant allele, which originated in west Africa and is also present in African-Americans. The absence of CRTAP or P3H1 protein in the patients was demonstrated by Western blot. Tandem mass spectrometry of proband collagen tryptic peptides revealed absence or significant reduction of prolyl-3-OH at the unique Pro986 site in the α1 chain of type I collagen. All probands collagen has overmodification of the helical region comparable to that found in collagen with a structural defect. Hydroxylation of α1(I)Pro986 or a chaperone function of the modification complex is apparently crucial for helix folding. CRTAP and P3H1 null probands have distinct features, although all have white sclerae. Probands with null CRTAP mutations have lethal OI with deficient long bone modeling, while probands with null P3H1 mutations have lethal to severe OI with extreme osteoporosis (DEXA ≤ −7) and growth deficiency. We demonstrated that prolyl 3-hydroxylation of type I collagen is crucial for bone formation; absence of prolyl 3-hydroxylation of types IV and V collagen may also be important in outcome of these probands.

Conflict of Interest: None declared

CC002

FOCAL OSTEOSCLEROSIS IN AN OTHERWISE OSTEOPENIC FEMALE PATIENT HARBORING A NEW HETEROZYGOUS GENETIC VARIANT (R714C) OF THE OSTEOPETROSIS GENE, PLECKSTRIN HOMOLOGY DOMAIN CONTAINING FAMILY M (WITH RUN DOMAIN) MEMBER 1 (PLEKHM1)

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The PLEKHM1 gene encodes for a non-secretory adaptor protein that localizes to osteoclast endosomal vesicles. A highly truncated Plekhm1 protein has previously been found in a patient with intermediate autosomal recessive osteopetrosis. Herein we describe a 39-years old female patient with a new heterozygous mutation of the PLEKHM1 gene, presenting with an unusual bone phenotype. This patient was admitted to our care unit with a diagnosis of osteopetrosis of the skull but, surprisingly, she had a low vertebral and femoral T score with areas of focal sclerosis at the frontal bone and at femoral neck. Routine laboratory tests were normal. Direct DNA sequencing showed that patient harbored a heterozygous C to T substitution (first codon) on cDNA position 2140 of the PLEKHM1 gene, leading to a predicted R714C variant. In vitro osteoclasts from the patient showed normal formation rate, morphology, number of nuclei, and actin rings, with lower TRAcP activity, and endosomal vesicles showing less acidic pH than control osteoclasts, but normal bone resorption. Patient had normal serum CTX, high PTH and TRAcP activity despite low TRAcP activity in cultured osteoclasts, suggesting increased release of the enzyme. Using overexpression studies, HEK293 cells transfected with wild type or Plekhm1-R714C-GFP showed no differences in localization between the two variants, which co-localized with Rab7 with equal efficiencies at the endosomal vesicles. However, HEK293 cells co-transfected with wild type or Plekhm1-R714C mutant and human TRAcP showed consistent less TRAcP activity in cells overexpressing Plekhm1-R714C compared to wildtype, suggesting that reduced enzyme activity is indeed associated with the plekhm1 mutation. In conclusion, our work provides evidence of a potential role of a new mutation of the PLEKHM1 gene in a complex bone disease characterized by a generalized osteopenia associated with focal osteosclerosis, in which major osteoclast anomalies are apparently linked to altered endosomal vesicle pH and TRAcP activity.

Conflict of Interest: None declared

CC003

ONCOGENIC OSTEOMALACIA CAUSED BY A FGF 23-PRODUCING HEMANGIOPERICYTOMA DETECTED WITH OCTREOTIDE SCAN

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Introduction. Tumour induced osteomalacia is a rare disorder characterized by inappropriate renal phosphate waste, severe hypophospatemia, and osteomalacia. Paraneoplastic production of fibroblast growth factor 23 (FGF 23) seems to be the main cause of renal phosphate leak and of impaired calcitriol production. In vitro studies demonstrated that some tumours express somatostatin receptors an, therefore, can be detected with octreotide scan.

Case report. A 40-yr-old Caucasian man was referred to our Centre in December 2005 with a 4-yr history of bone and muscle pain, progressive fatigue and fragility skeletal fractures. His illness began with foot pain in mid-2002. In December 2002 he developed spontaneous fracture of the right heel and of the left heel 3 months later. In the subsequent months he experienced progressively increasing pain also in the back, hip girdle, and upper legs, which was accompanied by generalized muscular weakness leading to difficulty in walking. He was treated with calcium and vitamin D supplements and bisphosphonates. In December 2004 he had a spontaneous fracture of left femoral neck. On referral he had a proximal myopathy being unable to rise from a chair, and severe pain in the upper left tight which was due to a subtrochanteric Looser's fracture. Investigation showed plasma phosphorus of 1.6 mg/dl (nr = 2.7–4.5) with urine excretion of 1000 mg 24-h (nr = 400–1300) and a maximum tubular resorption of 1.4 mg/dL GFR (nr = 2.5–5.4). Serum alkaline phosphatase was 1975 IU/L (nr = 80–280). Serum calcium, iPTH and 25-OH-D were within normal range, whereas serum calcitriol was inappropriately low: 13 pg/mL (nr = 10–50). Serum FGF 23 levels were 690 RU/mL (nr < 100). An octreotide scan showed an increase uptake in the right craniofacial location and a MR imaging revealed a large highly vascularized tumour in the right maxillary sinus, which resulted to be an hemangiopericytoma. After tumour removal FGF 23 serum levels normalised as well as the abnormal parameters of phosphate and vitamin D metabolism. After 6 months he returned to work.

Conflict of Interest: None declared

CC004

IDENTIFICATION OF A NOVEL MUTATION IN ACTIVIN RECEPTOR TYPE I (ACVR1) IN A FIBRODYSPLASIA OSSIFICANS PROGRESSIVA (FOP) PATIENT

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FOP is a rare, heritable, extremely debilitating condition involving progressive and extensive heterotopic ossification of skeletal muscles, fasciae, tendons and ligaments. Skeletal developmental defects are also apparent and the disease can be readily identified by the presence of malformed great toes. Recently the cause of FOP was determined as a point mutation (G617A) in Acvr1 in all patients studied (1). Here we report a further mutation in this gene in a FOP patient with some atypical clinical features.

DNA was prepared with ethical approval from blood samples of patients with clear clinical phenotypes of FOP and from the atypical patient. PCR products were directly sequenced by using an ABI3100 Prism automated sequencer. Restriction fragment length polymorphism was used to confirm the sequencing results. The previously described Acvr1 G617A mutation was confirmed in all patients with characteristic features of FOP but was not present in the atypical phenotype. In the latter, the novel heterozygous mutation, G605T, was found within the ACVR1 gene. Acvr1 is a member of the transforming growth factor-beta (TGF-beta) receptor superfamily of serine/threonine kinases. TGFbeta type I receptors can be regulated through the binding of fkbp12 to their GS domains. This inhibits activation of the receptor, blocking further cell signalling. The G617A mutation causes an arginine to histidine change within the GS protein domain and is proposed to confer constitutive activation, possibly by preventing fkbp12 binding and inhibition. The G605T mutation in the atypical patient causes an arginine to isoleucine change, also within the GS domain of the protein. The presence of isoleucine at position 202 of acvr1 may allow partial binding of fkbp12 and explain the less severe phenotype of this patient.

(1) Nature Genetics 38: 525–527, 2006. EM Shore, M Xu, GJ Feldman, DA Fenstermacher, T-J Cho , IH Choi, JM Connor, P Delai, DL Glaser, M Le Merrer, R Morhart, JG Rogers, R Smith, JT Triffitt, JA Urtizberea, M Zasloff, MA Brown, FS Kaplan.

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Conflict of Interest: None declared

P001-S

OSTEOCLASTS SECRETE NON-BONE DERIVED SIGNALS THAT INDUCE BONE NODULE FORMATION

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Patients with osteopetrosis caused by defective acidification of the resorption lacuna have severely decreased resorption, but increased numbers of TRACP positive osteoclasts with normal or even increased bone formation. This suggests that osteoclasts themselves are important for sustaining bone formation.

To investigate whether osteoclasts might contribute to the control of bone formation by production of bone anabolic signals, we collected conditioned media (CM) from human osteoclasts cultured on either bone or plastic, and tested their effects on bone nodule formation.

Human osteoclasts isolated by the CD14 magnetic bead procedure were grown on either plastic or bone in the presence of 25ng/ml M-CSF and RANKL. We ensured high quality of the osteoclast-conditioned medium by measuring TRACP activity and collagen type I fragment (CTX-I) levels. We tested whether the CM (30–80%) was able to induce bone nodule formation in MC3T3-E1 osteoblastic cells cultured in the presence of 50mg/ml ascorbic acid and 10mM b-glycerophosphate to allow osteoblast differentiation. We assessed bone formation by measuring alkaline phosphatase activity and performing Alizarin red and Von Kossa stainings.

We found that CM from osteoclasts grown on bone dose-dependently induced bone nodule formation to a maximal level of 400% at 60% CM. The negative control, non-conditioned osteoclast culture medium containing RANKL and M-CSF did not induce bone nodule formation. Furthermore, we found that CM from osteoclasts grown on plastic dose dependently induced bone formation by 200% at 60%.

In conclusion, we present evidence showing that osteoclasts, independent of their resorptive activity, secrete factor(s), which induce(s) preosteoblasts to form bone-like nodules in osteoblast cultures. Such a mechanism could provide a way by which osteoclast products contribute to the coupling of bone formation to resorption. It could also explain why some patients with osteopetrosis caused by defective acidification of the resorption lacuna have increased bone formation despite the lack of resorptive capacity.

Conflict of Interest: None declared

P002-M

LOSS OF ANION EXCHANGER 2 IN MICE RESULTS IN OSTEOPETROTIC LONG BONES, WHEREAS SKULL IS NOT AFFECTED

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Osteoclasts are multinucleated cells specialized in bone resorption. Central to this process is extracellular acidification by proton secretion, which results in a change of the internal pH of the cell. An anion exchanger (Ae) is thought to maintain the intracellular pH (pH_i) by exchanging bicarbonate for chloride ions. To evaluate the role of the exchanger present in numerous polarized cell types, Ae2, in osteoclastic bone degradation, mice were generated in which three of the five Ae2 transcripts were deleted (a, b1 and b2 transcripts, but not c1 and c2 transcripts). Skulls and long bones were examined by X-ray, microCT, light and electron microscopy. Osteoclasts generated in vitro from wildtype and $Ae2^{-/-}$ mice were tested for bone resorption, TRACP activity and intracellular pH regulation. X-ray and microCT showed a very high bone density in the long bones of the $Ae2^{-/-}$ mice, resulting in an osteopetrotic phenotype. The skulls, however, were normal. In the latter bone functional osteoclasts were present in $Ae2^{-/-}$ mice, but those in the long bones lacked a ruffled border and were inactive. Osteoclasts generated in vitro from $Ae2^{-/-}$ bone marrow contained lower numbers of multinucleated $TRACP^+$ cells after 6 days of culture; the majority of the $TRACP^+$ cells were mononuclear. After 17 days of culture normal numbers of $TRACP^+$ multinucleated cells were found. pH_i measurements demonstrated that these cells were impaired in pH regulation. Thus, in the absence of the Ae2 a and b isoforms, long bone osteoclasts lack the capacity to resorb bone, resulting in severe osteopetrosis. The pH_i is not properly regulated, and the formation of multinucleated cells in vitro is impaired. Osteoclasts of the skull, however, appear unaffected: they resorb bone and exhibit an extensive ruffled border. Our data indicate that the a and b Ae2 isoforms are essential for the activity of long bone osteoclasts, whereas skull osteoclasts appear to function without these isoforms. We hypothesize that skull osteoclasts make use of the c1 and/or c2 Ae2 isoforms.

Our findings indicate that osteoclasts of long bones differ from those present in the skull, thus strongly support the notion that bone-site specific differences exist among osteoclasts.

Conflict of Interest: None declared

P003-T

CLC-7 CHLORIDE CHANNEL EXPRESSION IS RATE LIMITING FOR THE RESORPTIVE ACTIVITY OF OSTEOCLASTS

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Loss of function mutations in the ClC-7 chloride channel abolish osteoclast activity leading to infantile malignant osteopetrosis in mice and humans, which often entails neurodegeneration. Dominant negative mutations, which statistically reduce the number of functional chloride channel dimers to 25%, evoke the milder Albers–Schoenberg form of osteopetrosis. Carriers of heterozygous recessive mutations do not have any overt skeletal phenotype. The strictly intracellular localisation of ClC-7 makes it inaccessible for direct measurements of channel function. According to the human osteopetrosis phenotypes ClC-7 becomes rate limiting for osteoclast resorptive activity if channel function is reduced by 50–75%. In order to more closely determine this threshold we used transgenic mice expressing ClC-7 driven by the osteoclast-specific TRAP-promoter to rescue the bone phenotype of Clcn7−/− mice. Three different mouse lines rescued the osteopetrotic phenotype to different degrees, which we correlated to the expression levels of the transgenic construct. All rescue lines still displayed neurodegeneration indicating that this complication is not secondary to the changes in bone mass and structure. Interestingly, the bone formation rate was not significantly altered even in rescue mice showing an almost three-fold increased bone mass. Thus, even a strong attenuation of osteoclast function by reduced ClC-7 expression did not impair the coupling process. These findings might have implications for the evaluation of ClC-7 as a drug target for antiresorptive therapy.

Conflict of Interest: None declared

P004-S

THE EXTRACELLULAR MATRIX (ECM) COMPONENT PRELP (PROLINE/ARGININE-RICH END LEUCINE-RICH REPEAT PROTEIN) INHIBITS OSTEOCLASTOGENESIS

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ECM is a complex set of molecules with different functional properties. They form tissue-specific networks, and regulate many cell functions. PRELP is a ubiquitously found basement membrane anchoring protein that binds types I/II collagens, heparin and heparan sulfate. Loss of function mutations of PRELP are involved in the pathogenesis of Hutchinson–Gilford progeria, characterized, among other symptoms, by severe osteoporosis. PRELP is highly expressed in cartilage and developing bone. Herein we demonstrate that a peptide corresponding to the entire heparin-binding domain of PRELP inhibits mouse osteoclast formation. Treatment with the PRELP-peptide decreased by 80% osteoclastogenesis and bone resorption in non-fractionated mouse bone marrow cultures exposed to 1, 25(OH)2VitD3, and in purified bone marrow macrophages treated with M-CSF and RANKL (IC50 = 7.3 microM). Pretreatment of mature osteoclasts with the PRELP-peptide decreased adhesion to substrate by 60%. In contrast, the peptide showed no activity with osteoclast precursors and neither their adhesion and number nor TRAcP activity were affected by the treatment. Consistently, PRELP-peptide inhibited osteoclastogenesis when added at the 4th day of culture, suggesting a late effect on prefusion osteoclast precursors. Early uncommitted precursors expressed surface heparan sulphate proteoglycans (HSPGs) which declined in culture with time. Although a terminally-tagged PRELP-peptide co-localized with HSPGs, they did not appear to physically interact as suggested by PRELP-peptide membrane retention when HSPGs vanished. Consistently, treatment with the heparan sulphate disrupting enzyme, heparinase, failed to prevent PRELP from inhibiting osteoclastogenesis. Whether the peptide activity depended on binding to the chondroitin sulphate chains of cell surface proteoglycans is not yet known. Finally, PRELP-peptide affected osteoclastogenesis and bone resorption only by a direct mechanism as real-time RT-PCR failed to detect any change in expression of cytokines promoting osteoclastogenesis in mouse calvarial osteoblasts. Accordingly, PRELP-peptide showed no effect on their alkaline phosphatase activity, nodule mineralization, gene expression and intracellular signalling protein phosphorylations. We conclude that PRELP is a direct negative regulator of osteoclast formation acting at the late stage of prefusion committed osteoclast precursor differentiation, with no apparent effect on the osteoblast lineage.

Conflict of Interest: None declared

P005-M

DIRECT RAB11–RAC1 INTERACTION REGULATES EXIT OF RECYCLING VESICLES FROM THE PERINUCLEAR COMPARTMENT IS INVOLVED IN CELLULAR MIGRATION

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Rab GTPases play many important roles in the regulation of vesicular transport. Rab11 has been shown to regulate the slow endocytic recycling pathway, especially involved in the late steps of vesicular targeting and fusion to the plasma membrane, and finally exocytosis. Here, we report that another small GTPase Rac1, which we have previously shown to bind to Rab7, directly interacts with Rab11. This was demonstrated in the bacterial two-hybrid system, pull-down experiments and co-immunoprecipitation assays. We further showed that Rab11 and Rac1 colocalized at perinuclear recycling compartment and punctuate recycling vesicles through the cytoplasm. Importantly, when we followed the transferrin uptake and recycling, we saw a clearly increased Rab11–Rac1 colocalization at the basolateral plasma membrane in Hela cells which expressed GFP–Rab11 and Dsred–Rac1, and also in primary resorbing osteoclasts. Colocalization could also be visualized at the endocytic recycling vesicles and perinuclear recycling compartment. Furthermore, an intensive triple colocalization of Rab11, Rac1 and transferrin at the leading edge of the migrating osteoclasts suggests that the direct Rab11–Rac1 interaction may be involved in cellular migration. In addition, we were able to show that only the GTP-bound and wild type forms of Rab11 but not GDP-bound form colocalized with Rac1 at recycling vesicles and plasma membrane in Hela cells after transferrin internalization. Moreover, after 30 min of transferrin internalization the perinuclear recycling compartment segregated into recycling vesicles revealing an increased triple colocalization of Rab11, Rac1 and transferrin receptor. Importantly, in the GDP Rab11 and Rac1 expressed Hela cells, the perinuclear structures were even more condensed than in non-treated cells. GDP Rab11 and Rac1 were partially overlapping and retained in the pericellular compartment preventing also the exit of transferrin receptor. Our data suggests that direct GTP Rab11 and Rac1 interaction is required not only for Rab11 controlled targeting and fusion to the plasma membrane, but it is also involved in the exit of vesicles exit from perinuclear recycling compartment.

Conflict of Interest: None declared

P006-T

CATHEPSIN K INHIBITORS PREVENT MATRIX-DERIVED GROWTH FACTOR DEGRADATION BY HUMAN OSTEOCLASTS

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Under normal circumstances, bone resorption and bone formation are coupled in such a way that, if bone resorption is inhibited, then so is bone formation. This coupling between bone formation and resorption creates therapeutic difficulties in osteoporosis: further bone loss can be prevented using antiresorptive therapy, but because suppression of resorption also inhibits bone formation, the condition is not reversed. Surprisingly, inhibition of resorption by cathepsin K inhibitors has recently been found to augment bone formation. Uniquely amongst resorption inhibitors, inhibitors of cathepsin K suppress degradation of the organic matrix while allowing demineralisation. We hypothesised that these characteristics might explain the unexpected capacity of cathepsin K inhibitors to enhance bone formation: they might prevent degradation not only of matrix collagen, but also other matrix-embedded proteins, including the growth factors that are known to be embedded in bone matrix. We tested this hypothesis using osteocalcin and IGF-I as examples of matrix-embedded proteins. We found that, when osteoclasts were incubated on bone, inhibitors of cathepsin K dramatically increased the concentrations of these matrix-derived proteins in supernatants. Other resorption inhibitors did not do this. Inhibition of cathepsin K appeared to act by protecting the proteins against intracellular degradation, rather than against their degradation in supernatant. We also found that protons are both necessary and sufficient to release IGF-I from bone matrix, and that recombinant cathepsin K is capable of the degradation of both marker proteins. In the presence of a cathepsin K-inhibitor, the amount of IGF-I released from matrix by osteoclasts substantially exceeded the amount they secreted. Moreover, the numbers of osteoblastic MC3T3-E1 were greater after co-culture with osteoclasts on bone with versus without cathepsin K-inhibitor, showing that osteoclasts release biologically significant quantities of growth factors from bone, and that the biological activity of the released growth factors is preserved by inhibition of cathepsin K. These results suggest a model in which cathepsin K inhibitors prevent degradation of growth factors released from bone matrix by osteoclast-derived protons, thus augmenting the anabolic component of the coupling between bone resorption and bone formation.

Conflict of Interest: Medivir UK, Consultancy

P007-S

SRC MEDIATED CBL–PI3KINASE INTERACTION REQUIRED FOR OSTEOCLAST FUNCTION

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The non-receptor tyrosine kinase Src and the proto-oncogene product Cbl, Src's major substrate in osteoclasts (OCs), are required for OC motility and bone resorption. Phosphatidylinositol 3' kinase (PI3K), which is also necessary for normal OC migration and function, associates with Cbl. Both the SH2 and SH3 domains of PI3K's p85 regulatory subunit have been implicated in mediating the Cbl–PI3K interaction. Cbl's tyrosine 731 occurs in a sequence (YEAM) that is consensus motif for phosphorylation by Src and, when phosphorylated, for binding the SH2 domain of p85. In the present study, we show that mutating tyrosine 731 to phenylalanine (Y731F), inhibiting Src kinase activity or mutating the Src-binding site on Cbl blocked the binding of PI3K to Cbl. Thus, the Cbl–PI3K association appears to require both the binding of Src to Cbl and the subsequent phosphorylation of tyrosine 731 of Cbl by Src. In contrast to Cbl, over-expression of kinase-dead Src had no effect on the interaction between p85 and Cbl-b. Although highly homologous to Cbl, Cbl-b lacks a Tyr residue in a motif homologous to Cbl Y731, possibly explaining the independence from Src phosphorylation. This suggests that Cbl and Cbl-b bind p85 at different sites and that binding of PI3K to the two Cbl proteins may be regulated differently. We then investigated the functional role of the Cbl–PI3K interaction in M-CSF-induced OC migration and in bone resorption, using Cbl−/− osteoclast-like cells (OCLs) reconstituted with Cbl proteins. M-CSF (50 ng/ml) induced a 60% increase in migration of WT OCLs in a Boyden chamber migration assay. In contrast, the intrinsically lower migration of untreated Cbl−/− OCLs was not increased upon treatment with M-CSF. As expected, expression of WT Cbl in Cbl−/− OCLs with the adenovirus system rescued the phenotype and M-CSF induced a 65% increase in migration, comparable to the response of WT OCLs to M-CSF. In contrast, expression of CblY731F failed to rescue the M-CSF-induced motility of the Cbl−/− OCLs, demonstrating that the Src-dependent phosphorylation of Y731 and the recruitment of PI3-kinase by Cbl are required for OC migration in response to M-CSF. Along with the impaired migration, the pit-forming capability of Cbl Y731F-expressing-OCLs was also reduced to 30% of control. Taken together these results indicate that the Src kinase-dependent interaction of Cbl with PI3K is important for OC migration and bone resorption.

Conflict of Interest: None declared

P008-M

DIPHYLLIN, A NOVEL POTENT V-ATPASE INHIBITOR, ABROGATES ACIDIFICATION OF THE OSTEOCLASTIC RESORPTION LACUNAE AND BONE RESORPTION

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Dissolution of the inorganic phase of bone by the osteoclasts, which is a prerequisite for bone resorption, is mediated through the V-ATPase and ClC-7. Inhibitors of ClC-7 or the osteoclastic V-ATPase are novel approaches for inhibition of osteoclastic bone resorption. By testing natural compounds in acidification assays, diphyllin was identified. We characterized diphyllin with respect to potency in a V-ATPase assay, an acid influx assay, lysosomal acidification in osteoclasts by acridine orange and in a human osteoclastic bone resorption assay, using the known V-ATPase inhibitor bafilomycin A1 as a control.

Diphyllin was tested in the V-ATPase and the acid influx assay using bovine chromaffin granules isolated from the medullae of bovine adrenal glands. Human osteoclasts were generated from CD14+ monocytes cultured with 25ng/ml M-CSF and RANKL. The effect of diphyllin on lysosomal acidification in human osteoclasts was investigated using the dye acridine orange. The effect of diphyllin on bone resorption by osteoclasts was measured as release of CTX-I and calcium into the supernatants, and by scoring pit area. The number of osteoclasts, the TRACP activity, and the cell viability were measured.

We found that diphyllin inhibited the V-ATPase with an IC50 value of 17nM, compared to 4nM for bafilomycin. In the acid influx assay, diphyllin potently inhibited influx with an IC50 of 0.6nM, which is similar to the effect of bafilomycin. Moreover, diphyllin dose dependently inhibited lysosomal acidification in human osteoclasts, with potency similar to that of bafilomycin. Finally, we found that Diphyllin inhibited human osteoclastic bone resorption measured by CTX-I and calcium release with an IC50 of 14nM, in face of increasing levels of TRACP activity, osteoclasts, and cell viability.

We have identified a natural compound that potently inhibits the V-ATPase and the lysosomal acidification in osteoclasts and thereby abrogates bone resorption. Since recent studies indicate that inhibition of the osteoclastic acidification leads to inhibition of resorption without inhibiting formation, we speculate that diphyllin is a potential novel treatment for bone disorders involving excessive resorption.

Conflict of Interest: None declared

P009-T

APPLICATION OF BIOLUMINESCENCE IN BONE FORMATION

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Current treatments for osteoporosis suppress bone resorption, thereby blocking further deterioration, but do not restore bone. Therefore, therapies stimulating bone formation would greatly enhance treatment of osteoporosis.

The bone forming osteoblast originate from the multipotent mesenchymal stem cells (MSCs). We have used the MSC-like cell line KS483, to obtain insight in the factors influencing recruitment and subsequent differentiation of osteoblasts from the stem cell population.

This cell line was genetically modified enabling efficient generation of isogenic stable cell clones by Flp-mediated homologous recombination by integrating 1 copy of a FRT-target site into the cell's genome. After we had tested the efficacy of the FRT site by the overexpression or knock-down of the RunX2 gene, it was used to insert a luciferase2 gene containing a His tag enabling us to follow the cell fate in vivo by bioluminescence after implantation in nude mice and the detection of cells ex vivo by immunohistochemistry using antibodies against the His tag. KSfrt HisLuc2 cells were used to perform a bone marrow ablation assay in which the cells were injected in the marrow cavity. Cell fate was followed by non-invasive bioluminescent imaging and X-rays. At several time points, mice were sacrificed and both tibias were isolated for analysis. In addition, to study their fate outside the bone forming environment and to study possible adverse effects, cells were also transplanted subcutaneously. We have followed cell fate non-invasively for 20 weeks and afterwards isolated the luciferase expressing tissue for immunohistochemical analysis.

Finally, we used the genomic FRT site for the insertion of gene reporter constructs, which respond to Wnt- or BMP-signaling. Isogenic stable cell lines were obtained that were highly sensitive for Wnt and BMP, respectively, and retained multipotency.

In conclusion, genetically modified KSfrt cells provide a simple and fast model to study MSC function. In combination with bioluminescent imaging, we will use this model to evaluate the effects of biomaterials on stem cell function in vitro and in vivo as a first step towards a bone replacement therapy for osteoporosis.

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Conflict of Interest: None declared

P010-S

HIGH-THROUGHPUT GENETIC EXPRESSION PROFILES AND PROTEOMICS APPROACHES FOR UNDERSTANDING THE BISPHOSPHONATE'S MODES OF ACTION ON BONE

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Osteocytes are the most abundant cell type in bone tissue. Low doses of Bisphosphonates (BPs) can prevent osteocytes' apoptosis through the activation of extracellular signal-regulated kinases (ERKs). However, BP effects at the molecular level are still unclear.

To address the issue, we performed a complete analysis that combines a high-throughput screening of gene expression profiling and proteomics approaches on mouse osteocytes (MLO-Y4 cell line). Messenger RNA was obtained from cells treated for 48h with Risedronate and Alendronate (10^{-7}M). After discrimination of the messengers on microarrays, expression data for each time point was compared to control. Using statistical analysis, 125 genes were found to be differentially expressed and a first analysis revealed a marked upregulation of messengers encoding zinc-binding proteins. Since Risedronate proved to be the most effective on the osteocytic cells in terms of transcriptome, we decided to analyse its effect on cells proteome. We therefore applied a combination of 2D gel electrophoresis-based and ShotGun proteomics to identify differentially expressed proteins isolated from MLO-Y4 cells treated for 1 hour and 48 hours with Risedronate, at the concentration of 10^{-7}M. Treatment of cells with bisphosphonates revealed three different species (vimentin, 78KDa-glucose regulated protein and endoplasmin) that were shown to be downregulated while only one (Histone 1, H2bb) showed a marked induction of expression. To improve the amount of information obtained, we performed the more sensitive ShotGun proteomics approach, on the same experimental set. Samples were analyzed by liquid chromatography–electrospray ionization tandem mass spectrometry. Analysis was performed by in-house developed software tools.

The results suggest that bisphosphonates are able to modulate osteocyte's proteome and transcriptome and may open new pharmacological perspectives in the treatment of osteoporosis.

Conflict of Interest: None declared

P011-M

BONE-DERIVED LIPIDS STIMULATE BREAST CANCER CELL GROWTH THROUGH A CROSSTALK BETWEEN FARNESOID X RECEPTOR AND ESTROGEN RECEPTOR: IN VITRO AND CLINICAL DATA

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Bone is the most common site of metastasis from breast cancer. Recent data indicate that bone tissue contains bile acids that are accumulated from serum and may promote the migration of breast cancer cells (Silva, J Lipid Res 2006). Primary bile acids are ligands for farnesoid X receptor (FXR), a nuclear receptor involved in the regulation of bile acid metabolism. FXR has been recently detected in breast cancer cell lines. In this study, we examined the effects of FXR activation by chenodeoxycholic acid (CDCA) on breast cancer cells (MCF-7 and MDA-MB-231) cultured in steroid-free medium, and examined its expression in human tumor specimens.

High levels of FXR mRNA and protein were found in both breast cancer cell lines. CDCA stimulated the proliferation of ER+ MCF-7 cells (EC50 = 15 μM, EC100 = 50 μM), while it had no effect on the ER- MDA-MB-231 cell line. Antiestrogens and FXR suppression completely abolished the mitogenic effect of CDCA. CDCA downregulated ER by 40% and stimulated the expression of progesterone receptor by 316% in MCF-7 cells. These effects were completely abrogated by antiestrogens. Finally, FXR immunoprecipitation and subsequent ER Western blotting revealed physical interaction between both receptors.

We also looked for FXR expression in metastatic and primitive breast cancer tissue samples by immunohistochemistry (nuclear staining was graded from 0 to 8, summing up percentage and staining intensity). FXR was expressed in 58% of bone metastatic tissue biopsies (mean score = 2.46, n = 36), while it was detected in 47% of visceral metastases (mean score = 2.40, n = 15). We currently study the predictive value of FXR for bone metastasis development. Initial data revealed that FXR was expressed (mean score = 4.32) in 100% of breast cancer tissue samples (n = 29) in patients developing bone metastases. By contrast, it was detected at a lower level (mean score = 3.06) in only 61% of primitive cancer specimens (n = 13) from patients who developed visceral secondaries.

Altogether, our data reveal a positive crosstalk between FXR and ER, which accounts for FXR-mediated ER activation and subsequent mitogenic effects of FXR agonists in MCF-7 cells. Because of significant FXR and ER expressions in bone metastases, bone-derived lipids might contribute to the vicious cycle of tumor-induced osteolysis. Finally, our preliminary clinical data seem to reveal an intriguing relationship between FXR expression in breast cancer and the propensity to develop bone metastasis.

Conflict of Interest: None declared

P012-T

ACCELERATED BONE RESORPTION PROMOTES TUMOUR GROWTH IN A MURINE MODEL OF BREAST CANCER BONE METASTASIS

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Background/aims: Breast cancer cells preferentially metastasize to bone and cause osteolytic lesions. In mouse models of bone metastasis, inhibition of osteoclastic bone resorption by bisphosphonate treatment or with osteoprotegerin (OPG) results in bone protection and inhibition of tumour growth. However, it is not known whether high pre-existing levels of bone resorption enhance breast cancer establishment and growth in bone. In this study, high bone resorption was induced in young rapidly growing mice by low calcium diet and the effects on the growth of breast cancer cells were evaluated.

Methods: Female nude mice (age: 4 weeks) were placed on a diet with normal (0.6%) (Normal-Ca) or low (0.1%) (Low-Ca) calcium content. Mice were concurrently treated with vehicle or recombinant OPG (1mg/kg/day sc) (Amgen) (n = 16/group). Three days later, 50,000 cells of the bone-seeking cell line MDA-MB-231 were implanted by intra-tibial injection (day 0). Serum PTH,

osteocalcin and TRAP5b were measured at day 0 and at sacrifice (day 17). Lytic lesion area, tumour area and osteoclast numbers were measured by radiography and histomorphometry.

Results: Low-Ca increased serum PTH and TRAP5b levels. OPG treatment also increased serum PTH, with the largest effect in mice receiving Low-Ca. OPG profoundly reduced serum TRAP5b levels in mice on either diet. Low-Ca alone increased lytic lesion area by 43% ($p < 0.01$) and tumour area by 24% ($p < 0.01$, compared to Normal-Ca) at day 17; this was associated with increased breast cancer cell proliferation (by 24%) (ki67 immunohistochemistry staining). OPG treatment completely blocked development of lytic lesions and reduced tumour area by 46% and 45% ($p < 0.01$) in mice on Normal-Ca or Low-Ca, respectively. OPG treatment increased breast cancer cell apoptosis (TUNEL staining) and decreased cell proliferation.

Conclusion: Increased bone resorption due to a low calcium diet promotes tumour growth in bone. Increased bone resorption, rather than hypocalcemia or secondary hyperparathyroidism, produced this effect, as OPG treatment reduced tumour growth while enhancing hypocalcemia and secondary hyperparathyroidism. These results support the concept that high bone resorption enhances breast cancer tumour establishment and growth in bone. Also, our findings may have clinical implications since many cancer patients, much like the general population, are deficient in calcium and vitamin D and may present with secondary hyperparathyroidism.

Conflict of Interest: None declared

P013-S

ZOLEDRONIC ACID RESISTANCE MECHANISMS OF OSTEOSARCOMA CELL LINES

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We recently demonstrated selective and original anti-tumor effects of zoledronic acid (Zol) on several osteosarcoma cell lines independently of their p53 and Rb status. Zol inhibits cell proliferation, migration, disrupts the cytoskeleton and increases atypical apoptosis. The present study investigated the potential Zol-resistance developed by osteosarcoma cells after prolonged treatment. After 4 to 8 weeks of culture in the presence of 1 μM Zol, the effects of high dose Zol (10 to 100 μM) were compared between the untreated rat (OSRGA, ROS) and human (MG63, Saos2) osteosarcoma cells and Zol-pretreated cells in terms of cell proliferation, cell cycle analysis, migration assay and cytoskeleton organization. Long-term treatment with 1 μM Zol reduced the sensitivity of osteosarcoma cells to high concentrations of Zol as measured by XTT assay, cell cycle analysis and cell migration assay. The molecular mechanisms involved in this reduced-Zol sensitivity were then analyzed. XTT assays demonstrated that the Zol-resistant cells were always sensitive to conventional anti-cancer agents such as methotrexate, mafosfamide and doxorubicin and that the resistance process was not associated with the multidrug resistance (MDR) phenotype. However, similar experiments performed in the presence of clodronate and pamidronate revealed that this drug resistance was restricted to the nitrogen containing bisphosphonates. This resistance was also correlated with a higher transcript level and enzymatic activity of farnesyl diphosphate synthase (FPPS), the molecular target of Zol, in resistant cell lines. To demonstrate the involvement of FPPS in the Zol-resistance mechanism, the Zol-resistant cells were transfected with a siRNA for FPPS. Inhibition of FPPS activity was then assessed indirectly, by Western blot analysis of the unprenylated form of the small GTPase Rap1A. The transfection of Zol-resistant cells with FPPS siRNA strongly increased their sensitivity to Zol. This study demonstrates the induction of metabolic resistance after prolonged Zol treatment of osteosarcoma cells, probably by selecting clones overexpressing FPPS. This study confirms the therapeutic potential of Zol for the treatment of bone malignant pathologies but reveals the possibility that the treatment regimen may be important in terms of duration and dose to avoid the development of drug metabolic resistance.

Conflict of Interest: None declared

P014-M

THE PPAR GAMMA AGONIST PIOGLITAZONE EXAGGERATES BONE LOSS, WHILE THE PPAR ALPHA AGONIST FENOFIBRATE MAINTAINS BMD AND BONE ARCHITECTURE IN OVARECTOMIZED RATS

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Peroxisome proliferator-activated receptors (PPARs) exist in three different isoforms (alpha, beta/delta and gamma) and belong to the steroid nuclear superfamily of receptors. PPARs are known to modulate the expression of genes involved in lipid metabolism and fat storage. The presence of PPARs has also been demonstrated in bone cells and a role in bone metabolism has been postulated. In this study we have examined the effect of the PPARalpha agonist fenofibrate and the PPARgamma agonist pioglitazone on bone mineral density (BMD) and bone architecture in ovariectomized rats.

Forty female Sprague Dawley rats 12 weeks of age were assigned to the following four groups: 1: sham operated rats given methocel (vehicle), 2: ovariectomized (ovx), given vehicle, 3: ovx, given fenofibrate (90 mg/kg), or 4: ovx, given pioglitazone (35 mg/kg), by daily intragastric gavage for four months. Body weight was registered throughout the study, and plasma osteocalcin was measured at the end of the study by an ELISA kit. BMD was measured by dual x-ray absorptiometry (DXA) and bone architecture of the femoral shaft and head was examined by μCT.

There was no difference in body weight between ovx control rats and the treatment groups at the end of the study, while the sham rats had significantly lower weight. Plasma osteocalcin levels were significantly higher in ovx fenofibrate treated group compared to sham operated group, while ovx pioglitazone treated group had significantly lower plasma osteocalcin compared to all the other groups. At the end of the study, both whole body and femoral BMD was significantly higher in sham rats and ovx rats receiving fenofibrate compared to the ovx control rats, whereas the ovx pioglitazone treated rats had significantly lower whole body and femoral BMD. Most parameters measured by μCT were significantly decreased in rats receiving pioglitazone, both compared to the sham and ovx control groups. Cortical volume, cortical thickness, trabecular volume and trabecular bone volume fraction were significantly higher in fenofibrate treated rats compared to ovx controls. In conclusion, treatment with the PPARalpha agonist fenofibrate prevented the ovariectomy induced bone loss and changes in bone architecture and increased plasma osteocalcin level, while the PPARgamma agonist pioglitazone exaggerated bone loss and affected bone architecture negatively in ovariectomized rats.

Conflict of Interest: None declared

P015-T

SYSTEMIC ADMINISTRATION OF THYROID STIMULATING HORMONE (TSH) RESTORES BONE VOLUME AND STRENGTH WITHOUT AFFECTING SERUM T3 AND T4 LEVELS IN AGED OVARIECTOMIZED RATS

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The loss of function studies have shown that TSH receptor haploinsufficient mice with normal circulating thyroid hormone levels have a reduced bone mass suggesting that TSH itself directly affects bone remodeling. We have demonstrated for the first time that the exogenous administration of low doses of TSH prevents bone loss and restores bone mass in aged OVX rats through both anabolic and anti-resorptive action on bone remodeling, thus enhancing trabecular and cortical bone quality and strength. Mechanism of action studies indicate that TSH promotes osteoblast differentiation by inducing the activity of alkaline phosphatase of bone marrow derived mesenchymal stem cells and stimulates osteoblast activity by enhancing matrix mineralization in osteoblast-enriched cultures. In addition, TSH inhibits RANKL-induced osteoclast differentiation of bone marrow derived hematopoietic stem cells and a premonocytic cell line RAW 264.7. The therapeutic effects of TSH in aged OVX rats was achieved with relatively low doses of the hormone (0.1 to 0.3 μg/rat) and the clearance of TSH was similar between control and OVX animals, with a mean half-life of 1.5 hours. Importantly, administration of these low doses of TSH did not induce acute (within 24 hr following administration) or chronic (3x/wk for 8 weeks of administration) state of hyperthyroidism since there was no significant elevation in serum T3 and T4 levels as determined during the course of the study. However, a high dose (100 μg/rat) of TSH significantly increased serum T4 levels. These data indicate that doses of TSH which result in beneficial effects on bone remodeling do not appear to change thyroid hormone homeostasis. Collectively, our results demonstrate low doses of TSH exhibit both anabolic and anti-resorptive effects on bone remodeling without affecting serum T3 and T4 levels, and thus may provide additional therapeutic benefits beyond the anti-resorption actions of bisphosphonates or SERMs and the anabolic actions of PTH.

Conflict of Interest: Rebecca Sendak, Michael Huff, Kerry Culm-Merdek, Susan C. Schiavi, John M. McPherson, and T. Kuber Sampath are employees of Genzyme.

P016-S

THE ACTIVIN A – FOLLISTATIN SYSTEM: POTENT REGULATOR OF EXTRACELLULAR MATRIX SYNTHESIS AND MINERALIZATION

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Bone quality is an important determinant of osteoporosis and osteoblasts play an important role in the control and maintenance of bone quality. Several members of the transforming growth factor-β (TGFb) superfamily including, TGFβ and bone morphogenic proteins are well known regulators of bone formation. Activins belong to the TGFβ superfamily and in this study we investigated the impact of activin signaling on human osteoblast function.

To study the role of activins in human osteoblasts we used two different human osteoblast mineralization models. Activin A strongly inhibited matrix mineralization in osteoblast cultures, whereas the activin antagonist follistatin increased matrix mineralization. Protein measurement showed that osteoblasts produce significant amounts of activin and follistatin. Moreover, osteoblasts produced activin A and follistatin in a differentiation-dependent manner resulting in suppressed endogenous activin signaling during stages of mineralization. Time-course studies revealed that activin A inhibits mineralization by actions in the pre-mineralization period. Following this, comparative activin A and follistatin gene expression profiling (Affymetrix Genechips) showed a strong regulation of a wide range of extra cellular matrix (ECM) genes in the pre-mineralization period. Finally, to take these mineralization effects to a broader context we showed that activin A also inhibited mineralization in a model for vascular mineralization using vascular smooth muscle cells.

In summary, the key findings are: 1) osteoblasts express activin A and its natural inhibitor follistatin to control activin signaling in a differentiation-dependent manner, 2) activin inhibits mineralization in a human bone formation model as well as in a model for vascular mineralization, 3) activin does so by changing the expression of a wide range of matrix proteins prior to the onset of mineralization leading to a matrix composition with no or reduced mineralizing capacity. This led to the conclusions that activin signaling is a potent regulator of matrix mineralization. Using activin A, follistatin, or analogs of these compounds mineralization can be controlled in two directions. As a consequence activin signaling and activin target genes are important therapeutic targets to control matrix mineralization in bone as well as mineralization in pathological conditions.

Conflict of Interest: None declared

P017-M

ROLE OF VASCULAR ENDOTHELIAL GROWTH FACTOR AND ITS RECEPTORS AS MEDIATORS OF VARIOUS PRO-FIBROGENIC ACTIONS OF PARATHYROID HORMONE-RELATED PROTEIN IN THE KIDNEY

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Recent evidence supports that parathyroid hormone-related protein (PTHrP) and vascular endothelial growth factor (VEGF) may act co-ordinately to modulate bone turnover. Both factors can promote cell survival and also affect cell matrix remodelling in various settings. We hypothesized that PTHrP and VEGF might interact to promote fibrogenesis in the kidney. We used an experimental model of unilateral ureteral obstruction (UUO) in normal (N) and transgenic (TG) mice overexpressing PTHrP in the proximal tubule. VEGF, VEGF receptor (VEGFR)-1 and 2 and fibronectin gene expression were analyzed by real-time PCR. alpha-Smooth muscle actin (alpha-SMA), integrin-linked kinase (ILK) and E-cadherin protein expression were assessed by Western blot and/or immunohistochemistry. Apoptotic cells were detected by TUNEL or flow cytometry (in vitro studies). VEGFR-1 gene was rapidly upregulated in the obstructed kidneys of N mice at 2 days, decreasing thereafter. However, this upregulation was maintained in TG mice up to 6–12 days after UUO. In contrast, VEGF and VEGFR-2 mRNA levels were downregulated in the obstructed kidneys of both types of mice. Renal fibronectin mRNA levels, and positivity for alpha-SMA and ILK (fibrosis markers) were increased at 6 days after UUO; and this increase was 2-fold higher in TG mice than in control littermates. An anti-VEGF (alpha-VEGF) antibody decreased the overexpression of these markers in both N and TG mice. In addition, the obstructed kidneys from the latter mice showed less apoptotic interstitial cells than those from their normal littermates. However, administration of alpha-VEGF increased cell apoptosis into the renal interstitium after UUO in both types of mice ($p < 0.05$). In vitro, PTHrP(1–36) increased (2-fold vs basal) alpha-SMA and ILK protein expression in tubulo-epithelial MCT and NRK 52E cells; and decreased the protein levels of E-cadherin. Moreover, PTHrP(1–36) significantly decreased apoptosis induced by ciclosporine A in MCT and NRK 52E cells, and also in renal fibroblastic NRK 49F cells. Both the fibrosis markers alterations and anti-apoptotic effects induced by PTHrP(1–36) in these cells were abolished by alpha-VEGF. SU5614, a VEGFR-2 inhibitor, significantly decreased the aforementioned effects of PTHrP (1–36) in NRK 52E cells, but not in MCT cells which do not express detectable levels of VEGFR-2.

Collectively, these findings indicate that VEGF and its receptors are important mediators of several pro-fibrogenic effects of PTHrP in the kidney.

Conflict of Interest: None declared

P018-T

GONADAL ANDROGENS AND PERIPHERAL AROMATIZATION AFFECT PERIOSTEAL BONE GROWTH IN MALE MICE DURING PUBERTY

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Androgens and estrogens are known to play a role in male skeletal growth and maturation. The aim of the present study was to determine if and to what extent peripheral aromatization of androgens into estrogens impacts on male trabecular and cortical bone development during puberty. To this end, male C57Bl6/J mice were either sham-operated (sham) or orchidectomized (orx) at start of puberty (3 weeks of age). Orx mice were treated for 5 weeks with either vehicle or aromatase inhibitor (anastrazole, 10 mg/kg/day p.o.). Tibial trabecular and cortical BMD and bone geometry were measured weekly by in vivo pQCT from 3 till 8 weeks of age. Also serum was collected weekly by tail bleeding for measurement of IGF-I and IGF binding protein 3 (BP3). Orx performed at start of puberty impaired radial bone expansion resulting in a significantly reduced cross-sectional area (CSA) (−11% vs. sham, $p < 0.005$), periosteal perimeter (Ps.Pm.) (−5% vs. sham, $p < 0.005$) and cortical thickness (−14% vs. sham, $p < 0.0001$) at 8 weeks of age. Interestingly, an additional decrease of pubertal radial bone expansion was observed in orx mice treated with the aromatase inhibitor; a significant reduction in CSA (−15% vs. sham, $p < 0.0001$) and Ps.Pm. (−8% vs. sham, $p < 0.0001$) was already observed at 5 weeks of age and was maintained till the end of the experimental period (8 weeks of age). Moreover, the impaired cortical bone expansion in orx mice treated with the aromatase inhibitor was associated with lower serum IGF-I levels at 4 weeks (−33% vs. sham, $p < 0.005$) and 5 weeks of age (−23% vs. sham, $p < 0.005$), and with reduced serum IGFBP3 at 4 weeks (−36% vs. sham, $p < 0.0005$) and 5 weeks of age (−42% vs. sham, $p < 0.0001$). Moreover, serum IGF-I levels were strongly correlated with Ps.Pm. ($r = 0.85$, $p < 0.0001$) in these mice. Orx also induced a sustained reduction of trabecular BMD during the entire experimental period (−44% vs. sham, $p < 0.0001$), which was not further affected by inhibition of aromatase activity. This finding indicates that trabecular bone modeling is mainly determined by testicular androgens, whereas aromatization of androgens into estrogens has no apparent effect. On the contrary, combined testicular androgen withdrawal and inhibition of aromatase activity result in an additional decrease of periosteal bone growth. We conclude that gonadal androgens and peripheral aromatization are both needed to obtain optimal stimulation of periosteal bone growth during male puberty.

Conflict of Interest: None declared

P019-S

KINETIC ANALYSIS OF CONFORMATIONAL CHANGES IN FARNESYL PYROPHOSPHATE SYNTHASE INDUCED BY NITROGEN CONTAINING BISPHOSPHONATES

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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 N-BPs inhibit the enzyme by a slow tight binding mechanism which involves conformational changes in the enzyme. Preincubating the enzyme with N-BP causes an apparent and large increase in inhibitory potency e.g. the Ki for Risedronate (RIS) shifts from 72nM to 0.34nM. This shift is observed with most N-BPs, with the Ki of Zoledronate (ZOL) shifting from 72 to 0.07nM, Ibandronate (IBN) from 195 to 3.6nM and Alendronate (ALN) from 416nM to 57.2nM. We have endeavoured to quantify the extent of the conformational change (known as an isomerisation constant – ICON) and calculated the preincubated Kis for a wide range of N-BPs for which in vivo potency is known. We have correlated the

preincubated Kis with in vivo potency which reveals that the overall inhibition of FPPS by N-BPs is extremely closely linked ($R = 0.98$ $p = 0.0001$) to antiresorptive efficacy.

The magnitude of the ICON for clinically relevant N-BPs covered a large range of values, the greatest being ZOL 1244, RIS 226, IBN 53, and the least being ALN 6.3. The larger the isomerisation constant, the greater the enzyme inhibition and correlation with in vivo potency ($R = 0.76$ $p = 0.05$), with the most potent N-BPs having the largest ICON. This order also matches the order of the IC50s which are 4.1, 5.7, 25.4 and 330nM respectively. The fact that RIS and ZOL exhibit more closely related IC50s also suggests that isomerisation is likely to be a major component in enzyme inhibition by highly potent compounds versus weaker inhibitors such as IBN and ALN.

In conclusion it is apparent that the overall ability of the BP to inhibit the enzyme is very closely linked to the in vivo potency, however the ability of a BP to hold the enzyme in the isomerised state also plays a crucial role in the overall potency.

Conflict of Interest: J. Dunford, Proctor and Gamble pharmaceuticals, Grant research support

P020-M

STRONTIUM RANELATE COUNTERACTS THE DRAMATIC INCREASE OF ENDOCORTICAL RESORPTION IN MICE WITH A SEVERE OSTEOPOROSIS

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The increased endocortical bone resorption contributes to the bone fragility observed in post-menopausal women. Mice overexpressing Runx2 in osteoblastic lineage, a murine model of severe osteoporosis with spontaneous fractures, like osteoporotic women exhibit an expansion of the marrow cavity due to endocortical bone resorption resulting in both a substantial cortex thinning and a decrease in cortical density. We showed previously that part of the strontium ranelate efficacy in preventing vertebral fracture in these mice was due to increase in bone mass and improvement in the trabecular microarchitecture. We present here the strontium ranelate effect on cortical compartment.

Female mice overexpressing Runx2 (7-week-old) were randomized to receive either strontium ranelate (1800 mg/kg/d) or vehicle (VEH) for 9 weeks once daily by gavage. At the end of treatment, strontium ranelate-treated mice had a plasmatic strontium concentration in the same range than the one observed in patients receiving the therapeutic dose.

We showed previously using histomorphometric analysis that at the end of the experiment vertebrae cortical thickness increased significantly (VEH, 60.2 ± 4.0 μm vs strontium ranelate, 70.5 ± 4.7 μm;$p < 0.001$). At the same time-point in-vivo pQCT measurements of the tibia at the metaphysis and diaphysis level were also carried out. Strontium ranelate significantly increased cortical thickness at both metaphysis (VEH, 44 ± 27 μm vs strontium ranelate, 136 ± 31 μm;$p < 0.0001$) and diaphysis (VEH, 168 ± 34 μm vs strontium ranelate, 203 ± 15 μm;$p = 0.018$). Furthermore we showed using histomorphometric analysis that strontium ranelate decreased significantly the marrow cross sectional area, i.e., the internal cross sectional area of the bone (VEH, 0.087 ± 0.020mm2 vs strontium ranelate, 0.065 ± 0.012mm2;$p = 0.0223$), and the marrow diameter, i.e., the bone internal diameter (VEH, 332 ± 39 μm vs strontium ranelate, 286 ± 28 μm;$p = 0.0227$) at the diaphysis. The same trends were observed at the metaphysis of the tibiae

These data show that strontium ranelate effect in osteoporotic mice overexpressing Runx2 concerns also on the cortical bone. Strontium ranelate reduces the endocortical bone resorption associated with osteoporosis in long bone. This is important in a biomechanical view point, since internal bone diameter decrease contributes highly to the bone strength increase.

Conflict of Interest: None declared

P021-T

SRP3 AND SRP9 POLYMORPHISMS IN THE SOST GENE ARE ASSOCIATED WITH PERIMENOPAUSAL BONE MASS AND EARLY POSTMENOPAUSAL BONE LOSS

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The SOST gene encodes sclerostin that participates in bone metabolism by inhibiting bone formation. Moreover, loss-of-function mutations in this gene are related to the rare disorders of sclerosteosis and Van Buchem's disease, characterized by an overgrowth of the skeleton. The SOST gene has therefore been suggested as a candidate gene for the genetic influence on bone mass and fracture risk.

In this study we have examined the effect of two common polymorphisms in the SOST gene, SRP3 and SRP9, located in the promoter region and in the region deleted in Van Buchem's disease, on body composition, BMD, BMC, and fracture risk in 1714 perimenopausal women participating in the Danish Osteoporosis Prevention Study.

Genotype frequencies were 41% del-del, 45% del-GGA and 14% GGA-GGA for the SRP3 polymorphism, and 32.2% AA, 49.4% AG and 18.4% GG for the SRP9 SNP.

Women carrying the 3 bp insertion of the SRP3 polymorphism had lower fnBMD 0.791 ± 0.115 g/cm^2 vs. 0.802 ± 0.114 g/cm^2 in non-carriers ($p < 0.05$). Similar results were found at thBMD. BMC was also significantly reduced in carriers of the insertion fnBMC 4.1 ± 0.7 g vs. 4.2 ± 0.7 g in women without; lsBMC 46.9 ± 8.3 g vs. 47.9 ± 8.5g, $p < 0.05$. Moreover, the carriers of the insertion had significantly higher levels of serum bone alkaline phosphatase. No effect of the SRP3 polymorphism on early postmenopausal bone loss or fracture risk could be demonstrated.

The SRP9 polymorphism is also associated with BMD. Women carrying at least one G allele had significantly higher wbBMD 1.079 ± 084 g/cm^2 vs 1.070 ± 083 g/cm^2 in non-carriers ($p < 0.05$). Statistical significance was maintained after correcting for weight. The polymorphism also affected postmenopausal bone loss: 5 years after menopause, women carrying the G allele had a greater decrease in BMD compared to those with the AA genotype: wbBMD -1.94 ± 3.3 % in women carrying the G allele vs. -1.73 ± 3.1 % in women with the AA genotype ($p < 0.01$); thBMD -4.29 ± 4.7 % vs. -3.93 ± 4.7 %, $p < 0.01$. The polymorphism was not associated with fracture risk in these early postmenopausal women.

In summary, SRP3 and SRP9 polymorphisms in the SOST gene are associated with perimenopausal BMD and BMC. Moreover, the SRP9 polymorphism is associated with increased early postmenopausal bone loss.

Conflict of Interest: None declared

P022-S

NO EVIDENCE OF ASSOCIATION BETWEEN CODING LRP5/6 POLYMORPHISMS AND BMD AND FRACTURE RISK IN THREE INDEPENDENT LARGE-SCALE POPULATION BASED STUDIES OF YOUNG AND ELDERLY MEN

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The LRP5/6-Wnt signalling system is of importance for regulating osteoblastic activity, which became clear after findings that inactivating mutations in LRP5 cause severe osteoporosis, whereas activating mutations cause osteosclerosis. Besides known mutations the gene for the LRP5 and 6 harbours naturally occurring coding polymorphisms causing amino acid substitution. Of these polymorphisms the rs4988321 (V667M) and the rs3736228 (A1330V) in the LRP5 gene and the rs2302685 (I1062V) in the LRP6 gene have been reported to influence bone mineral density (BMD) in cohort studies.

The aim of this study was to investigate the association between LRP5 (rs4988321 and rs3736228) and 6 (rs2302685) polymorphisms and BMD in three independent large-scale population based cohorts of elderly and young men from Sweden and Hong Kong; the MrOS Studies in Sweden ($n = 3016$, aged 70–80 years) and in Hong Kong ($n = 2000$, aged > 65 years) and the Swedish GOOD Study ($n = 1068$, aged 18–20 years). We also aimed to study the association between the LRP5 and 6 polymorphisms and fracture risk in the Swedish MrOS Study.

BMD was measured at femoral neck, total hip and lumbar spine by Lunar and Hologic equipments and standardised according to circulating phantoms. Standardised BMD was calculated in the Swedish MrOS Study using previously reported algorithms. Spine X-ray analyses were performed in the Swedish MrOS Study in 1349 randomly selected individuals for vertebral fracture assessments, where 16.2% ($n = 218$) of the subjects were reported to have at least one prevalent vertebral fracture. All genotypes in the three study populations were determined by allelic discrimination using the 5′ nuclease Taqman assay.

The genotype frequencies were similar to frequencies previously reported and all polymorphisms were in Hardy–Weinberg equilibrium. Notably, the

rs4988321 SNP was not polymorphic in the Hong Kong population. The statistical models showed clearly that there are no independent correlations between BMD at any site and the LRP5/6 polymorphisms in either study cohorts. Moreover, when studying gene–gene interaction no evidence of interaction between LRP5 and LRP6 polymorphisms were observed.

No evidence of association between the polymorphisms and risk of vertebral fractures were seen in the Swedish MrOS Study.

In these three large cohorts, with a combined number of >6000 individuals, there are no correlation between polymorphisms in the LRP5 and LRP6 genes and BMD or risk of vertebral fractures.

Conflict of Interest: None declared

P023-M

VARIATIONS IN THE LRP5 AND LRP6 GENES: ASSOCIATIONS WITH BONE MINERAL DENSITY IN SCOTTISH POSTMENOPAUSAL WOMEN

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Bone mineral density (BMD) is highly heritable trait, and the most important clinical predictor of fracture risk in osteoporosis. Osteoporosis is a complex polygenic disease with many genes making a relatively small contribution to BMD.

By studying monogenic bone diseases, low-density lipoprotein receptor-related protein 5 (LRP5) and its close homologue low-density lipoprotein receptor-related protein 6 (LRP6) were identified as candidate genes for bone regulation.

Gain-of-function mutations within the LRP5 gene cause a high bone mass phenotype whereas loss-of-function mutations cause osteoporosis pseudoglioma syndrome (OPPG), a low bone mass phenotype. Human and mouse studies indicate LRP5 and LRP6 regulate bone metabolism via the Wnt signalling cascade.

In this study, we investigated the relationship between SNPs of the LRP5 and LRP6 genes, and BMD, bone loss and fracture in a large population based study of 2940 women who comprise the Aberdeen Prospective Osteoporosis Study (APOSS) from the North East of Scotland. A longitudinal study, data was available for BMD and other clinical data at the baseline visit and at the follow-up visit approximately 6.3 years later. Genotyping was performed for V667M (rs4988321) and A1330V (rs3736228) in LRP5 and I1062V (rs2302685) in LRP6.

Associations between LRP5/6 SNPs and bone phenotypes were analysed using GLM-ANOVA, correcting for confounding factors such as age, weight, height, menstrual status, HRT use, smoking and physical activity level.

We found a highly significant association between lumbar spine BMD (LSBMD) at follow-up and LRP5 A1330V ($p=0.008$). Trends for association were observed at the femoral neck (FNBMD) at baseline ($p=0.031$) and follow-up ($p=0.060$). Suggestive association was also found for LRP5 V667M with LS BMD ($p=0.063$) and FN BMD ($p=0.044$) at follow-up. No association with change in BMD or fracture was observed.

No association was found between LRP6 I1062V and BMD at any site, bone loss or fracture.

In conclusion, our results suggest that the LRP6 gene does not make an important contribution to the regulation of BMD in postmenopausal women. By contrast, in this population, variants of the LRP5 gene are an important source of variation in BMD, especially at the lumbar spine.

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Conflict of Interest: None declared

P024-T

VARIATION IN THE BONE MORPHOGENETIC PROTEIN 2 GENE: BODY COMPOSITION AND BONE MINERAL DENSITY IN YOUNG AND ELDERLY WOMEN

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Introduction: Bone morphogenetic protein-2 (BMP2) plays a critical role in osteoblastogenesis and adipogenesis from osteoprogenitor cells. The favoured pathway depends on complex interactions including BMP2 concentration and the presence of other regulators. BMP2 gene variants may contribute to osteoporosis risk. To date their effect on adiposity and muscle mass has not been studied. Since body size is itself a risk factor for osteoporosis and body mass index is heritable, the aim of this study was to investigate the relationship between BMP2 gene variants, body composition and BMD in two population-based cohorts of Swedish women.

Methods: We studied 4 single nucleotide polymorphisms (SNP) in the vicinity of the BMP2 gene, reflecting haplotype structure in that region of chromosome 20. These were: Ser27Ala; rs235710; rs235767 and rs235754. We analysed PEAK-25 young women aged 25 ($n=993$) and OPRA elderly women aged 75 ($n=1001$). Total body fat mass (tbFM), lean mass (tbLM) and BMD were measured by DEXA.

Results: In the elderly women, we found no relationship between variation in the BMP2 gene, body composition or BMD. In the young women however we observed an effect on lean mass, but not fat mass or BMD. Regression analysis identified rs235767 (A/C) as a predictor of tbLM ($p=0.018$) and an association was observed whereby tbLM values decreased from A to C homozygotes, with a gene dose effect ($p=0.028$). Ser37Ala showed the opposite trend, tbLM increasing in individuals carrying the rare allele, however this was not significant. A haplotype derived from these SNPs (Ser-C), occurred in 36% of the population. Individuals carrying 2 copies had the lowest tbLM values, compared to 0 copies (highest) and 1 copy (intermediate) $p=0.042$. Although these SNPs were not significantly associated with tbFM or LSBMD, the directions of the relationships were similar to those observed for tbLM.

Conclusions: Our data suggests that the BMP2 gene may be a pleiotropic genetic factor contributing to both bone mass and body composition, at least in young women. Functional variants within the gene appear to have opposing effects on lean mass, fat mass and bone mass, with the direction of the relationship identical for all measurements. This relationship between BMP2 and body mass may explain why we previously observed effects on bone quality rather than bone density in this population.

Conflict of Interest: None declared

P025-S

IDENTIFICATION OF SEX-SPECIFIC EFFECTS OF TNFRSF11B POLYMORPHISMS ON THE RISK TO DEVELOP PAGET'S DISEASE OF BONE

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Background: Juvenile Paget's disease is caused by mutations in *TNFRSF11B* encoding osteoprotegerin. Although mutations in this gene have never been found in typical PDB patients, there are indications that SNPs in *TNFRSF11B* might contribute to the risk to develop PDB.

Methods: We recruited a population of 131 sporadic Belgian PDB patients and 171 controls. By means of the HapMap 17 tagSNPs and 4 multi-marker tests were selected. In order to replicate the findings observed in the Belgian study population, genotyping data of SNPs generated in a UK population (312 cases and 378 controls) (1) were reanalysed.

Results: When analysing the entire Belgian study population, for two SNPs associations were found: rs11573871 ($p=0.040$) and rs1485286 ($p=0.044$). When subsequently analysing males and females separately, both these associations turned out to be driven by females (p-values 0.038 and 0.005 respectively, 56 cases and 78 controls). In addition, 3 other tag SNPs and 1 multi-marker test turned out to be associated in females only. These were: rs2073617 (C950T, $p=0.010$), rs6415470 ($p=0.037$), rs11573869 ($p=0.009$) and the multi-marker test giving information on rs1032129 ($p=0.002$). The haplotypes formed by the associated SNPs in the female population also turned out to be associated with the disease ($p=0.007$). Reanalysis of the data reported by Daroszewska et al. (1) indicated that the associations found for SNPs C950T and C1181G in the UK population were also only driven by females (p-values 0.026 and 0.007 respectively, 146 cases and 216 controls). Finally, we combined the genotyping results of the 2 female populations for SNPs C950T and C1181G and used the Cochran–Mantel–Haenszel test to calculate common odds ratios. This meta-analysis indicated that the T allele of C950T is associated with a 1.520 times increased risk to develop PDB in females (95%CI: 1.176–1.965, $p=0.002$). Although SNP C1181G did not show association in the Belgian study population, meta-analysis on the combined data set provided proof of the involvement of this SNP in PDB ($p=0.003$, OR = 1.497, 95% CI: 1.158–1.937).

Conclusions: We have shown for the first time that SNPs influencing the risk to develop Paget's disease of bone could be sex-specific. Further research is necessary to identify the causative variants in *TNFRSF11B* and to elucidate the molecular pathogenic mechanism.

(1) Daroszewska et al. (2004) JBMR, 19(9): 1506–1511.

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Conflict of Interest: None declared

P026-M

THIGH MUSCLE AREA CORRELATES WITH BONE MINERAL DENSITY (BMD) IN HEALTHY MEN AT THE TIME OF PEAK BONE MASS – RESULTS FROM THE ODENSE ANDROGEN STUDY

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Background: Body weight (BW) correlates to Bone Mineral Density (BMD). BW, however, consists of fat mass and lean body mass and it is uncertain whether BW per se, lean body mass or fat mass are associates with bone mineral density. Aim: We hypothesize that lean body mass, abdominal muscle area and thigh muscle area are associated with peak bone mass in men. Participants and design: The Odense Androgen Study is a population-based, prospective, observational study on endocrine status, body composition, muscle function, and bone metabolism in young men. In brief, 3000 males aged 20–30 years were randomly selected from the civil registration database in Funen County, Denmark, and invited by mail to participate in the study. A total 783 gave written informed consent to participate. BMD of the spine, hip and whole-body was measured using a hologic-4500a densitometer. The area of subcutaneous fat on thigh, thigh muscle, abdominal subcutaneous fat, intra-abdominal fat and abdominal muscle were measured by MRI (Magnetom Open Viva, Siemens) using a T1-weighted gradient echo sequence. Results: The relationship between thigh-muscle area, abdominal muscle area, subcutaneous fat on thigh and abdomen and intra-abdominal fat as tested using multiple regression analysis is shown in the table as partial correlation coefficients. Thigh muscle area and intra-abdominal fat were significant predictors of BMD in spine, hip and whole-body. Conclusion: Our results suggest that the correlation between BW and BMD in young men is mediated by the association between muscle mass (thigh muscle area) and BMD explaining 11–31% of the variation in this parameter. Intra-abdominal fat is a negative independent predictor of BMD explaining 0.8–3% of the variation in BMD.

Table: Data are shown as partial correlation coefficients

BMD	Spine	Hip	Whole body
Thigh Muscle	0.42***	0.56***	0.33***
Subcut. Fat Abdomen	NS	NS	NS
Subcut. Fat Thigh	NS	NS	NS
Muscle Abdominal	NS	NS	0.08**
Intraabd. Fat	−0.13**	−0.09**	−0.17***
Overall Model	0.43***	0.56***	0.50***

Conflict of Interest: None declared

P027-T

ADIPONECTIN IS A PREDICTOR OF PEAK BONE MASS IN MEN

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Introduction: Body weight (BW) is positively associated with bone mineral density (BMD) and inversely correlated to fracture risk. BW corresponds to the sum of lean body mass (LBM) and whole body fat mass (WB FM). Variations in adipocytokines have been proposed as explanation of the relationship between BW and BMD. Serum levels of adiponectin (AN), a cytokine expressed in adipocytes, are negatively correlated with WB FM and Body Mass Index (BMI). The aim of this study was to examine the potential inverse relation between AN, FM and peak bone mass (PBM).

Methods: The Odense Androgen Study is a population-based, prospective, observational study regarding the interrelationship between endocrine status, body composition, muscle function, and bone metabolism in young men. 3000 males aged 20–30 years were randomly selected from Central Office of Civil Registration and invited by mail to participate in the study. Each received a questionnaire which was returned by 68%. 783 gave written informed consent to further participation in the clinical follow-up. The study population was equivalent to the background population on socio-economic status, BMI, and educational level. BMD, WB FM, and LBM were measured by use of DXA scans on hip, lumbar spine, and whole body (WB). MR scans were used to estimate the total abdominal FM as well as the cross sectional area of the femoral shaft.

Results: In the spine, no age-related change was detected. In the hip, however, maximum BMD was achieved at the age of 22. AN was significantly correlated to hip BMD (rho: −.12, p = .002) and bone marrow size (rho: .13, p = .014) whereas no correlation was found in the lumbar spine (rho: −.07, p = .06) or cortical bone of the femoral shaft (rho: −.02, p = .70). In the hip, an inverse association between BMD and AN remained after adjustment for age, smoking, alcohol, sporting activities, total WB FM and LBM in a multiple regression analysis (R2 = .24; r = −.09; p = .019). In a similar analysis, only AN and LBM were significantly associated with bone marrow size (R2 = .13; r = .30 and r = .19; p < .0001). AN was negatively associated with WB, subcutaneous and visceral FM (rho = −.19, −.18, −.16, p < .001) and LBM (rho = −.10; p = .001). In partial correlation analysis, WB FM and not LBM was significantly associated with AN (r = −.13; p = .001).

Conclusion: AN was inversely correlated with WB FM and BMD in the hip.

Conflict of Interest: Brixen K, Eli Lilly, Consultant Brixen K, Eli Lilly, Speakers bureau Andersen M, Ipsen, Grant/Research Support Andersen M, Ipsen, Speakers Bureau Brixen K, MSD, Grant Research Support Brixen K, Novartis, Consultant Brixen K, Novartis, Speakers Bureau Andersen M, Novo Nordisk, Speakers Bureau Andersen M, Pfizer, Speakers Bureau Brixen K, Servier, Consultant Brixen K, Servier, Speakers Bureau

P028-S

INTRINSIC BONE QUALITY IN FRAGILITY HIP FRACTURE PATIENTS: ALTERED MINERALISATION AND MICRODAMAGE ACCUMULATION

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The quality of the bone in the ageing human skeleton depends on the amount of bone, geometry, microarchitecture, material properties of the bone (including the degree of mineralisation and microdamage accumulation), and the molecular and cellular regulation of bone turnover and repair. A modification at one or more of these hierarchical levels in the bone may result in a deterioration of the bone's mechanical performance and thus compromise bone strength. This study aimed to identify material and structural factors that contribute to bone quality in fragility hip fracture patients (Fx) and age-matched controls (C). Intertrochanteric bone cores were obtained from patients undergoing hemi-arthroplasty surgery for a non-traumatic subcapital femoral fracture (7f, 5m, mean age 80 [67–91] years), and from controls at autopsy (9f, 4m, 77 [65–88] years). Samples were *en bloc*-stained in basic fuchsin and resin-embedded, for quantitative backscattered electron imaging of the degree of mineralisation, and histomorphometric assessment of bone architecture, resorption, and microdamage. Trabecular bone volume, architectural parameters, and indices of bone resorption were not different between groups. Both groups showed normal distributions of percent calcium; however, the fracture cohort was less mineralised (mean % calcium: Fx: 24.2%, C: 24.9%). Linear microcrack parameters were similar between groups. Whereas diffuse microdamage was increased in bone from fracture patients (DxV/BV[%]: Fx: 1.51(0.19–4.67), C: 0(0–0.33), $p < 0.01$ [median(quartiles)]). The ratio of microdamage (cracks and diffuse) density to resorption site density was higher in the fracture group compared to controls (Mdx.Dn/Rs.Dn: Fx: 0.44(0.10–0.86), C: 0(0–0.31), $p < 0.01$), which is suggestive of an unrepaired microdamage burden in the fracture cohort. Interestingly, an inverse association between diffuse microdamage and the degree of mineralisation was observed in the fracture cohort ($p < 0.05$). Microdamage accumulation in bone from fragility hip fracture patients may be a consequence of altered bone matrix properties and/or defective damage repair mechanisms. Collectively, these data suggest that increased fragility fracture risk is associated with under-mineralisation and microdamage accumulation rather than changes in bone volume or architecture. Inclusion of bone material property data together with other bone quality measures may hold the key to better fracture risk assessment and treatment efficacy.

Conflict of Interest: NL. Fazzalari, Eli Lilly, Grant Research Support NL. Fazzalari, Procter and Gamble, Grant Research Support JS. Kuliwaba, Procter and Gamble, Grant Research Support P. Sutton-Smith, Eli Lilly, Grant Research Support

P029-M

PARATHYROID HORMONE IS AN INDEPENDENT PREDICTOR OF FIBROBLAST GROWTH FACTOR-23 IN HEALTHY MEN: MROS SWEDEN

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Fibroblast Growth Factor-23 (FGF23) is a circulating factor that regulates serum levels of inorganic phosphate (Pi). The importance of FGF23 is seen in several diseases, such as XLH, ADHR and TIO, where increased levels of FGF23 result in severe hypophosphatemia and rickets or osteomalacia. The hypophosphatemia is mainly due to altered kidney expression of the sodium-phosphate co-transporter 2a (NPT2a) which results in a decrease in Pi-reabsorption, and the skeletal changes is thought to be due to endocrine or auto/paracrine actions of FGF23, although the exact mechanisms is not fully understood. The objectives of the current study was to investigate the association of circulating FGF23 to Pi, calcium, PTH as well as other biochemical variables and to determine a reference range for serum intact FGF23 levels in a population-based cohort of elderly men. We used a population-based epidemiological prospective study; the Swedish MrOS Study in Uppsala. In total, 1000 men aged 70–80 years were randomly selected from population registries. Serum levels of intact FGF23 were measured with ELISA (Kainos Laboratories Int'l; Tokyo, Japan). Routine serum biochemistries, i.e. Pi, calcium, albumin, GFR (calculated from cystatin c), were assessed by standard protocols and PTH levels were analyzed using the Immulite 2000 Intact PTH Assay (Diagnosic Products Corporation, CA, USA). In this group of elderly men, FGF23 levels correlated to serum PTH ($p < 0.0001$), glomeral filtration rate (GFR) ($p < 0.00001$) and age ($p < 0.00001$). These variables also remained as independent predictors of FGF23 levels in a multi-regression analysis. Importantly, no correlation between FGF23 and Pi or calcium was observed. Further, intact serum FGF23 showed a normal distribution and mean values were 44.1 pg/mL. These are slightly but significantly higher FGF23 values compared to the previously established normal reference range for this ELISA as determined by Yamazaki and colleagues in 104 healthy controls (28.9 ± 1.1 pg/mL) of younger age. In conclusion, this large-scale population-based study indicates that PTH is an important predictor of intact serum FGF23 levels, independent of renal function, in a population-based cohort of elderly men. This suggests a co-regulation of PTH and FGF23 as well as the existence of a bone/parathyroid axis yet to be defined.

Conflict of Interest: None declared

P030-T

BLOOD LEAD LEVELS, RATES OF BONE LOSS AND INCIDENT FRACTURES: THE STUDY OF OSTEOPOROTIC FRACTURES (SOF)

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BACKGROUND AND METHODS: Lead disturbs neuromuscular functions, affects bone turnover, and decreases growth and stature in children. Lead is stored in the skeleton; post-menopausal women have higher Blood Lead Level (BLL) than pre-menopausal women. Whether lead is associated with bone loss and fracture risk in older women is not established. To test the hypothesis that women with higher BLL experience faster rates of bone loss, and higher fracture rates, we measured BLL in 533 white women aged 65–87 years at 2 SOF clinical centers in 1990–91 by atomic absorption spectrophotometry. Total hip Bone Mineral Density (BMD) was measured twice by dual energy x-ray absorptiometry 3.3 years apart. Incident nonspinal nontraumatic fractures were identified over 10 years of >95% complete follow-up. Fractures were confirmed by radiographic report. We used ANCOVA to compare BMD and annualized (%) bone loss across 3 strata of BLL. Proportional hazards models were used to calculate the Hazard Ratio (HR) and 95% Confidence Intervals of fracture; stratum 1 (low) formed the referent group. We adjusted for age, clinic, education, diabetes, hypertension, alcohol consumption, smoking, health status, physical activity, body mass index, estrogen, calcium, Vit D use, history of fractures in mother and baseline BMD.

RESULTS: The mean blood lead level BLL was 5.3 μg/dl ± 2.3 and ranged from 1–21 μg/dl. Women in the highest BLL category (≥ 8 μg/dl) were older, had lived more years after menopause, had a lower body weight, height, and BMI, than women with lower BLL. Baseline total hip BMD was 7% lower in the higher BLL category compared to the lowest BLL ($p < 0.02$). The annualized rate of hip bone loss was 3 times greater among women with the highest BLL (Table) (p trend = 0.04). A total of 163 incident fractures were reported. Women with the highest BLL had a 72% higher risk of fracture, independent of BMD and other covariates.

CONCLUSIONS: We conclude that BLL may be an independent environmental risk factor for osteoporotic fractures.

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Table:. Total hip BMD annualized rates of total hip bone loss a

	Blood Lead (μg/dl)			p-value (trend)
	Strata 1 (≤ 3) N = 122	Strata 2 (4–7) N = 332	Strata 3 (≥ 8) N = 79	
Total hip BMD (g/cm^2)	0.77	0.76	0.72	0.02
Annualized % BMD change +	−0.29	−0.46	−0.83	0.04
Fracture incidence (per 1000 py)	35.3	32.9	54.5	
HR (95% CI) + +	1.0	1.15 (0.75–1.79)	1.72 (1.00–2.93)	0.03

+ Multivariate adjusted + + BMD + multivariate

Conflict of Interest: J Cauley, Merck & Co; Eli Lilly & Co; Pfizer Pharm; Novartis Pharm, Grant/Research Support; Merck & Co, Speakers Bureau L Morrow, Psychology Software Tools, Consultant

P031-S

EFFECT OF ONCE-YEARLY INFUSION OF ZOLEDRONIC ACID 5 MG IN POSTMENOPAUSAL WOMEN WITH OSTEOPOROSIS

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METHODS: The HORIZON-PFT is a multinational, 3-year, randomized, double-blind, placebo-controlled trial evaluating the potential of once-yearly zoledronic acid (ZOL) 5 mg, infused over 15 minutes, to decrease risk of fracture in 7736 postmenopausal osteoporotic women 65–89 years of age.

RESULTS: Treatment with ZOL 5 mg resulted in significant relative risk reductions in morphometric vertebral fracture of 70% vs PBO (3.3% vs 10.9%; 95% CI [62%, 76%]) and in hip fracture of 41% vs PBO (1.4% vs 2.5%; 95% CI [17%, 58%]). Secondary endpoints, non-vertebral (excluding finger, toe, and facial), clinical vertebral, and any clinical fracture (including non-vertebral, hip, and clinical vertebral), were significantly reduced by 25%, 77%, and 33% (all $P < .0001$), respectively. Bone mineral density increased significantly in ZOL vs PBO at total hip (6.0%), lumbar spine (6.7%) and femoral neck (5.0%) ($P < .0001$). While transient increases in serum creatinine ≥ 0.5 mg/dL over preinfusion levels were seen in a small fraction (1.3%) of patients in the ZOL 5 mg group; no cumulative impact on renal function was demonstrable. Hypocalcemia (serum calcium < 2.075 mmol/L) was observed in 2.3% of patients. Virtually all events occurred after the first infusion of ZOL and all were asymptomatic and transient. Adverse events occurring ≤ 3 days after infusion were more frequent after first infusion (44.7% ZOL vs 14.7% PBO) but declined markedly on subsequent infusions. There were more atrial fibrillation serious adverse events in ZOL vs PBO (1.3% vs 0.5%). Two cases of osteonecrosis of the jaw (1 in PBO, 1 in ZOL) were identified on adjudication; both resolved with antibiotic therapy and limited debridement.

CONCLUSION: Once-yearly infusion of ZOL 5mg over 3 years achieves a highly significant decrease in vertebral, hip, and other fracture risk and is generally safe and well tolerated.

Conflict of Interest: D. Black, Novartis, Grants/Research support; P. D. Delmas, Novartis, Grants/Research support, Consultant R. Eastell, Novartis, Grants/Research support I. Reid, Novartis, Grants/Research support, Consultant S. R. Boonen, Novartis, Grants/Research support, Consultant J. A. Cauley, Novartis,

Grants/Research support Z. Man, Novartis, Grants/Research support S. Cummings, Novartis, Grants/Research support, Consultant

P032-M

HISTOMORPHOMETRIC AND MICRO-CT ANALYSIS OF BONE BIOPSIES AFTER 3 ANNUAL INFUSIONS OF ZOLEDRONIC ACID 5 MG: EVIDENCE FOR PRESERVATION OF BONE STRUCTURE AND REMODELING CAPACITY

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The HORIZON Pivotal Fracture Trial in 7736 postmenopausal women demonstrated significant antifracture efficacy of 3 annual infusions of zoledronic acid (ZOL) 5 mg on vertebral, hip and non-vertebral fractures. In this study, 153 individual bone biopsies were obtained at the final visit at month 36, 1 year after the last infusion. The biopsies were subjected to micro-CT (μCT) analysis to assess biopsy quality and bone structure, 143 biopsies (76 ZOL, 67 placebo) had at least one μCT parameter measured. Following micro-CT analysis the biopsies were sent for histomorphometry. A total of 152 (82 ZOL, 70 placebo) were available for qualitative analysis of tetracycline labeling by inspection and 111 for quantitative histomorphometry (59 ZOL, 52 placebo). Novartis, the study sponsor, conducted the statistical analysis.

Micro-CT analysis of bone structure revealed higher trabecular bone volume (BV/TV) (median 16.6 vs 12.8 %, $P = 0.0200$), higher trabecular number (Tb.N) (median 1.36 vs 1.22/mm, $P = 0.0081$), decreased trabecular spacing (Tb.Sp)(median 0.72 vs 0.80 mm, $P = 0.0105$), and a strong trend toward improvement in connectivity density (median 4.40 vs 3.30/mm3, $P = 0.062$) in biopsies obtained from patients on ZOL indicating preservation of trabecular bone structure vs placebo. Tetracycline label was demonstrable in all but 1 of 82 biopsies obtained in patients treated with ZOL; the 1 biopsy without label was incomplete and fragmented. All 70 biopsies obtained from placebo patients had label. Biopsies obtained from patients treated with ZOL exhibited reduction in activation frequency (Ac.F) by 63% (median 0.10 vs 0.27/y, $P < 0.0001$), mineralizing surface (MS/BS) (median 0.45 vs 4.79%, $P < 0.0001$), volume referent bone formation rate (BFR/BV) (median 0.05 vs 0.15 mm2/mm2/y, $P < 0.0001$). Mineral appositional rate (MAR) was significantly higher in patients on active treatment (median 0.60 vs 0.53 μm/d, $P = 0.0002$), indicating increased activity of individual osteoblasts vs placebo. No woven bone formation, marrow fibrosis, or mineralizing defect was detected.

In conclusion, yearly infusions of ZOL 5 mg over 3 years preserved trabecular bone structure as assessed by μCT in women with postmenopausal osteoporosis. Dynamic histomorphometry demonstrated a 63% reduction of bone turnover in ZOL patients compared to placebo, but increased formative activity at the level of individual osteoblasts. No sign of excessive suppression of bone turnover or other bone pathologies was detected.

Conflict of Interest: R. Recker, Novartis, Research grants, Consultant, Speaker P. Delmas, Novartis, Consultant, Speaker I. Reid, Novartis, Research grants, Consultant, Speaker S. Boonen, Novartis, Research grants, Consultant

P033-T

IBANDRONATE-INDUCED REDUCTION OF BONE TURNOVER MARKER PREDICTS ANTIFRACTURE EFFICACY

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Suppression of bone turnover markers (BTM) by antiresorptive therapy appears to predict antifracture efficacy independently of bone mineral density (BMD) gains.[1] One of the most commonly used BTM is CTX (C-telopeptide crosslinks of type I collagen), levels of which have been shown to correlate strongly with fracture risk reduction in trials of antiresorptive therapy.[2] The current analysis examines the relationship between urinary CTX (uCTX) and vertebral fracture risk reduction for ibandronate. uCTX and fracture data were obtained from the per-protocol populations in two randomised, double-blind, placebo-controlled fracture studies in women with postmenopausal osteoporosis (PMO): BONE in which women were randomised to oral daily (2.5mg) or intermittent (20mg) ibandronate[3] and a study of intermittent intravenous (i.v.) ibandronate injections (0.5mg or 1mg every 3 months).[4] Outcome measures included moving average plots, logistic regression and surrogate marker analyses. For the BONE study, the moving average plot showed a clear separation between placebo and ibandronate for 6-month relative change in uCTX vs reduction in vertebral fracture rate and logistic regression analyses showed that changes in uCTX at month 6 were significant predictors of the risk reduction in vertebral fractures. It was estimated that every 1% change in uCTX at month 6 accounts for a 1.8% risk reduction in vertebral fractures at year 3. Surrogate marker analysis, for the BONE study, indicated that relative change in uCTX at month 6 predicted 41% of the treatment antifracture effect at year 3. The strength of the relationship between vertebral fracture reduction and change in uCTX was comparable to that seen between fracture reduction and change in BMD in the BONE study.[5] The changes in uCTX at month 6 in the i.v. ibandronate study were not found to be significant predictors of the risk reduction in vertebral fractures, probably due to suboptimal dosing for intermittent administration as reflected by BTM and BMD changes.[4] In conclusion, bone turnover reduction at 6 months, as assessed by change in uCTX, can predict antifracture efficacy of ibandronate at 3 years.

1. Hochberg MC, et al. J Clin Endocrinol Metab 2002;87: 1586–1592.
2. Bjarnason NH, et al. Osteoporos Int 2001;12: 922–930.
3. Chesnut CH, et al. J Bone Miner Res 2004;19: 1241–1249.
4. Recker RR, et al. Bone 2004;34: 890–899.
5. Wasnich R, et al. Osteoporos Int 2003;14(Suppl. 7): S76 (Abstract P272).

Conflict of Interest: PD Miller, F. Hoffmann-La Roche Ltd, GlaxoSmithKline, Merck, Lilly, P+G, Amgen, grant/research support, consultant R Blackburn, F. Hoffmann-La Roche Ltd employee R Grant, F. Hoffmann-La Roche Ltd employee RR Recker, F. Hoffmann-La Roche Ltd, GlaxoSmithKline, Merck, Lilly, Wyeth, P&G, Amgen, Novartis and NPS Allelix, grant/research support, consultant

P034-S

IBANDRONATE-INDUCED BONE MINERAL DENSITY GAINS ARE RELATED TO VERTEBRAL ANTIFRACTURE EFFICACY

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Evidence supports a relationship between bone mineral density (BMD) gains with antiresorptive agents and antifracture efficacy, [1] but the strength of this relationship remains controversial.[2] This analysis explored the connection for ibandronate. BMD and vertebral fracture data from randomised, double-blind, placebo-controlled fracture studies were used: one evaluated daily (2.5mg) and intermittent (20mg) oral ibandronate;[3] the other, i.v. ibandronate (0.5 or 1mg every 3 months).[4] The relationship between BMD change (% from baseline) and vertebral fractures was assessed by moving average plots and logistic regression analyses. BMD gains were also analysed for their validity as surrogate markers for vertebral fracture risk reduction (RR). In the i.v. ibandronate study, BMD increases were significantly associated with vertebral fracture RR with ibandronate at years 1, 2 and 3 at the total hip ($p = 0.0228$, $p = 0.0035$ and $p < 0.0001$, respectively) and at years 2 and 3 at the lumbar spine ($p = 0.0104$ and $p = 0.0008$, respectively). In the oral ibandronate study, 3-year increases were also associated with 3-year vertebral fracture RR ($p = 0.0084$ for total hip; $p = 0.0565$ for lumbar spine BMD). In a pooled analysis of these two studies, lumbar spine (L2–L4) and total hip BMD changes from baseline at 3 years explained a substantial proportion of the antifracture effect (Table), indicating the substantial value of ibandronate-induced BMD gains as a potential surrogate for the related effects on fracture risk. The demonstrated relationship between ibandronate-induced BMD gains and vertebral fracture RR supports the usefulness of BMD change as a contributing surrogate marker for vertebral antifracture efficacy.

1. Hochberg MC, et al. J Clin Endocrinol Metab 2002;87: 1586–1592.
2. Delmas PD, Seeman E. Bone 2004;34: 599–604.
3. Chesnut CH, et al. J Bone Miner Res 2004;19: 1241–1249.
4. Recker RR, et al. Bone 2004;34: 890–899.

Table: 3-year antifracture effect explained by change in BMD

	Year	Fracture effect explained
Change (%) from	1	14%
baseline total	2	24%
hip BMD	3	37%
Change (%) from	1	6%
baseline lumbar	2	23%
spine BMD (L2–L4)	3	27%

Pooled analysis

Conflict of Interest: S Adami, F. Hoffmann-La Roche Ltd/GlaxoSmithKline, grant research support and consultant PD Miller, F. Hoffmann-La Roche Ltd/GlaxoSmithKline, Merck, Lilly, P+G, Amgen grant research support and consultant K Patel, F. Hoffmann-La Roche Ltd employee RC Schimmer, F. Hoffmann-La Roche Ltd R Grant, F. Hoffmann-La Roche Ltd RR Recker, F. Hoffmann-La Roche Ltd/GlaxoSmithKline, Merck, Lilly, Wyeth, P&G, Amgen, Novartis and NPS Allelix grant research support and consultant

P035-M

EFFICACY AND SAFETY OF A NOVEL TRANSDERMAL LOW-DOSE ESTRADIOL DELIVERY SYSTEM (MENOSTAR) FOR THE PREVENTION OF POSTMENOPAUSAL BONE LOSS COMPARED WITH RALOXIFENE (EVISTA): A 2-YEAR RADOMIZED CLINICAL TRIAL

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To investigate the efficacy and safety of a novel transdermal low-dose estradiol delivery system for the prevention of vertebral bone loss with reference to raloxifene 60 mg/day.

The study was a multicenter, randomized, double-blind, double-dummy, active-controlled non-inferiority comparison trial. Participants were 500 osteopenic, postmenopausal women aged 55 to 80 years. Patients were randomized to receive either a weekly transdermal patch delivering 0.014mg estradiol daily (Menostar®, Schering AG) together with placebo capsules in lieu of the active oral therapy, or oral treatment with raloxifene 60mg daily (Evista®, Eli Lilly), together with a weekly placebo patch for 2 years. The primary efficacy endpoint was the percentage change from baseline in BMD at the lumbar spine (L2–L4) after 2 years. Secondary efficacy endpoints reported herein were the proportion of patients with no loss of lumbar spine BMD and the percentage change in bone turnover markers (CTX-I, D-Pyr, Osteocalcin, BSAP). Safety assessments included careful assessment of endometrial effects (bleedings/endometrial thickness/biopsy), changes of breast density, laboratory parameters, and self-reported adverse events.

In the full analysis set addressed by the LOCF approach, the percentage change from baseline at the lumbar spine for the estradiol (n=233) and raloxifene (n=241) groups were 2.3 (95% CI 1.8–2.7) and 2.7 (95% CI 2.2–3.1) respectively. Thus, the difference between estradiol and raloxifene were -0.39 % (non-inferiority test p=0.005). The proportion of patients with no bone loss at the lumbar spine at the final visit was 77.3 % and 80.5%, respectively. Changes of the different bone resorption and bone formation markers indicated comparable degree of inhibition by the two treatments. Number of subjects who discontinued therapy in the estradiol and raloxifene groups was 56 (22.1%) and 41 (16.4%), respectively. Incidence of serious adverse events was low; 4.9% and 5.4%, respectively. There were no clinically significant signs of endometrial or breast stimulation by either treatment.

In the growing era of ultralow-dose estradiol therapies, the herein described transdermal delivery system offers a useful option for the prevention of vertebral bone loss with efficacy and safety comparable with that of Evista.

Conflict of Interest: Matthias Schäfers and Christoph Muysers are full-time employees of Schering AG

P036-T

STRONTIUM RANELATE DEMONSTRATES VERTEBRAL AND NON-VERTEBRAL ANTI FRACTURE EFFICACY INCLUDING HIP FRACTURES OVER 5 YEARS IN POST MENOPAUSAL OSTEOPOROTIC WOMEN

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Objective: The objective of this work was to describe the long term antifracture efficacy of strontium ranelate over 5 years of treatment in TROPOS1 study.

Materials and methods: Strontium ranelate is a new anti-osteoporotic treatment with a unique mode of action both increasing bone formation and decreasing bone resorption.

In the TROPOS (TReatment Of Peripheral OSteoporosis) phase III study, performed in 75 European and Australian centres, 5091 Caucasian women with postmenopausal osteoporosis have been enrolled with mean age (SD) of 76.7(5) years and with mean femoral neck BMD T-score (SD) of -3.1(0.6). The patients were randomly assigned to receive strontium ranelate 2g/day or placebo for 5 years. All patients received supplementation with calcium and vitamin D adapted to their needs. The main statistical analysis was performed over 3 years of treatment and demonstrated efficacy of strontium ranelate at vertebral and non-vertebral sites. Moreover, a significant decrease in hip fracture risk was demonstrated in patients at risk over the same treatment period.

Results: The 5-year results of TROPOS established the long term vertebral and non-vertebral antifracture efficacy of strontium ranelate, with a 24 % reduction in vertebral fracture (RR=0.76; 95% CI[0.65;0.87] Cox model: p<0.001) and a 15% reduction in non-vertebral fracture (RR=0.85; 95% CI[0.77;0.99] Cox model: p=0.03) in the intent-to-treat population (n=2479 in strontium ranelate group and 2453 in placebo group). In addition, hip antifracture efficacy of strontium ranelate was assessed in women at particular high risk (age > 74 years with lumbar and femoral neck BMD T-score < −2.4 according to NHANES normative values). In these patients, (n= 1128, mean age (SD) 79.2(4.4), mean lumbar T-score = −4.2, mean femoral neck T-score = −3.0), strontium ranelate demonstrated a 43% reduction of the risk of hip fracture (RR=0.57; 95% CI[0.33;0.97] Cox model: p=0.036).

Conclusion: These results demonstrate, uniquely for an anti-osteoporotic treatment, that strontium ranelate provides sustained efficacy over five years against vertebral , non-vertebral and hip fractures.

1. Reginster JY et al (2005) J Clin Endo Metab. 90: 2816–2822.

Conflict of Interest: None declared

P037-S

BONE MINERAL DENSITY RESPONSE TO 24 MONTHS OF TERIPARATIDE (RHPTH 1–34) IN PATIENTS WITH INADEQUATE RESPONSE TO PRIOR ANTIRESORPTIVE TREATMENT

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The prospective, controlled, randomized EUROFORS trial was designed to compare 3 sequential treatment regimens of teriparatide (TPTD) in postmenopausal women with osteoporosis. Main results were reported previously (1, 2). Here we report the results of a subgroup analysis of women treated with TPTD for 24 months after an inadequate clinical response to prior antiresorptive therapy (Inad AR Resp), which was defined as either (a) 1 or more new clinical fragility fracture(s) after >12 months, or (b) a low T-score <= −3 SD or (c) a BMD decrease of 3.5% or more after >24 months of prior antiresorptive drugs, at either spine, hip, or femoral neck. In this analysis, we present the BMD responses of Inad AR Resp who fulfilled criterion (a) only, those who fulfilled criterion (c) only, and those who fulfilled both criteria (a+c), as well as the BMD response of the total subgroup of those Inad AR Resp who were assigned to receive 20 μg/d TPTD continuously for 2 years, plus daily supplements of 500 mg calcium and 400–800 U vitamin D.

Of the 865 patients originally enrolled, 310 met the above criteria and were included in this analysis. The Table lists the final BMD results after 24 months of TPTD [mean change from baseline in g/cm^2 (group level % change from baseline)]. An intention-to-treat approach was used for this analysis; for 16 randomised patients who discontinued the study before reaching the final visit, the last observation was carried forward.

In conclusion, treatment with TPTD for 24 months is associated with statistically significant BMD increases at the lumbar spine, hip, and femoral neck in patients with inadequate clinical outcomes following prior antiresorptive treatment, regardless whether this outcome consists of a new fracture, a further loss of BMD, or both.

(1) Eastell R et al. 2006; JBMR 21 Suppl 1: S70.
(2) Obermayer-Pietsch B et al. 2006; JBMR 21 Suppl 1: S43.

Table:. BMD gains after 24 months of TPTD in Inad AR Resp

Subgroup (n)	Lumbar Spine	Total Hip	Femoral Neck
All Inad AR Resp (310)	0.066*** (+9.8%)	0.013*** (+1.9%)	0.021*** (+3.7%)
Fracture criterion (a) only (141)	0.066*** (+9.4%)	0.009** (+1.3%)	0.020*** (+3.3%)
BMD loss criterion (c) only (25)	0.062*** (+9.7%)	0.015* (+2.2%)	0.030*** (+5.5%)
Both criteria combined (119)	0.067*** (+10.2%)	0.017*** (+2.6%)	0.021*** (+3.8%)

*P < .05, **P < .01, ***P < .001

Conflict of Interest: K. Brixen, Eli Lilly, Grant Research Support T. Nickelsen, Eli Lilly, Full-time employee F. Marin, Eli Lilly, Full-time employee C. Barker, Eli Lilly, Full-time employee B. Obermayer-Pietsch, Eli Lilly, Grant Research Support C. Glüer, Eli Lilly, Grant Research Support, Speakers Bureau J. Farrerons, Eli Lilly, Grant Research Support M. Audran, Eli Lilly, Grant Research Support S. Boonen, Eli Lilly, Grant Research Support, Speakers Bureau R. Eastell, Eli Lilly, Grant Research Support, Consultant, Speakers Bureau

P038-M

TERIPARATIDE REDUCES THE RISK FOR NEW ADJACENT VERTEBRAL FRACTURES

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OBJECTIVES: Vertebral fractures (VFX) are known to increase the risk of new VFX, but no studies have reported the risk of new VFX adjacent to existing VFX. Moreover, although several therapies have been shown to reduce VFX risk among postmenopausal women, little is known about their ability to influence the risk of new adjacent VFX.

MATERIALS AND METHODS: In a post-hoc analysis, data from women in the placebo group of the Fracture Prevention Trial (N = 398) and two year data from women in the placebo group of Multiple Outcomes of Raloxifene Evaluation trial (N = 828) with prevalent VFX at baseline were examined. Placebo group data were analyzed to determine the distribution of prevalent VFX, and new adjacent and non-adjacent VFX across vertebral levels. New adjacent VFX and new non-adjacent VFX risk was determined and stratified by number and severity of prevalent VFX and compared using logistic regression analysis. The effects of teriparatide on new adjacent VFX and new non-adjacent VFX risk was determined using pooled doses (20 mcg/day and 40 mcg/day) of teriparatide (N = 793) versus placebo (N = 398) group data from the Fracture Prevention Trial.

RESULTS: New adjacent fracture risk in untreated women was approximately 2-fold higher than non-adjacent VFX risk, 4.03% vs. 1.59%, respectively. The incidence of new adjacent VFX was higher than the random fracture rate at four vertebrae (T8, T12, L1, and L3) and new adjacent VFX risk increased with the number and severity of prevalent VFX. Teriparatide reduced the risk for any new VFX, new adjacent VFX and new non-adjacent VFX by 72%, 75% and 70%, respectively, versus placebo. In the pooled teriparatide groups versus placebo, adjacent VFX risk in women with two or more baseline prevalent VFX was reduced 81% and 68%, adjacent VFX risk was reduced 62% in women with a prevalent mild and moderate VFX, and reduced 86% in women with a severe prevalent VFX.

CONCLUSION: New adjacent VFX are common in mid-thoracic and thoracolumbar regions of the spine and increase with both the number and severity of prevalent VFX. Teriparatide reduced the risk for new adjacent VFX associated with increasing number and severity of osteoporotic fractures in the Fracture Prevention Trial.

Conflict of Interest: P Chen, EV Glass, BH Mitlak are employees of Eli Lilly and Company

P039-T

EFFICACY OF PTH (1–84) ON VERTEBRAL AND NON-VERTEBRAL FRACTURES IN POSTMENOPAUSAL WOMEN WITH A HIGH RISK OF VERTEBRAL FRACTURES

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Introduction: The Treatment of Osteoporosis with PTH (TOP) study, an 18-month, multinational, randomized, double-blind, placebo-controlled trial, that assessed the effect of recombinant human PTH(1–84) on vertebral fracture incidence in postmenopausal women with osteoporosis, proved PTH(1–84) to be efficacious and safe. The overall risk of new vertebral fractures was low in the TOP study. The present abstract reports the result from a high-risk sample from the TOP study.

Methods: Analysis was by intent to treat (ITT), which included all randomized subjects with evaluable spine X-rays at 18 months. Non-spine fractures were collected as adverse events but were not further reviewed or adjudicated. Subjects included postmenopausal women with low bone mass defined as T-score of the lumbar spine BMD < -3 and with prevalent vertebral fracture at baseline. All subjects received calcium (700 mg per day) and vitamin D (400 IU per day) supplements.

Results: 231 out of 2532 patients enrolled in the TOP study qualified for the analysis. 112 patients received PTH (1–84) and 119 patients received placebo. At 18 months, the incidence of new vertebral fractures was 13.4% in placebo-treated subjects vs 5.4% in PTH (1–84)-treated subjects, a relative risk reduction (RRR) of 60.2% (p-value (Fishers exact) = 0.044). At 18 months, the incidence of non-vertebral fractures was 10.9% in placebo-treated subjects vs 6.3% in PTH (1–84)-treated subjects, a relative risk reduction (RRR) of 42.8% (p-value (Fishers exact) = 0.25).

Conclusion: We conclude that PTH (1–84) therapy significantly reduces the incidence of vertebral fractures in a population of postmenopausal women with a high risk of vertebral fractures.

Conflict of Interest: C Roux, Nycomed company, consultant Dr. Jesper Clausen, International Medical Affairs, Nycomed Denmark Dr. Oscar Illera Martin, Nycomed company, consultant

P040-S

THE INFLUENCE OF A ONE-YEAR MODERATE INTENSITY SCHOOL-BASED PHYSICAL ACTIVITY PROGRAM ON BONE DENSITY, MUSCLE STRENGTH, BODY COMPOSITION AND PHYSICAL PERFORMANCE IN PRE-PUBERTAL GIRLS –DATA FROM THE PEDIATRIC OSTEOPOROSIS PREVENTION (-POP) STUDY

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Background: Several exercise interventions have examined the influence of specially designed exercise programs on muscle and bone health, but few have evaluated whether a general physical activity (PA) program incorporated into the school curriculum can improve BMD, lean mass (LM), fat mass (FM), muscle strength and physical performance. The aim of this 12 month study was to evaluate whether a similar program could improve bone mass, body composition, muscle strength and performance in pre-pubertal girls as a possible strategy to reduce the long-term risk of fracture. Methods: Fifty-three girls aged 7–9 yrs were included in a school curriculum based general PA program for 40 min/d during school (200 min/wk). Fifty age-matched girls in three neighboring schools, assigned to the general Swedish school curriculum of PA (60 min/wk), served as controls. All girls remained prepubertal (Tanner stage 1). Total body, FN and L2–L4 BMC and aBMD, and LM and FM were measured by DXA. Isokinetic peak torque (PT) of the knee extensors (Ex) and flexors (Fl) (60 and 180°/sec) were evaluated using the Biodex system and reported relative to body weight (Nm/kg). Physical performance was assessed using vertical jump height (VJH). Results: No differences were found in the baseline characteristics between the groups, except for organized PA outside of school (hr/wk), VJH and peak torque (all measures) which were greater in the controls ($p = 0.06$ to < 0.001). Thus, all analyses were adjusted for baseline age and organized PA, and for PT or VJH their respective baseline measures. The annual gain in weight was similar between the groups, but there was a greater increase in LM ($p < 0.05$) and FM ($p < 0.01$) in the intervention group. As previously reported, the gain in L2–L4 BMC and aBMD were 2.8 to 4.7% ($p < 0.001$) higher in the intervention group; no differences were found for the changes at the FN or TB. Mean gains in PTEx60 and PTEx180 were also greater (7.0–7.6%, $p < 0.05$–< 0.001) in the exercise group, which remained after also adjusting for annual gains in LM or FM. No significant differences were detected in VJH (11 vs 5%, $p = 0.14$). Conclusion: Increasing the amount of general school based PE to at least 3 hr/wk provides a feasible strategy to enhance bone mass, muscle force and lean mass in pre-pubertal girls. This supports the view that moderate PA can play an important role in enhancing musculoskeletal health during the pre-pubertal period which may help to prevent future fractures.

Conflict of Interest: None declared

P041-S

INCIDENCE OF NUTRITIONAL AND HEREDITARY RICKETS AMONG CHILDREN LIVING IN DENMARK

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The incidence of rickets in Denmark is unknown. We therefore undertook a study to determine the incidence of nutritional and hereditary rickets among children living in Denmark. We also described ethnic origin and clinical characteristics of patients with nutritional rickets.

Methods: All possible cases of rickets were identified from medical records from hospitals in Southern Denmark in the period 1985 to 2005. Cases fulfilling the diagnostic criteria based on clinical signs, biochemical and/or radiological changes compatible with rickets were included. Patients aged more than 15 years at time of diagnosis or with secondary rickets were excluded. In addition,

questionnaires were sent to all GPs and paediatricians in Southern Denmark to identify all patients with rickets in 2005.

Results: Nutritional rickets: A total of 111 cases with nutritional rickets were included. Of these 76% were of non-Danish ethnic origin. As 5% of the children in the background population of Southern Denmark were immigrants this corresponds to an estimated incidence of 2.4 pr. year pr. 100.000 children living in Denmark. There was a seasonal variation as 75% of the cases presented from January to June. The age at diagnosis was divided into two incidence peaks. In the first group 63 (57%) children presented before 4 years of age, median 1.3 years. In 52 of these cases data on vitamin D supplementation were available. In 42 of 52 cases (82%) insufficient or no vitamin D supplementation was given. The young children displayed the typical clinical signs of rickets. In the second group 48 (43%) cases presented after age 4 years, median 12.6 years. These children displayed few clinical signs and experienced unspecific symptoms. Hereditary rickets: A total of 18 cases were hereditary rickets and the incidence was estimated to 0.4 pr. year pr. 100.000 children. Median age of presentation was 1.7 years. The response rate from the questionnaire to GPs and paediatricians was 75% and no children with nutritional rickets were treated without admission to hospital during 2005.

Conclusion: Nutritional rickets is rare and in Denmark largely restricted to immigrants. In this study the estimated incidence of nutritional rickets were 2.4 pr. year pr. 100.000 children and for hereditary rickets 0.4 pr. year pr. 100.000 children. Main risk factors were low intake of vitamin D, especially in young children, in combination with inability to synthesize vitamin D from sun exposure during winter months.

Conflict of Interest: None declared

P042-M

NANOSTRUCTURE OF GROWING BONES IN BISPHOSPHONATE TREATED PIGS

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Bisphosphonates (BP) are widely used for the treatment of osteoporosis. The positive effects of BPs in osteoporosis trials have made doctors search for other possible applications. In children BPs are currently being used to treat diseases like osteogenesis imperfecta (OI) and juvenile idiopathic osteoporosis. Despite the fact that BPs today are used as a standard treatment of OI, there are still concerns about the long term effect of BPs use in children; mainly for two reasons. First BP administration may impair the growth of long bones from endochondral growth plates resulting in shorter stature. Secondly, potent BPs may inhibit bone resorption to an extent, where it changes the mechanical properties of the bone. A blockage of the bone turnover by BPs could in term result in hypermineralized bone, which is stiffer but also more fragile. Therefore, we have applied scanning small angle X-ray scattering (scanning SAXS) to reveal any possible effect of BPs (alendronate) on the bone nanostructure near the proximal femoral epiphysis in a pig model. Small angle X-ray scattering (SAXS) gives information on the orientation and thickness of the hydroxyapatite nanocrystals in bone. The studied animals were female Danish landrace pigs that had undergone surgery in the spine at 3 months, been treated with alendronate (10 mg/day or placebo) for 3 months and sacrificed after an additional 3 three months. A total of 12 pigs, 6 receiving BP and 6 controls, were included in the study and bones from all animals were studied. A total of 3148 individual SAXS measurements on bone were performed. The data were analyzed by a newly developed curve fitting approach (Birkedal, Bünger & Skov Pedersen, to be published). We found several general features in both groups that provide new insights into the nanostructure of growing bone: A significant fraction of the mineral plates are in fact oriented perpendicular to the growth plate in the calcified cartilage zone. The thickness of the crystallites increases with distance from the mineralization front in a manner reminiscent and saturate at a value of around 2.5 nm at a distance of about 3 mm from the mineralization front. Possible effects of alendronate treatment are small if at all present, a detailed statistical analysis will be discussed. The present analysis provides new detailed data on bone growth and illustrates how modern nanotechnological experimental approaches can provide new insights into bone biology.

Conflict of Interest: None declared

P043-T

INDICATION OF THINNER MINERAL PLATES IN NEW BONE OF RATS TREATED WITH SRCL2

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Strontium salts have attracted strong interest as a possible anabolic drug for the treatment of osteoporosis. Sr2+ substitutes Ca^{2+} in the apatite crystal lattice. However, detailed knowledge about possible effects of Sr treatment on the bone nanostructure is still missing. Therefore, we have applied scanning small angle x-ray scattering (sSAXS) to investigate the influence of SrCl2 on HA mineral plate thickness and orientation in a rat osteoporosis model.

The effect of SrCl2 on the treatment of ovariectomy induced osteoporosis was examined in twelve 6-month-old female Wistar rats. The animals were treated with 4 mmol SrCl2(aq)/kg/day or placebo for a period of 140 days and labelled with fluorochromes at days 7, 126 and 136. Cross sections from the midshaft femur from three animals in each group (−ovx/-Sr, +ovx/-Sr , −ovx/+Sr and +ovx/+Sr) were studied in detail using fluorescence microscopy and scanning electron microscopy including element mapping by energy dispersive X-ray analysis (EDAX) and sSAXS. The sSAXS investigations comprised a total of 5500 measurement points in new and old bone in the three samples from each of the four groups. The effect of SrCl2, ovariectomy and new/old bone on mineral plate thickness was evaluated using a three way ANOVA model including an individual sample effect.

The new bone, identified by fluorescence microscopy, was found to contain increased levels of Sr by EDAX analysis in the two +Sr animals. The model estimates of the average plate thickness ranged between 25.63 Å (24.37–27.09) in new bone in the +ovx/+Sr group to 29.77 Å (27.94–31.94) in the old bone in the −ovx/-Sr group. The largest difference in mineral plate thicknesses was between new and old bone in the −ovx/+Sr group, where the model estimate of mineral plate thickness was 25.04 Å (23.85–26.41) in new bone and 28.59 Å (26.94–30.53) in old bone. In the ANOVA analysis this effect tended to be significant with $p = 0.07$.

The results indicate that Sr treatment has an effect on mineral plate thickness in new bone. Sr treatment may stimulate appositional bone growth resulting in a large amount of "immature" new bone with relative thin mineral plates. However, it is also possible that Sr inhibits the growth of the HA mineral plates during secondary bone mineralization.

Conflict of Interest: None declared

P044-S

OSTEOSPHERES: A NOVEL APPROACH FOR SCAFFOLD-ENHANCED EX VIVO OSTEOGENESIS

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Bone formation is the sine qua non of the osteoblastic phenotype. However, there is a lack of in vitro assays to assess bone formation potential of osteoblastic cells. Three-dimensional (3D) culture provides a temporally and spatially regulated microenvironment suitable for exploring complex biological processes. Thus, we present a highly reproducible platform for the generation of ex vivo 3D culture of human mesenchymal stem cells (hMSC), termed here osteospheres, which recapitulate in vivo bone formation. Osteogenic differentiation factors induced osteospheres with a central core of quiescent cells expressing bone related proteins (e.g. alkaline phosphatase, type I collagen, osteonectin and osteocalcin) and a cortical layer of slowly dividing cells. Crystalline hydroxyapatite–tricalcium phosphate (HA/TCP) scaffold was osteoconductive in the presence of differentiation factors leading to specific upregulation of genes for osteogenic factors; homeobox transcription factors msx-2, dlx-5 and transcription coactivator TAZ. Also, a temporal sequence similar to that observed for in vivo bone formation was detectable with high levels of expression of genes specific for both chondrocytes and osteoblasts in early phase cultures, followed by dominance of osteoblastic gene expression. Interestingly, geometry influenced cell differentiation; concavities contained mature osteoblastic cells lining the HA/TCP surface expressing osteocalcin, biglycan and phospho-AKT, plus multi-layers of TAZ positive cells. Finally, the cells in the osteospheres formed lamellar bone-like collagen matrix, as evidenced by strong birefringence of polarized light in the cortical layer and concavities. Osteospheres are thus a good model to study molecular control of the differentiation sequence of hMSC and may be used in biotechnological applications for refining bone tissue engineering and for screening novel anabolic agents for the treatment of bone loss states including osteoporosis.

Conflict of Interest: None declared

P045-M

A METHACRYLATE POLYMER CONTAINING IODINATED MONOMER USABLE AS BONE CEMENT: RADIO-OPACITY, IN VITRO AND IN VIVO BIOCOMPATIBILITY

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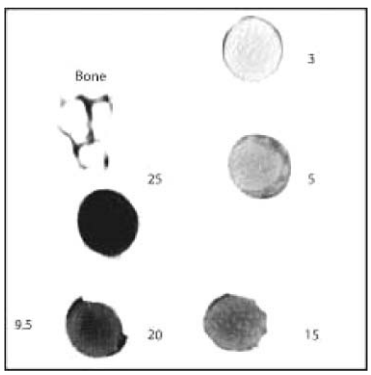
Poly(methylmethacrylate pMMA) is used as dental or bone cement. A major drawback of the polymer biomaterials is that they are radiolucent since they hardly absorb Xray radiation due to the absence of heavy elements in their structure. The well-known solution is the incorporation of inorganic particles such as barium sulphate or zirconium dioxide. However, these particles diminish the mechanical properties (fatigue life) due to the interfaces between polymer matrix and particles.

We have developed copolymers with monomers with covalently bound iodine, which ensure radio-opacity: 2-[2′,3′,5′-triiodobenzoyl]oxoethyl methacrylate (TIBOM). TIBOM was synthesized and copolymerized with MMA in various amounts. The microstructural details (i.e., distribution of monomer units along the macromolecular chain) of the copolymers and the reactivity ratios of the two monomers were determined by the integral and differential equations. The copolymers were analyzed by microCT to measure the radio-opacity. The cytocompatibility was appreciated by an in vitro assay with osteoblast-like cells. Polymer cylinders were implanted subcutaneously in rats to evaluate biocompatibility.

The reactivity ratios were determined and the composition diagram is typical for a practically non-homopolymerisable monomer (TIBOM) and a very reactive monomer (MMA). MicroCT revealed that the copolymer had a suitable radio-opacity, even at low concentration. Biocompatibility tests found that copolymers are not toxic and were well tolerated. TIBOM containing copolymers could be used as radio-opaque dental or bone cements.

Conflict of Interest: None declared



P046-T

VIABILITY OF OSTEOCYTES IN BONE AUTOGRAFTS HARVESTED FOR DENTAL IMPLANTOLOGY

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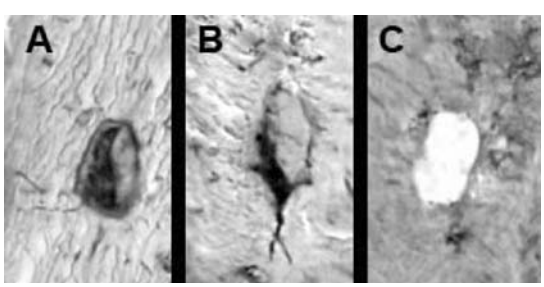
Bone autograft remains a very useful and popular way for filling bone defects. In maxillofacial surgery or implantology, it is used to increase the volume of the maxilla or mandible before placing dental implants. Because there is a noticeable delay before harvesting the graft and its insertion in the receiver site, we evaluated the morphologic changes in bone cells at the light and transmission electron microscopy levels.

Five patients having an autograft (bone harvested at the chin) were enrolled in the study. A small fragment of the graft was immediately fixed after harvesting and a second one was similarly processed at the end of the grafting period when bone has been stored at room temperature for a 20 min period in saline. Samples were embedded undecalcified and semithin sections were stained for the histomorphometric analysis of osteocyte's lacunae with a normal osteocyte A; an altered cell B, empty lacuna C. Thin sections were observed by TEM. A net increase in the number of osteocyte lacunae filled with cellular debris was observed (+41.5%) after storage. However, no cytologic alteration could be observed in the remaining osteocytes. The viability of these cells is known to contribute to the success of autograft in association with other less well identified factors.



Conflict of Interest: None declared

P047-S

ACTIVATED PLATELETS STIMULATE DIFFERENTIATION AND PROLIFERATION OF PRIMARY HUMAN BONE CELLS

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In bone tissue engineering approaches, the expansion of bone cells is an essential part. Cell cultures have traditionally been supplemented with fetal bovine serum (FBS) to support proliferation and attachment; however FBS is a potential source of xenogeneic protein contamination. In recent years the search for an appropriate alternative to FBS in the ex vivo expansion process has increased. Our study demonstrates that human platelet rich clot releasate (hPRCR) could be an appropriate alternative.

The effects of hPRCR on bone cell cultures derived from 5 different human donors were analyzed with respect to morphology, proliferation, apoptosis and gene expression. Cells were isolated as described previously in Clausen et al (1). Five different hPRCR concentrations were tested; 1%, 5%, 10%, 20%, and 40%. Light microscopy analysis was done after three and nine days and population doubling (PD) values were calculated for each concentration.

The gene expression of alkaline phosphatase, collagen type 1, osteocalcin, bone sialoprotein and osteopontin were analysed with RT-PCR. 10% FBS cultures were used as controls. With 10% hPRCR the cell morphology resembled the control cultures, however the PD value was significantly higher ($p < 0.01$). Concentrations of 20 –and 40% had a clear cytotoxic effect, observed with LM analysis. Accordingly apoptosis analysis by flow cytometry (propidium iodide assay), demonstrated a significant apoptotic cell fraction. hPRCR had a potent effect on the expression of osteogenic markers and resulted in a concentration dependent upregulation.

We demonstrate that human bone cells derived from the maxillary alveolar ridge can be cultured in medium containing 10% hPRCR instead of FBS. The addition of hPRCR results in higher proliferative capacity and upregulation of osteogenic markers. These results indicate that FBS could be avoided in future tissue engineering approaches using bone cells from this anatomic site, providing a new and safer cell culture system for cell therapy.

1. Clausen, C., Hermund, N. U., Donatsky, O., Nielsen, H. (2006). Characterization of human bone cells derived from the maxillary alveolar ridge. Clin Oral Implants Res. 17: 533–540.

Conflict of Interest: None declared

P048-M

TERIPARATIDE USE TO ACCELERATE FRACTURE REPAIR IN DELAYED HEALING OF COMPLEX FRACTURES IN MILITARY PERSONEL: A CLINICAL CASE

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Teriparatide use to accelerate fracture repair.

INTRODUCTION: The patient was injured in combat area. He suffered of transverse apophysis fractures (L1 L2, L5), multi-fragmentary unstable fracture of right ilium wing, stable fracture of the left ilium wing, fracture–luxation of the coccyx. Fractures cannot be treated by surgical or non-surgical means, apart from bed-rest, provoking a total disability of patient.. Two months later there was no sign of improvement in fracture healing and disability parameters. After careful evaluation of bone metabolism, general conditions, CT scan and literature survey, the patient started Teriparatide treatment for 2 months.

GOAL: To accelerate the fracture healing because the rehabilitation program was negatively affected by the impossibility to load the muscle–skeletal apparatus.

PATIENT and METHOD: On May 18th a white, 30 years, Caucasian male presented multiple, severe fractures after being involved in an explosion while aboard an armored vehicle. 27 days later he was still on forced clinostatism. First examination showed an important hypotrophy of anti-gravitation muscles.. On June 16th a CT –scan showed almost no improvement in fracture healing. A CT scan on day +64 showed amelioration of the left ilium wing only. The patient started Teriparatide, 20mcg/day. After 33 days on Teriparatide (Aug. 21st) a CT scan showed progression of fractures healing in all sites. After 7.4 weeks on Teriparatide the patient begun walking without support. After 2 months on Teriparatide, treatment was interrupted. Safety parameters were within normal values.

CONCLUSIONS: This case has limitations, including lack of controls due to its exceptional nature. Animal models and case reports suggest Teriparatide accelerate fracture healing. This case suggests that this capability and its safety

profile, may be of value for short-term treatment of severe, complex fractures to improve physical rehabilitation by accelerating fracture healing.
Conflict of Interest: None declared

P049-T

PHYSICALLY COMPETENT WOMEN DECLINE IN KINETIC PARAMETERS DURING AGING

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AIM: The purpose of this study was to analyze kinetic parameters of locomotor system in Greek women.

METHODS: Ninety pre and postmenopausal women nonosteoporotic women, without a history of a neuromusculoskeletal disorder, aged 20–79 years (yrs), were included in the study. Women were separated according to age decade: 20–29 yrs (n = 12), 30–39 yrs (n = 7), 40–49 yrs (n = 14), 50–59 yrs (n = 22), 60–69 yrs (n = 13) and 70–79 yrs (n = 22). We studied anthropometric (weight, height, BMI) and kinetic parameters (jump height, velocity, force, power). All women performed two leg jumping mechanography (Leonardo platform, Novotec, Pforzheim, Germany) for the measurement of the objective parameters of movement . This mechanographic device measures forces (N) applied to the plate over time, calculates through acceleration the vertical velocity (m/sec) of centre of gravity and jump height (m) and using force and velocity it calculates power (Watt) of vertical movements. We also calculated the personal power after weight adjustment, meaning Power/Weight parameter. Jumping was performed as counter-movement jump with freely moving arms.

RESULTS: Height and all kinetic parameters (except force, $p = 0.85$) were statistically decreased ($p < 0.001$) during ageing. Body weight and BMI were gradually increased ($p = 0.01$).

DISCUSSION: The results suggest that in physically competent pre and postmenopausal women a decline in the kinetic parameters is expected. Possible reasons are changes in body composition, reduction of skeletal mass and tendons properties. The personal power parameter value could be a tool to assess the physical performance. Jumping mechanography gives to the clinician additional information about locomotor system.
Conflict of Interest: None declared

P050-S

BONE MINERALISATION DENSITY AND MARROW SPACE ORIENTATION VARY BY SITE IN THE EQUINE THIRD METACARPAL CONDYLE

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Condylar fracture of the third metacarpal bone (Mc3) is a common cause of racetrack fatality in Thoroughbred horses. Fractures propagate through subchondral bone (SCB) of the distal Mc3 condyle, typically originating in parasagittal groove (PSG) articular calcified cartilage. Bone microstructure in the Mc3 is likely to influence fracture propagation. Preconditioning exercise (PX) may affect bone development in young horses and could protect against later athletic injury, but its effects are poorly understood. Twelve Thoroughbred horses were raised at pasture in New Zealand. Six were given PX from 10 days old. IV injections of calcein were given 19 and 8 days prior to euthanasia at 18 months old. Osteochondral specimens were cut from the distal Mc3 in the dorsal and palmar oblique frontal planes, imaged with DXA, fixed in ethanol and embedded in PMMA. Surfaces were finished to a diamond polish and imaged using quantitative backscattered electron scanning electron microscopy (qBSE). Montages of 56–120 images (each 4.46mm field width) were made of entire specimen surfaces. Specimens were then diamond ultramilled and imaged with confocal scanning light microscopy (CSLM) and qBSE. Montages were repeated on the ultramilled surface, and 13 defined sites imaged at higher magnification (445 μm FW). CSLM images were registered to matching qBSE images with in-house software. Montaged image sets were used to measure bone volume fraction (BVF); bone mineralisation density (Dm); mean volumetric density (VDm); marrow space Feret diameter (MSFD) and marrow space orientation (MSO). Linear accretion rate (LAR) and inter-label mineralisation density (ILDm) were determined in registered image pairs. All measurements were made in 13 identical sites. No significant effect of PX was found in any parameter ($p < 0.05$). All parameters except MSFD, LAR and ILDm varied significantly by site ($p < 0.05$). LAR and ILDm were negatively correlated (Pearson -0.328, $p < 0.001$). DXA bone mineral density (BMD) was positively correlated with BVF and VDm (Pearson 0.851 & 0.881, $p < 0.001$) and negatively correlated with Dm (Pearson -0.155, $p < 0.001$). MSO was highly variable in the PSG and was related to MSFD. Bone structure and composition in the distal Mc3 condyle was not detectably altered in PX. Individual variation exists in Mc3 condylar microstructure, which may result in an increased fracture risk for some horses. Supported by the Horserace Betting Levy Board and the Global Equine Research Alliance.
Conflict of Interest: None declared

P051-M

THE RELATION BETWEEN MECHANICAL STIMULUS AND CELL RESPONSE IN TRABECULAR BONE REMODELING

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The ability of bone to adapt to a changing mechanical environment is the result of a mechanically controlled remodeling process. Bone is preferentially removed where the local mechanical load is low, and added where the local increase in bone mass helps to reduce the mechanical stresses. Over the last thirty years the mechanosensory system has been intensively investigated using in vivo, in vitro and in silico models. Two important unknowns are how the mechanosensitive cells process the information of their local loading environment resulting in what is called a mechanical stimulus. A further unknown is then the remodeling rule, i.e., the phenomenological rule which connects the local mechanical stimulus to the probability for resorption and deposition by osteoclasts and osteoblasts respectively, at the bone surface. An advantage of computer models is that different influencing factors contributing to the mechanical feedback loop can be analyzed separately by a specific variation of only one of these factors. In our computer simulations we focused on the influence of the processing of the mechanosensitive cells and the remodeling rule. The model employed for the work is characterized by an efficient mechanical assessment [1] allowing the study of the time evolution of trabecular bone in different scenarios. Concerning the remodeling rule, simulations with changes in the osteoclast activity indicate that the mechanosensory system in bone contains a threshold value above which the deposition of new bone by osteoblasts is increased significantly. Simulated microstructures further correspond best to real trabecular bone architecture when the mechanosensitive cells respond to a mean value of the mechanical environment surrounding them.

[1] R. Weinkamer, M. A. Hartmann, Yves Brechet, P. Fratzl, Phys. Rev. Lett. 93, 228102 (2004).
Conflict of Interest: None declared

P052-T

APPLICATION OF PHOSPHOPHORYN/COLLAGEN COMPOSITE TO HORIZONTAL BONE LOSS IN PERIODONTAL DEFECTS IN VIVO

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Objective: The purpose of this study was to examine the regeneration of periodontal tissue after the application of phosphophoryn/collagen composite to horizontal circumferential defects in vivo.

Methods: Thirty six mandibular premolars in 6 adult beagle dogs were subjected to experimental periodontal breakdown created by a round bur under the flap surgery. The exposed root surface was curetted by a hand scalar to remove the cementum and then treated by citric acid for 1 minute. The distance between the bone crest and cement–enamel junction was approximately 5 mm. In the test group, phosphophoryn/collagen composite was placed onto the defect area in order to cover the root surface. In the control group, collagen sponge without phosphophoryn was placed in the same manner. At 4, 8 and 12 weeks after surgery, microscopic and immunohistochemical investigations were performed to characterize the regenerated periodontal tissues.

Results: Calcified tissue formation was observed in the healing connective tissue of the test sites at 4 weeks post surgery. At 12 weeks in the test sites, new cementum with Sharpey's fibers was observed on the treated root surface. Histomorphometrical evaluation showed that the amount of new bone was significantly higher in the test sites than in the control sites at 12 weeks post surgery ($p < 0.05$). In immunohistochemistry, an excellent amount of Vimentin and SMA positive cells were observed in the regenerative periodontal ligament.

Conclusions: These results indicate that phosphophoryn/collagen composite has potent roles in promoting periodontal tissue regeneration during the healing process and could readily achievable methods of treatment for horizontal circumferential defects in periodontal disease.
Conflict of Interest: None declared

P053-S

MECHANICAL AND MATERIAL PROPERTIES OF CORTICAL BONE FROM nNOS NULL MICE

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Nitric oxide (NO) is an important signalling molecule in many tissues. Neuronal nitric oxide synthase (nNOS) is one of the enzymes responsible for NO production in bone. nNOS knock out (KO) mice have been found to have high bone mineral density (BMD) and reduced bone turnover indices when compared to wild type (WT) controls. The mechanical properties of the tibia and femur from 5 month old male mice (6 KO, 10 WT) were measured using three-point bending. The density (by immersion) and speed of sound (ultrasound) of the cortical bone were measured and the elastic modulus calculated. Bones were then ashed to determine composition as water, organic and mineral content. The ablation of nNOS increased the mechanical properties of cortical bone. Tibial stiffness (101 (KO) vs. 77 (WT) N/mm $P = 0.003$), load at and work to the failure point (18.8 N (KO) vs. 13.6 N (WT), $P < 0.001$ and 2.65 (KO) mJ vs. 1.47 (WT) mJ, $P = 0.004$) were all significantly greater. Loads at the yield and fracture points show a similar pattern with values for the knock out group being significantly greater (13.0 (KO) vs. 9.8 (WT) N, $P < 0.001$ and 8.5 (KO) vs. 3.7 (WT) N, $P < 0.001$). The same trend was seen in the femur, although the differences between the two groups were less marked. No differences were found, however, between KO and control groups in the material properties measured in femur or tibia with P values being 0.35 or more. When ashed, tibiae from KO mice contained more water (30.9 (KO) vs. 27.9 (WT) %) and mineral (54.3 (KO) vs. 40.8 (WT) %) and less organic material (14.8 (KO) vs. 31.3 (WT) %) than bones from WT animals (P values 0.045, < 0.001 and < 0.001 respectively). Again, data for femur showed the same trend with differences being less marked. Bones from nNOS knock out mice are stronger, stiffer and require more energy to break than WT controls, probably due to an increase in mineral content. The differences between the groups are more marked in the tibia than the femur. This confirms the importance of nNOS in the development of bone strength.

Conflict of Interest: None declared

P054-M

ULTRASOUND MACHINE ASSISTS DECALCIFICATION

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Background: Decalcification refers to processes of removing hydroxyapatite (HA) from the collagen fibers in bone, which is a technique used for processing bone specimen for pathologic diagnosis or producing surgical graft material. However, it usually takes a long period of time (e.g. weeks or months) for a bone specimen to be completely decalcified. The long period of time for decalcification could cause delay in obtaining experimental results, loss of signals as well as reduce staining qualities. This invention incorporates ultrasonic waves into the decalcification process so as to shorten the period required for decalcification.

Methods: Eight femurs from male Sprague–Dawley rats at 12 weeks old, around 450g in weight were used. They were assigned randomly in equal number (4 in each group) into either control (using traditional decalcification), or ultrasonic decalcification group. Bone mineral density (BMD) of the midshaft and the distal region of specimens before decalcification and every 24 hours during decalcification were measured by using peripheral quantitative computed tomography (pQCT). The end point of decalcification was estimated by X ray. Decalcified specimens were stained with Haematoxylin and eosin for morphological studies. Paired t test was used in comparing the total BMD between Day 0 (before decalcification) and Day 1 (24 hours after decalcification). Independent t test was also used in analyzing the percentage loss of calcium at different regions between the ultrasonic decalcification group and the control group on Day 0 and Day 1 ($\alpha = 0.05$).

Results: Bones treated with ultrasonic waves decalcify much faster than those without as monitored by pQCT. The amount of boss loss in ultrasound treated femur and control femur were 30% and 15% respectively after decalcified for 24 hours. After decalcified for 96 hours, no mineral component could be detected in ultrasound treated femurs while the decalcification was still incomplete for those femurs in the control group. Results on H&E stained sections indicated that decalcification of bone did not result in any architectural changes or distortion in morphology as compared with tissues processed in the conventional fashion.

Conclusion: The proposed machine could accelerate decalcification thus greatly reduce the period of time required for decalcification to few days or few hours without introducing defects on the morphological structures of the specimens.

Conflict of Interest: None declared

P055-T

EXTRACORPOREAL SHOCK WAVE TREATMENT OF ATROPHIC NONUNIONS – AN IN VIVO STUDY USING AN ANIMAL MODEL

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The delayed healing or nonunion of bone is a common problem as it occurs in 5–10% of all fractures. The treatment of the aseptic, atrophic nonunion is a clinically relevant problem, because of its biological inactivity. The current treatment is accompanied by an extended surgical procedure including resection of necrotic bone and bone grafting and so causing a large amount of stress, hospitalization and a long time work incapacity for the patient. The Extracorporeal Shockwave Therapy (ESWT) is a possible non-invasive treatment, as recent clinical trials show. In basic research the application of extracorporeal shock waves has shown to enhance new bone formation in healthy bone. It was also shown in vitro and in vivo, that ESWT is able to release local acting bone and vascular growth factors as TGFb1, BMP's 2, 3, 4 and 7 and VEGF. Therefore we investigated the in vivo effect of a single dose shock wave treatment on nonunions in a rabbit model.

16 female NZW rabbits were taken into the study. After performing the osteotomy to the tibia and fixing it with an external fixator the animals were kept for 8 weeks before shock wave treatment with 0.5 mJ/mm2 was applied to the treatment group, the control group received a sham treatment. In the course of the following 4 weeks there were measurements of the bone metabolism using scintigraphy, X-rays were taken, bone growth factors and mineralization markers (TGFb1, bFGF, VEGF, Osteocalcin) measured in the serum and finally the histological examination.

The treatment group showed a significant increase in bone metabolism in the scintigraphy, the radiological and histological classification showed especially for the formation of callus a significant difference between the control and the treatment group. Concerning the blood samples we saw a clear increase in VEGF up to 7 days after shock wave treatment, which was scarcely not reaching significance, the other blood parameters just showed tendencies to a elevated production of growth factors in the treatment group.

We could show for the first time that extracorporeal shock waves are able to induce bony healing in a standardized atrophic non-union model. This was supported by a strong increase in bone metabolism, measured by scintigraphy. So in our opinion extracorporeal shock waves should be the first choice to treat non unions and in the future more clinical studies are needed to bring this method into the mind of trauma surgeons.

Conflict of Interest: None declared

P056-S

EFFECT OF TEMPERATURE AND FLUORIDE ON AMELOBLASTS IN VITRO

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Tooth development occurs by the close interaction of the oral epithelial tissue and mesenchyme, and several factors and external agents may alter this epithelial–mesenchymal interaction. Fluoride is well known as a specific and effective caries prophylactic agent and its systematic application has therefore been recommended widely. Excess fluoride may, however, promote disturbances in enamel development and mineralization of teeth known as dental fluorosis. A systemic condition during tooth development, such as high fever, may also affect the mineralization process and produce a pattern of enamel defects in the dentition. We have examined the effect of fluoride and heat, alone or in combination, on protein and gene expression in murine ameloblasts. Murine ameloblasts (LS-8) has been C for 24 h) and various concentrations of sodium fluoride°exposed to heat (40 (40 μg/ml and 200 μg/ml), alone or in combination, for 1, 3 and 7 days. Cell culture medium and cells have been harvested at each time point. mRNA was isolated using Dynal beads (Dynal, Norway), and mRNA expression of various genes were quantified by real-time PCR. Secretion of cytokines and interleukins to the medium has been measured using 25-plex kit (BioSource) and Luminex technology. We observed no significant toxic effect of fluoride or heat on the cells, measured as lactate dehydrogenase activity (LDH) in the cell culture medium. Alkaline phosphatase (ALP) activity in the culture medium was not affected by the treatments, neither was osteocalcin mRNA expression. Amelogenin mRNA expression was significantly enhanced, whereas the secretion of vascular endothelial growth factor (VEGF), monocyte chemoattractant proteins (MCP-1) and interferon inducible protein 10 (IP-10) to the medium was reduced by 80% of control with fluoride. The effects were time- and dose-dependent. A stimulatory effect on amelogenin expression, a protein critical for the structural organization of apatite crystals during enamel mineralization, as well as a reduction in vascular signalling factors with high dosages of fluoride may be mechanisms involved in fluorosis. These factors were not affected by heat treatment.

Conflict of Interest: None declared

P057-M

A TWO-YEAR MODERATE INTENSE EXERCISE INTERVENTION PROGRAM IN PRE-PUBERTAL CHILDREN INFLUENCE BONE MINERAL ACCRUAL BUT NOT HIP STRUCTURE: TWO-YEAR PROSPECTIVE DATA FROM THE PEDIATRIC OSTEOPOROSIS PREVENTION (POP) STUDY

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Background. Exercise during growth has positive effect on bone mineral accrual. Most prospective exercise intervention studies in children have evaluated bone mass, few bone structure. Furthermore, most have included volunteers and used specifically designed osteogenic exercise programs. In addition, before the POP study, only one report exceeds 12 months. The aim of this study was to evaluate the effects of two years general school-based exercise intervention program on femoral neck structure and bone mass in a population based cohort of pre-pubertal children.

Methods. Forty-nine healthy girls and 80 boys aged 7–9 years at base line were included in the intervention program comprising 40 minutes of general physical activity per school day (200 minutes/ week). Fifty healthy age-matched girls and 57 boys who participated in the general Swedish physical education curriculum (60 minutes/week) served as controls. Bone mineral content (BMC) and areal bone mineral density (aBMD) were measured by dual X-ray absorptiometry of the total body, the third lumbar vertebra (L3), and the femoral neck (FN). Hip strength analysis (HSA) was performed to evaluate the structural properties of FN. All children remained in Tanner stage 1 throughout the study.

Results. No differences between the groups were found at baseline in age, anthropometric or bone parameters. In girls, the annual gain in L3 BMC was mean 0.23 standard deviations (SD) higher ($p = 0.0002$), in L3 aBMD mean 0.12 SD higher ($p = 0.02$) and in L3 width mean 0.16 SD higher ($p = 0.003$) in the intervention group than in the control group. In boys, the annual gain in L3 BMC was mean 0.13 SD higher ($p = 0.009$) and the annual gain in L3 width mean 0.15 SD higher ($p = 0.002$) in the intervention group than in the control group. In contrast, in neither girls nor boys where there were any significant differences in the annual changes of the FN skeletal traits, when comparing the intervention and the control group.

Conclusions. A two-year school-based exercise program within the general curriculum for pre-pubertal children enhance BMC accrual and bone width in lumbar spine, but does not influence the structural changes or the bone mineral accrual in the FN.

Conflict of Interest: None declared

P058-T

MOLECULAR ANALYSIS OF OSTEOGENESIS BY HUMAN OSTEOBLASTS ON A 3D COLLAGEN–GAG SCAFFOLD

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INTRODUCTION: A composite collagen–GAG porous scaffold is been developed in our laboratory as the basis for a novel tissue engineered bone graft. This project aims to investigate the molecular analysis of osteogenesis using the model osteogenic human cell line hFOB 1.19 under optimal conditions on this scaffold.

METHODS: Initially, we assessed the attachment and proliferation of hFOB 1.19 on a collagen scaffold. 3 million cells/ml were seeded onto a 10x10x3mm collagen–GAG scaffold and incubated for 2, 4, and 7 days. Samples were histologically prepared prior to H&E staining. An Alamar blue assay was optimised to access viability of the hFOB cells within the scaffold. To do this a standard curve of cell viability was created. The standard curve was prepared under two conditions; the first used cells alone, the second used cells seeded onto scaffold. For the cells alone experiment cell densities (0.5–2.5 million cells/ml) ($n = 3$) were cultured on a 96-well plate for 48 hours in medium containing 10% alamar blue. In the cell seeded std curve, cell densities of 1–5 million cells/ scaffold were seeded ($n = 3$) and incubated for 48 hours, medium was replaced with that containing 10% alamar blue for 24 hours. Percentage dye reduction was obtained by reading absorbance at 540 and 620 nm. An optimal cell seeding density curve was determined by seeding 1–5 million cells/scaffold ($n = 3$) and incubating for 48 hours. Cell viability was also assayed using a standard Trypan blue exclusion method for comparison.

RESULTS: Following H&E staining it was evident that hFOB cells do attach and proliferate throughout the scaffold at 2, 4, and 7 days. Increased incubation period led to increased cell infiltration. Using alamar blue it was found that the higher the cell density the more reduced the dye becomes. Results show optimal cell attachment to the scaffold after initial seeding with 4 million cells.

CONCLUSION: We have shown that these human osteoblasts do in fact attach and infiltrate into the collagen–GAG scaffold. Standard graphs produced using alamar blue provide us with a reference of viability for future experiments. This provides us with a real time non-toxic viability assay for use on our scaffolds. The optimal cell seeding value ensures that we are starting the graft with the most number of cells attached as possible. Long term experiments investigating the effect of growth factors and cytokines on osteogenesis in the scaffold are underway.

Conflict of Interest: None declared

P059-S

PROSTAGLANDINS DIFFERENTIALLY AFFECT OSTEOGENIC DIFFERENTIATION OF HUMAN ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELLS

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Adipose tissue-derived mesenchymal stem cells (AT-MSCs) are currently used for bone tissue engineering. AT-MSCs undergoing osteogenic differentiation, as well as mature bone cells, respond to mechanical loading with increased cyclooxygenase-2 gene expression, a key enzyme in prostaglandin synthesis. Prostaglandins (PGs) are potent multifunctional regulators in bone, exhibiting stimulatory and inhibitory effects on bone formation and resorption. Mature bone cells produce PGE2, PGI2, and PGF2α in response to mechanical loading. PGE2, but not PGI2 or PGF2α, has been shown to recruit osteoprogenitors from the bone marrow space and to influence their differentiation. This led to the hypothesis that PGE2, PGI2, and PGF2α may differentially regulate osteogenic differentiation of human AT-MSCs.

Human AT-MSCs were obtained from waste material after elective surgery and donated upon informed consent of 3 donors (age 28–49). The ethical review board of the Vrije Universiteit approved the protocol. AT-MSCs were incubated with 0.01–10 mM of PGE2, PGI2, and PGF2α for 4 days. Osteogenic differentiation of AT-MSCs was measured as alkaline phosphatase activity and gene expression of osteopontin and α1(I)procollagen. Gene expression data were normalized for house keeping (18S) gene expression.

Four days of treatment with 0.01–10 μM PGE2, PGI2, and PGF2α affected osteogenic differentiation, but not proliferation of AT-MSCs. Only PGF2α (0.1 μM) increased alkaline phosphatase activity by 1.5-fold. PGF2α (0.01–0.1 μM) and PGI2 (0.01 μM), but not PGE2, upregulated osteopontin gene expression by 2–2.4-fold. Only PGF2α (0.01 μM) upregulated α1(I)procollagen gene expression by 1.3-fold. Higher prostaglandin concentrations (1 or 10 μM) did not affect OPN and COL1A1 gene expression by AT-MSCs at day 4.

We conclude that PGE2, PGI2, and PGF2α differentially affect osteogenic differentiation of AT-MSCs, and PGF2α appears to be the most effective prostaglandin in stimulating differentiation of AT-MSCs towards the osteogenic lineage. Thus, locally produced PGF2α might be beneficial in promoting osteogenic differentiation of AT-MSCs, resulting in enhanced bone formation for bone tissue engineering.

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Conflict of Interest: None declared

P060-M

VIRTUAL HISTOLOGY OF SOFT AND CALCIFIED TISSUES WITH A NOVEL MUTI-LENGTH SCALE CT, WITH RESOLUTION FROM MM TO SUB 30 NM

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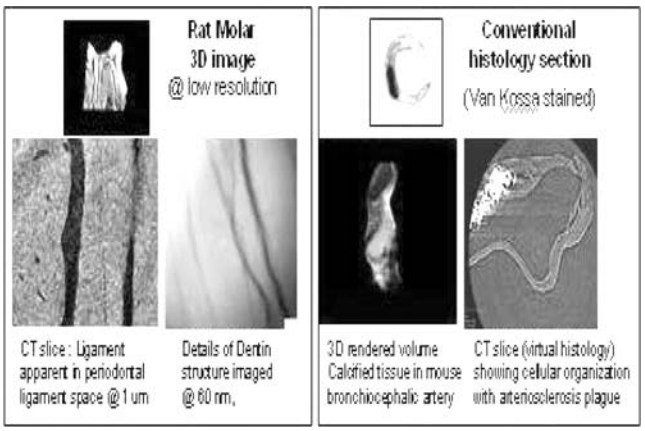
Background: One of the current challenges in characterizing soft and calcified tissue using conventional imaging tools such as optical and electron microscopy is that tedious sample preparation is required to visualize their internal 3D arrays and morphology. Sample preparation for histology sectioning can be difficult, requires staining and is very time consuming. It is also difficult to quantify 3D structures from 2D images. Conventional microCTs have spatial resolution limitations (ranging from several microns to tens of microns) and have very poor contrast for soft tissue. We describe a novel x-ray computer tomography (CT) system for rapid non invasive virtual histology of internal structures of soft or calcified tissue and biomaterials, capable of multi-length scale imaging from mm to sub 30 nm spatial resolutions and which requires little or no sample preparation or staining. Methods: The key to the novel multilength scale CT technology lies in utilizing proprietary x-ray optics with Fresnel Zone plates, and innovative high resolution, high contrast detectors. Depending on the spatial resolution required, sample thickness can range from several mm to several microns for virtual histology in 3D. Results/Discussion Examples showing 3D reconstructed images and their corresponding CT slices for calcified and soft tissues (without contrast agents), being imaged at progressively higher resolution is described. Conclusions The novel multilength scale CT provides an

alternative technique for quantitative histopathological characterization and can provide a more efficient means for disease, cancer, drug efficacy and tissue engineering research.



Conflict of Interest: None declared

P061-T

TEXTURE ANALYSIS OF COMPUTED TOMOGRAPHIC IMAGES IN OSTEOPOROTIC PATIENTS WITH SINUS LIFT BONE GRAFT RECONSTRUCTION

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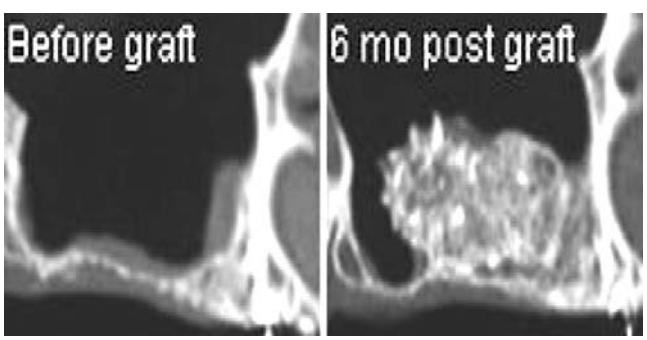
Bone implants are now widely used to replace missing teeth. Unfortunately, after prolonged period of being edentulous, the alveolar ridge becomes atrophic and sufficient bone may not be present for implant screwing. It is even possible that the sinus cavity can drift close to the ridge of the jawbone so far that there is insufficient surface area for the placement of implants. Bone grafting is a very useful way to increase the bone volume of the maxilla and the technique is referred as ''sinus lift''. There is a noticeable delay necessary for the receiver grafted site to heal (about 6 mo) before the implants can be placed. Computed tomography is a useful method to measure the amount of remaining bone before implantation and also to evaluate the quality of the receiver bone at the end of the healing period. Texture analysis of X-ray images is a non-invasive method that is useful to characterize bone microarchitecture on images coming from different X-ray devices.

Three patients in which a sinus lift surgery of both sides was necessary before implant were analyzed in the present study. All had a bone reconstruction with a combination of a biomaterial (beta-TCP or pyrolized bone) and autograft bone harvested at the chin. Computed panoramic tomographic images were obtained before and at one and 6 months post graft. Images were analyzed by the Mazda software offering texture analysis facilities for MRI and scanner images. The regions of interest were manually drawn on the images at the grafting area. Run lengths analysis was used.

A significant increase of texture parameters at 1 month was found and reflected a gain of homogeneity due to the graft. At 6 months, parameters were slightly but non-significantly decreased versus 1 month. This indicated a loss of homogeneity due to the remodeling of the graft and the replacement by bone tissue.

Texture analysis identified changes during the healing of the receiver site. The method was found to correlate with microarchitectural changes in bone and could be a useful approach to characterized osseointegrated grafts.



Conflict of Interest: None declared

P062-S

BRIDGING OF RABBIT TIBIA NONUNION PROMOTED BY EXTRACORPOREAL SHOCKWAVE

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Background. Extracorporeal shock wave (ESW) has been applied for treatment of nonunion fracture both clinically and experimentally. Little is known about the underlying mechanisms by which ESW promotes bone bridging of the nonunion. This study was designed to explore how the ESW stimuli evoke cellular events, which result in the bony bridging of the nonunion.

Methods. A tibial nonunion model was produced in each rabbit 12 weeks after diaphyseal osteotomy. Twelve rabbits were randomized with equal numbers in the control group and the experimental group, the latter receiving an ESW treatment with 1000 pulses at 0.54 mJ / mm2 and 1Hz frequency. During the post treatment period, bony changes of the nonunion region were monitored with sequential radiographs and polychrome sequential labeling for 10 weeks. The tibiae with nonunion were harvested for histological evaluation at the end of the experiment.

Results. It demonstrated a significantly higher rate of bony bridging of the nonunion gap in the EWS treatment group (Fisher exact test, $p < 0.05$). The ESW-promoted bony bridging was achieved via endochondral bone formation in the nonunion gap, with little periosteal callus formation. The histological findings suggest that the pattern and the process of ESW-evoked bony bridging of the nonunion are distinct from those of typical primary or secondary fracture healing.

Conclusions. ESW evoked bone bridging of the nonunion was via endochondral ossification from the islands of cartilage in the gap first, followed by remodeling of the transected cortices and intramedullary callus formation.

Conflict of Interest: None declared

P063-M

EXPRESSION OF WNT SIGNALING MOLECULES AT THE INJURED GROWTH PLATE CARTILAGE IN YOUNG RATS

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The growth plate cartilage is responsible for longitudinal growth of the skeleton. However, after injury, the growth plate is often repaired by bony tissue which causes bone growth deformities. Using a rat model, our previous studies have shown sequential inflammatory, fibrogenic, osteogenic and bone remodeling responses involved in the bony repair of the injured growth plate. However, molecular pathways regulating these cellular events remain unknown. One pathway that could be potentially important in regulating the bony repair of injured growth plate cartilage is the Wnt signaling pathway, which has been shown by many studies to be important in regulating mesenchymal stem cell proliferation and bone cell differentiation. Using quantitative real time RT-PCR analysis, this study examined mRNA expression profiles of the Wnt signaling components in the injured proximal tibial growth plate 1, 4, 8, 14 and 25 days after injury. Compared to uninjured control group, expression of Wnt-5a was significantly decreased during all phases of the bony repair, particularly on days 4 (fibrogenic), 8 (bone formation) and 14 (bone maturation). Levels of LRP-5 were significantly decreased on days 8, 4 and 14, where no changes were observed for LRP-6. A steady increase in SFRP-1 was observed after injury till day 25 and there was an increase on day 8 for SFRP-4 ($P > 0.05$). These results suggest a potential involvement of Wnt signaling particularly from Wnt-5a and LRP-5 in growth plate injury responses and during bony repair.

Conflict of Interest: None declared

P064-T

CHONDROGENIC DIFFERENTIATION OF HUMAN ADIPOSE-DERIVED MESENCHYMAL STEM CELLS

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Cartilage has limited capacity for self repair. Cell therapy has therefore been considered promising for the treatment of cartilage defect. Adult pluripotent mesenchymal stem cells (MSC) have recently been contemplated as potential reparative cells for cartilage tissue engineering. Whilst bone-marrow derived MSC have been extensively used for tissue engineering, adipose tissue recently deserves consideration owing to its high accessibility. Chondrogenic differentiation of human adipose tissue-derived MSC (hATSC) can be induced by the combination of enriched culture medium and a three dimensional (3D) environment. Given that cartilage is an avascular tissue, oxygen tension has also been suggested as a regulatory factor of chondrocyte differentiation. In this context, our work aimed at determining the effect of hypoxia on the chondrogenic differentiation of hATSC in 2 and 3D culture. hATSC were isolated from lipoaspirate and cultured for 5, 10, 20 and 30 days either in 2D (monolayer) or in 3D (high cell density pellets), in control or supplemented chondrogenic medium and under low (5%) or normal (20%) oxygen tension. Cell differentiation was monitored at the level of mRNA by real-time quantitative PCR. The production of sulfated glycosaminoglycans (GAG) and type II collagen were respectively determined by Alcian blue staining and immunohistological detection. Our results indicated that type II collagen expression was markedly induced by hypoxia in 2 and 3D culture. On the other hand, aggrecan expression was induced by the presence of chondrogenic medium in 2 and 3D culture whatever the oxygen tension. Histological analysis after a 30 days culture period showed the presence of GAG as evidenced by the positive Alcian blue staining in chondrogenic medium whatever the oxygen tension. The presence of type II collagen in the matrix of pellets was detected only when hATSC were exposed to chondrogenic medium and hypoxia. Our results highlight the major role of hypoxia and 3D environment in the chondrogenic differentiation of hATSC. To reinforce these data, additional in vivo experiments based on the transplantation of hATSC in subcutaneous site in nude mice are under investigation. Whether our findings may be promising for the cell-based therapy of cartilage should be paid further attention.
Conflict of Interest: None declared

P065-S

ULTRASTRUCTURAL AND CHEMICAL ANALYSES OF APATITE CRYSTAL IN HARD TISSUE OF CONODONT FOSSIL

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There were few reports that examined the ultrastructure and the property of the hard tissue in conodont fossil. The purpose of the present study is to examine the nature of apatite crystal of the hard tissue in conodont fossil. The tooth apparatus of conodont fossil (Yokogurayama Formation, Silurian, Kochi, Japan and Hushpuckney shale, Carboniferous, Missouri, USA) were used in this study. The specimens were observed 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. On the lower magnification of TEM, the hard tissue of condont consisted of 2 layers where the organization varied the size of crystal. The outer layer consisted of bigger crystals, and the inner layer consisted of smaller crystals. Higher magnification showed that the crystals were observed the lattice of (100) and did not present the central dark lines. On the electron diffraction, (002) plane was detected, which indicated the c-axis of apatite crystal. Results of EPMA showed that Ca, P, and F were detected in the crystal. 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 fluoraptite. This study was performed under the cooperative research program of Center for Advanced Marine Core Research (CMCR), Kochi University (06B004).
Conflict of Interest: None declared

P066-M

TITANIUM ORTHODONTIC MINIPLATES IN DOGS: ANCHORAGE EVALUATION WITH BONE MINERAL DENSITY MEASUREMENT

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Orthodontic titanium miniplates are new devices allowing temporary skeletal anchorage in particular indications like distalization of an entire arch, maxillary protraction and molar intrusion. The present animal study evaluated bone mineral density (BMD) around 80 orthodontic miniplates in order to define the conditions underlying their anchorage efficiency. Two miniplates were inserted in each jaw quadrant of 10 dogs. Two weeks later, coil springs generating a 125g force were fixed between the miniplates of one upper quadrant of each dog and between those of the contralateral lower quadrant. The other anchors remained unloaded. The dogs were sacrificed 7 or 29 weeks after surgery. Successful anchorage, analyzed with Kaplan Meyer test of the survival functions, was achieved in 53% of miniplates and was significantly ($p < 0.05$) higher for maxillary than for mandibular anchors, but not affected by loading. Peripheral quantitative computed tomography (pQCT) showed bone contact around most stable implants. BMD was higher around the mandibular than maxillary implants, particularly in the animals sacrificed after 29 weeks (paired t test, $p < 0.05$), without effect of loading. Failure occurred 4.9 ± 2.8 (mean $\pm$ SD) weeks after surgery, i.e. at the transition time between primary and secondary stability. Stability of titanium orthodontic miniplates depends on rigorous hygiene conditions, as well as on anatomical features of the receptor site, such as the amount of attached mucosa and bone architecture.
Conflict of Interest: None declared

P067-T

DISSOLUTION PRODUCTS FROM BIOMATERIALS USED FOR BONE REGENERATION

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Bioactive materials are frequently used as bone graft substitutes in the repair of bone defects. The bioactivity is related to surface reactions or dissolution products and interactions with surrounding tissues. The aim of this study was to evaluate the dissolution products of three different biomaterials used for bone replacement therapies; 45S5 Bioactive glass®, Bio-Oss® and Frios®.

Algipore®. The biomaterials were immersed (1 % w/v) in a-MEM cell culture medium, and after 24, 72 and 168 h. The media were thereafter analyzed for concentrations of calcium, phosphorus and silicon with ICP-OES. Our results demonstrated a small insignificant increase in calcium ion concentration, a significant decrease in phosphorus concentration and significant increase over time of silicon in the immersion media from 45S5 glass. The dissolution products for Bio-Oss® and Frios® Algipore® have not been reported earlier. Of particular interest is our finding that the bovine bone mineral Bio-Oss® released high concentrations of silicon while both calcium and phosphorus concentrations decreased significantly over time. The immersion of Frios® Algipore® also resulted in a significant time-dependent decrease in both calcium and phosphorus ion levels in the medium. The ICP results suggest formulation of Ca/P precipitates on the biomaterial surface which may be critical for the bone formation around these materials demonstrated in vivo.
Conflict of Interest: None declared

P068-S

TYPE OF PHYSICAL ACTIVITY AS A DETERMINANT OF BMC IN ADOLESCENT WOMEN: A 7-YEAR FOLLOW-UP

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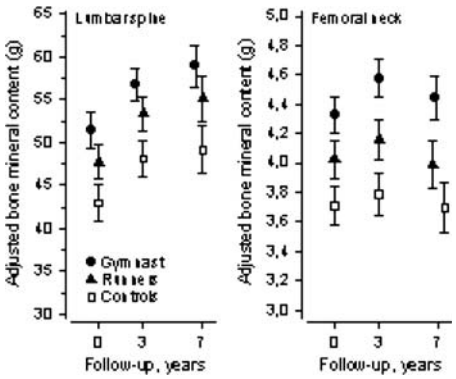
To investigate the effect of gymnastics and running on bone mineral content (BMC) at lumbar spine and femoral neck in comparison with age-matched sedentary controls.

A total of 142 Caucasian healthy peripubertal girls were included. 52 girls were competing gymnasts, 46 competing runners and 44 non-athletic controls. Weight, height, and type and amount of physical activity (MET hours/week) were recorded at 6-month intervals over 3 years and after seven years. BMC of lumbar spine and femoral neck were measured by the dual energy x-ray absorptiometry (DXA, Hologic) at baseline, 3-year and 7-year visits.

The median MET value (first, third quartile) of gymnasts was significantly higher compared with runners and controls at baseline [80.0 (55.5, 90.0), 42.5 (16.0, 66.7), 10.0 (6.0, 19.0), respectively, $p = 0.001$]. The gymnasts had started training significantly earlier than runners (6.4y vs. 4.6y, $p < 0.001$). The corresponding median MET values were [63.0 (25.5, 90.0), 24.0 (12.0, 75.0), 17.0 (9.7, 23.6)] at 3-year follow-up and [24.0 (12.0, 43.8), 27.8 (7.5, 50.0), 13.5 (5.2, 20.0)] at 7-year follow-up. The development of the adjusted (for years from menarche at 7-year follow-up, height, weight) bone mineral contents in lumbar spine and femoral neck are described in Figure 1. In both time and group the difference of

BMC was significant ($p < 0.001$). Group-by-time interaction was significant only at femoral neck ($p = 0.048$).

The type of sports was a significant factor of BMC. The benefits acquired by gymnastics persisted 7 years even after a clear reduction in physical activity levels after 3-year follow-up.



Conflict of Interest: None declared

P069-M

COMPARING THE EFFECTS OF EMDOGAIN (EMD), AMELOGENIN (AMEL) AND AMELOBLASTIN (AMBN) ON BONE GROWTH ONTO TITANIUM IMPLANTS

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The developing mammalian enamel matrix contains 90% amelogenin, ameloblastin and other matrix and serum proteins. Enamel matrix derivative (EMD), is clinically used in treatment of destructive dentoalveolar periodontitis to stimulate bone formation. The aim of the present study was to compare the effect of EMD, recombinant AMEL and AMBN on wound healing and bone attachment of titanium implants in vivo and osteoblast differentiation in vitro.

Coin shaped implants (n = 24) were placed onto pre-made defects with either EMD (10 μg/ml), AMEL (10 μg/ml), AMBN (10 μg/ml) or carrier medium randomly applied in tibia of rabbits. Tissue fluid and coins were analyzed after eight weeks. The effect of EMD, AMEL and AMBN were tested on murine preosteoblasts (MC3T3-E1) and primary human osteoblasts (Cambrex) cultured on titanium and plastic. Gene expression of bone markers and secretion of proteins to culture medium were tested after 24h, 48h and 72h.

AMEL enhanced the number of attached cells (measured as DNA content) by more than 2.5-fold compared to AMBN, EMD and carrier control in vivo. Alkaline phosphatase (ALP) activity in the tissue fluid was significantly enhanced ($p < 0.001$) after AMEL (2.6 fold) and EMD (4.8 fold) treatment, whereas AMBN had no effect. These observations were confirmed in vitro. Whereas the expression of osteocalcin in human primary osteoblasts was enhanced after all types of enamel protein treatments (EMD, 2.6 fold; AMBN, 3 fold; AMEL, 4 fold), only AMEL had an stimulatory effect on Runx2, CD44, OPG and leptin mRNA expression.

In conclusion, pre-treatment of bone defects with EMD and AMEL enhances osteoid mineralization (ALP activity) as well as cell growth on titanium implants. Moreover, recombinant AMEL seems to be more potent than the complete EMD formulation and AMBN in stimulating cell growth and differentiation of bone cells on titanium implants suggesting that EMD also contains other components that modulate bone formation.

Conflict of Interest: None declared

P070-T

SCAFFOLD GEOMETRY REGULATES TISSUE GROWTH IN VITRO

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The differentiation and proliferation of osteoblasts is known to be strongly influenced not only by biochemical signals but also by the physical characteristics of their substrate including roughness, elasticity, pore size and geometry. However, a quantitative evaluation of the influence of pore size and geometry in thee-dimensional tissue growth is missing.

For this purpose we established an in vitro model system, which allows microscopic observation and quantification in a thee-dimensional environment. We used hydroxylapatite plates containing perforations of various cross-sections (triangular, squared, hexagonal and round) and various sizes normalized to the perimeter (U = 3.14 mm, 4.71 mm and 6.28 mm). In each channel tissue growth was quantified over a period of six weeks with phase contrast microscopy combined with image analysis. Confocal laser scanning microscopy was used to visualize the tissue formation modus into the depth of the pores as well as cell orientation within the tissue.

Observations revealed a strongly favoured growth in the corners of the pores, which led to the formation of a tissue structure maintaining a round central channel. Tissue formation within the thee-dimensional pores clearly showed a striking influence of the perimeter. Doubling the perimeter from 3.14 to 6.28 mm reduced the amount of tissue at a defined time point to half or less. Whereas the different pore shapes within one size had no effect on total tissue area. The derived growth kinetics is consistent with a biphasic modus in the formation of new tissue.

Moreover, we found evidence for the development of a pattern of mechanical forces in the tissue. Stress fibres in the corners were typically directed towards the walls, while around the central channel, stress developed a ring-like pattern reminding of the "purse-string" found in embryonic wound healing. We speculate that the development of this mechanical interaction between cells is responsible for the change in growth kinetics in the biphasic model. The data of this project demonstrate that also physical parameters are essential in the process of tissue formation in vitro.

Conflict of Interest: None declared

P071-S

RELATIONSHIP BETWEEN WHOLE BODY, FOREARM AND AP SPINE DENSITY IN A POPULATION OF HEALTHY CHINESE ADOLESCENTS

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Bone density at different scan sites consists of different proportions of trabecular and cortical bone which in the growing child may vary even more than in the adult. This study evaluated the relationship between densities at largely cortical sites (whole body and proximal forearm) and sites that consist to a large extent of trabecular bone (distal forearm and AP Spine).

A normal population of 165 male and 73 female Chinese children between 13 and 17 years of age underwent evaluation of AP Spine, Proximal and Distal Forearm and Whole Body using the Norland XR-36. All scans were audited by a co-author to assess that the scans were free of methodological fault.

Analysis of the studies shows linear regressions between bone density at different scans sites as follows:

Body to Distal Forearm $y = 0.4484x - 0.0335$
Body to Proximal Forearm $y = 0.6653x + 0.1019$
Body to AP Spine $y = 1.1041x - 0.0506$
Distal to AP Spine $y = 1.4858x + 0.3551$
Proximal to AP Spine $y = 1.234x + 0.0638$

The studies also showed the densities at different sites were significantly ($p < 0.0000001$) correlated.

In conclusion, the results show that in this population of normal adolescent Chinese, the bone density at one site is highly correlated with bone density at other sites – even when the cortical and trabecular composition of the sites differs substantially.

Table: Correlation of bone density between scan sites

	Whole Body	Distal Forearm	Proximal Forearm	AP Spine
Whole Body	1.0000	0.7156	0.7585	0.8178
Distal Forearm		1.0000	0.7803	0.6896
Proximal Forearm			1.0000	0.7897
AP Spine				1.0000

Conflict of Interest: TV Sanchez, Norland – a CooperSurgical Company, Employee JM Wang, Norland – a CooperSurgical Company, Employee

P072-M

SEX-DEPENDENT EFFECT OF ALPHA-KETOGLUTARATE ADMINISTERED POSTNATALLY ON RIBS DEVELOPMENT IN PIGLETS PRENATALLY TREATED WITH DEXAMETHASONE

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The study was performed to determine whether alpha-ketoglutarate (AKG) administered during 35 days of postnatal life to male and female piglets reduces negative effect of dexamethasone (Dex) on the development of ribs when administered to sows during 45 last days of pregnancy.

The study was carried out on 5 control sows and 5 experimental sows administered with Dex i.m. at the dosage of 3 mg/sow/48h during the last 45 days of pregnancy. Piglets chosen randomly just after the birth from the control sows were assigned to the control group. Piglets born by sows treated with Dex were divided into two groups: Dex/AKG group was administered orally AKG at the dosage of 0.4 g/kg BW/d and Dex/Cont group was administered orally saline instead of AKG up to 35 days of postnatal life. Each group of piglets consisted of 10 males and 10 females. Bone mineral density and the mechanical properties with a use of three-point bending test were evaluated. Growth hormone (GH) was determined in serum. The heaviest and the longest ribs had females from Dex/AKG group, followed by males from the same group. The shortest and the lightest ribs had males from Dex/Cont group. The weight of ribs from females of Dex/Cont group was two-fold higher than in males from Dex/Cont group and about 30 % from control males. BMD of ribs in female piglets from Dex/AKG group (0.172 ± 0.03 g/cm^2) increased by 123 %, when compared with males from Dex/Cont group. BMD of ribs in control males and females were 0.121 ± 0.003 g/cm^2 and 0.110 ± 0.002 g/cm^2, respectively. The value of ultimate strength of ribs from female Dex/AKG group reached 118 ± 3.8 N and was higher by 55 % and by 207 %, when compared to male piglets from Dex/AKG and Dex/Cont groups, respectively. The value of ultimate strength of ribs from females and males in control group reached 90 ± 4 N and 103 ± 1.9 N, respectively. The highest level of GH was in females from Dex/AKG group (8.43 ± 0.2 ng/ml) and the lowest in males from Dex/Cont (3.01 ± 0.3 ng/ml). The level of GH in male and female controls reached 5.28 ± 0.3 ng/ml and 4.01 ± 0.3 ng/ml, respectively.

Oral AKG administration to piglets during their 35 days of postnatal life, born by sows exposed to dexamethasone treatment during the last 45 days before the delivery, reduced its negative effect. Effect of AKG depended on sex being essentially higher in females than in males. AKG may be applied therapeutically in newborns to diminish negative effects in bones caused by prenatal action of dexamethasone.

Conflict of Interest: None declared

P073-T

ARTERY WALL AS A SOURCE OF OSTEOGENIC CELLS AND ITS APPLICATION FOR GUIDED BONE REGENERATION

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BACKGROUND: As arterial calcification is a common finding in ageing, atherosclerosis and other pathological conditions, we hypothesize that artery wall has an intrinsic osteogenic potential and therefore the ability to heal bone defects when arteries are used as guided tissue regeneration membranes

AIMS: a) "in vitro" characterization of media layer cells and demonstration of their osteogenic potential; b) "in vivo" use of cryopreserved arteries as guiding membranes to regenerate bone defects

METHODS: Cryopreserved and fresh rabbit thoracic aorta specimens were used: a) Medial layer was separated from adventitia and intimae by physical methods and cultured as explants in standard medium; cells were harvested and added to biological scaffold in osteogenic medium. b) To test its ability in healing bone defects, 10 cryopreserved arterial grafts were applied as guiding membranes around 10 bone defects (10 mm in-length) in rabbit radius, and the obtained bone regeneration was compared with 10 uncovered control defects

RESULTS: a) Cultured cells showed smooth muscle features: inmunofluorescence with anti-smooth muscle alpha actin, anti-calponin and anti-vimentine antibodies. Two subpopulations were identified: polygonal cells and elongated cells, in which appeared nodular condensations after confluence. When a non-cloned population of cultured cells was added to a 3-D matrix (biological scaffold) they showed chondrogenic and osteogenic differentiation as they stained positive for type II and type X collagen, alkaline phosphatase and Von Kossa.

b) Radius defects surrounded by cryopreserved arteries as guiding membranes showed total regeneration in 9 of 10 cases, confirmed by radiological and histological analysis. Density and percentage of regeneration increased monthly during the first six months ($p < 0{,}05$). No tissue layer was found between the bone and the artery. Wide calcification areas between elastic laminae (confirmed by Von Kossa staining) were observed. No histological healing or radiological significant differences were detected in the control group.

CONCLUSION: These findings both suggest the ability of medial arterial cells to undergo endochondral ossification in a proper environment and the possible role of those cells in healing long bone defects when cryopreserved arteries are used as guided tissue regeneration membranes.

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Conflict of Interest: None declared

P074-S

PATHOLOGICAL OA CHONDROCYTES ARE PRIMED FOR DESTRUCTION COMPARED TO THAT OF HEALTHY CHONDROCYTES BY EXCESSIVE INDUCTION OF AGGRECANASE ACTIVITY IN RESPONSE TO PRO-INFLAMMATORY CYTOKINES

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Background: Pathological chondrocytes have altered protein expression profiles. However, whether these chondrocytes respond differently to cytokine stimulation, and thereby are primed for pathological excessive protease induction remains to be investigated. We investigated the response of either normal OA or osteoarthritic injured chondrocytes with respect to MMP and aggrecanase induction in response to pro-inflammatory cytokines.

Methods: Human articular cartilage was obtained from patients undergoing total knee arthroplasty and divided the material into normal OA versus eroded damaged osteoarthritic cartilage for investigation of the endogenous protein profile in response to stimulation *in vitro*. As triggers of cartilage degradation IL-1α, IL-1β or the combination of OSM and TNF-α was used in articular cartilage explants cultures. Cultures were kept for 3 weeks with refreshment of the conditioned medium every second to third day. Fragments released to the conditioned medium were quantified by ELISA, thus MMP-derived fragments of collagen type II and aggrecan were measured by CTX-II and ^{342}FFGV-G2 ELISA, respectively. Aggrecanase derived fragments were quantified by the ^{374}ARGS-G2 ELISA. The release of sulphated glycosaminoglycans (sGAG) and hydroxyproline (OHpyr) were also evaluated in the conditioned medium. As a measure of the retained proteins in the cultured cartilage explants, the explants were subsequently pulverized and the proteins resuspended into an aqueous solution, and all biomarkers were measured in the protein extracts.

Results: Stimulation of normal OA cartilage resulted in a modest 1 fold significant induction of aggrecanase activity. In contrast, stimulation of pathological chondrocytes resulted in a 3000% induction of aggrecanase activity. MMP mediated collagen type II degradation (CTX-II) and MMP mediated aggrecan degradation were comparable in the normal OA cartilage.

Conclusion: Therapies blocking a single dominant cytokine have been shown to relieve cartilage destruction in rheumatoid arthritis, and expectations are high that mechanism-based treatments could also be developed for patients with OA. Articular chondrocytes with a normal appearance from OA patients was compared to the injured osteoarthritic chondrocytes from the same patient, and we found that OA cartilage explants respond differently to various stimuli of cytokines. This aspect is important to consider when investigating new potential treatments for OA.

Conflict of Interest: None declared

P075-M

REGIONAL DIFFERENCES IN P2X7 RECEPTOR REGULATION OF BONE METABOLISM IN P2X7 KNOCK-OUT MICE

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The purinergic receptor P2X7 is an ATP-gated ion channel, involved in bone remodeling. The P2X7 receptor is primarily expressed in cells of the hematopoietic lineage, and mediates intercellular signaling between osteoblasts and osteoclasts. The P2X7 receptor is involved in modulating osteoclast activity at least partially through regulation of osteoclast apoptosis. The aim of the study was to investigate the role of the P2X7 receptor in bone in vivo.

Mice harboring a knockout (KO) of the P2X7 gene were compared to wildtype (WT) littermates. Two and 10 days before sacrifice (4 months old), the mice were injected with calcein and tetracycline, respectively. DXA scanning, blood collection for bone marker analysis (C-telopeptide collagen type I fragment, alkaline phosphatase, and osteocalcin), and bone collection (vertebrae and tibiae) for histomorphometry were performed.

KO mice had significantly lower BMD (5,7%) than the WT. In contrast DXA scanning of the femur showed that the KO animals had 6% higher BMD in the femur as compared to the WT. The level of bone formation and resorption markers in the serum, were not significantly different between the groups, but when determined as a formation/resorption ratio the KO mice had a 4,7–14,8% higher ratio than the WT mice. Bone histomorphometric analyses revealed that trabecular thickness (Tb.Th) was significantly lower (17,7%) in the vertebrae of the KO mice than in the WT mice. In contrast, Tb.Th in the tibiae was 19% higher in the KO than in WT. The other indices investigated showed no sig-

nificant difference in the vertebrae, while in the tibiae the bone volume fraction (BV/TV%) was doubled in the KO mice, and the cortical thickness (C.Th) was increased with 36% in the KO as compared to the WT. The eroded surface (ES/BS) and the mineralizing surface (MS/BS) in % of total surfaces were not significantly different among groups for either region. Interestingly, the ratio between the MS/BS and ES/BS were significantly higher in the KO mice than in the WT in both regions (25,4–25,7%).

In conclusion, the P2X7 receptor has different significance in different regions of the skeletal system and as it has been shown to be involved in the response to mechanical stimulation/physical activity the differentiated action of the receptor might account for some of these differences. Further studies are warranted to determine the background for the differentiated receptor function.
Conflict of Interest: None declared

P076-T

PRENATAL PROGRAMMING OF SKELETAL DEVELOPMENT IN THE OFFSPRING: EFFECTS OF MATERNAL TREATMENT WITH 3-HYDROXY-3-METHYLBUTYRATE (HMB) ON FEMUR PROPERTIES IN GROWN PIGS

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Alteration in fetal growth and development in response to prenatal environmental conditions such as nutrition has long-term or permanent effects during postnatal life. The aim of this study was to investigate effects of 3-hydroxy-3-methylbutyrate (HMB) treatment of sows during the last two weeks of pregnancy on programming of skeletal development in the offspring. The study was performed on 141 pigs born by 12 sows of Polish Landrace breed. Two weeks before delivery, pregnant sows were divided into two groups. The first group consisted of control sows ($n = 6$) that were treated with placebo. Sows that were orally treated with 3-hydroxy-3-methylbutyrate ($n = 6$) at the dosage of 0.05 g/kg of body weight per day belonged to the second group. Newborn piglets were weighed and subjected to blood collection for the determination of serum levels of growth hormone (GH), insulin-like growth factor-1 (IGF-1), insulin, leptin, glucose and bone alkaline phosphatase (BAP) activity and lipid profile. At the age of six months, the piglets were slaughtered and femur was isolated for analysis. The effects of maternal administration with HMB on skeletal properties in the offspring were evaluated in relation to bone mineral density and geometrical and mechanical properties. Maternal treatment with HMB increased serum levels of GH, IGF-1 and BAP activity in the newborns by 38.0%, 20.0% and 26.0%, respectively ($P < 0.01$). HMB administration significantly increased volumetric bone mineral density of the trabecular and cortical bone of femur in the offspring at the age of six months ($P < 0.001$). The weight of femur and geometrical parameters such as cross-sectional area, second moment of inertia, mean relative wall thickness and cortical index significantly increased after HMB treatment ($P < 0.05$). Furthermore, HMB induced higher values of maximum elastic strength and ultimate strength of femur ($P < 0.01$). The obtained results showed that maternal administration with HMB has positive long-term effects on bone tissue and improves volumetric bone mineral density, geometrical and mechanical properties of femur in the offspring. These effects were connected with increased concentrations of GH and IGF-1 in the newborns suggesting involvement of somatotropic axis function in prenatal programming of skeletal development in pigs.

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Conflict of Interest: None declared

P077-S

BONE-LIKE HYDROXYAPATITE FORMATION IN HUMAN BLOOD

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The morphology, structure and chemical content of calcificate hearth valves have been studied by transmission electron microscopy (TEM) and X-ray analysis with energy dispersion (EDS). According to the TEM data, the calcificate aggregates of the cardiac valves consist of primary particles of hydroxyapatite (HAP) different in size, morphology and structural ordering. The major part of the valve HAP consists of 10–100 nm microcrystals similar to the bone apatite (-axis in the crystal plane). Large grains normally consist of disoriented blocks with the sizes from 5 to 20 nm. The largest crystals comprising a small part of the volume in the samples of different mineralization degrees have a plate-like shape and are about 5 nm thick and 1 to 5mkm in cross sizes. A supposition on a possibility of formation of calcium phosphate mineral in plasma has been tested next. Solid rests of plasma of patients with calcinosis (4 samples), lymph of the patient with disseminated sclerosis (1sample) and blood healthy donors (7 samples) for the purpose of control have been studied. Organic parts of solid rests of blood have been removed.

We have discovered the HAP crystals (10–70nm), which are structurally and morphologically similar to the bone apatite and the fine fraction of the cardiac valve calcification, both in the solid residue of the blood plasma of patients with calcinosis, and in the blood of healthy donors. Furthermore, in the lymph of the patient with disseminated sclerosis we found plate-like crystals up to 2.5mkm in size similar to the microcrystals with (0001) planes in cardiac valves. It also contains needle-like microcrystals, but they differ from the microcrystals of the fine fraction of valve HAP. Hydroxyapatite of the solid residue of blood and lymph was identified by electron diffraction and X-ray spectra (EDS). The significance of these results for an explanation of the mineralization mechanism of soft tissue of an organism will be discussed.
Conflict of Interest: None declared

P078-M

MENOPAUSE-RELATED CHANGES IN THE RATE OF CORTICAL BONE RESORPTION INCLUDE CONSECUTIVE PHASES OF INCREASE, STABILISATION AND DECREASE

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Estimates of cortical bone resorption rates were derived by studying a population after accidental intake of large amounts of ^{90}Sr with river water contaminated by radioactive discharges from the Mayak plutonium production complex (Southern Urals, Russia) in the early 1950s. Repeated measurements of the ^{90}Sr-body burden obtained individually during long periods of observation (from 6 to 23 years) allow the estimation of individual cortical resorption rates and the study of age and gender features in the cortical resorption in the population over a wide age range (30–80 years old). Measured levels of 90Sr-body burden (5–150 kBq) did not result to an increase in the rate of cortical resorption. Longitudinal studies were conducted for 412 men (3509 measurements) and 365 women (3526 measurements). A procedure was developed to average individual cortical resorption rates obtained over long-term periods according to calendar age and/or age relative to menopause for women. It was found that women manifested statistically higher cortical resorption rates compared to men over the entire age period (30–80 years). The median rate of cortical resorption remains unchanged in men and women of reproductive age. Further, the rate increases in men after the age of 55 years from 2.8% y^{-1} to 3.1% y^{-1}. Subsequent increase with increasing age is slower reaching 3.7% y^{-1} by the age of 70–80 years. In women, evident menopause-related changes in the rate of cortical bone resorption were revealed allowing the selection of characteristics periods of sharp increase in cortical bone resorption rate from 3.1% y^{-1} to 6.9% y^{-1} during the first 1–2 years after the menopause; stabilization of cortical bone resorption rate up to 12 years after the menopause; subsequent decrease in resorption rate in the period of 12–13 years after the menopause from 6.9% y^{-1} to 6.1% y^{-1} and stabilization of the resorption rate up to 25 years after the menopause. The analysis of the influence of diseases affecting bone turnover showed individual changes in the resorption rate but this was not statistically significant. In a sample of the general population and in a sample without bone-threatening diseases or medication the cortical resorption rates were similar. This work has been funded by the Russian Foundation for Fundamental Investigations (Projects 04-04-96085).
Conflict of Interest: None declared

P079-T

THE EFFICACY OF TERIPARATIDE (TPTD) IN THE RECOVERY OF DIAPHYSIAL MIDDLE PSEUDO-ARTRHROSIS OF FEMOUR IN PATIENT AFFECTED BY NO RESPONDER SEVERE OSTEOPOROSIS (OPM)

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Woman of 70y, with no responder severe OPM, is submitted to surgical operation of osteosynthesis with steel implants internal synthesis for traumatic diaphysial middle fracture of the femur dx (Fig. 1). The patient had been treated in past with Clodronato (100mg/w for 3y) and, to the moment of the fracture, took on Alendronato 70mg/w + ca.& vit.D for 5y. The OPM is demonstrated clinically at the age of 60y with spontaneous fractures of the ribs and subsequently it is complicated also with the fracture of D6 at the age of 65y. The values of the BMD, indicatives of severe OPM (T-lumbar score = −4,2DS and T-score FN = −2,3DS), were you rhymed substantially constant during the period of treatment. To the radiological control of the femur, performed to distance of about 5 months to the operation, it is emphasized evolution pseudo-arthrosis of the injury, characterized from persistence of the rhyme of fracture and from presence of meager callus of type hypertrophy, for that you are shown the

necessity of a further operation (Fig. 2). On the escort of how much restored recently in literature, it has decided to delay at the moment at the surgical operation, of to treat the patient with TPTD & of to program a successive radiological control to 3 months to the beginning of the administration of the medicine. To the follow-up is observed the entire recovery radiographic of the injury with total disappeared some rhyme of fracture pseudo-arthrosis and consolidation of the fracture (fracture repair) (Figs 3 and 4).

Conclusions: this clinical case confirms the osteoinductor effect of TPTD across a most rapid recovery of the also not spinal breaks in patients with severe OPM and, besides, to have a potential role in the treatment of some shapes of pseudo-arthrosis.

Conflict of Interest: None declared

P080-S

THE EFFECT OF RHBMP-4 INCORPORATED COLLAGEN HYDROGEL ON OSTEOGENESIS OF NON-DECORTICATED POSTERIOR SPINAL FUSION

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Background: The finding that rhBMP-4 can induce non-marrow derived mesenchymal cells to differentiate into osteogenic cells encouraged the using of rhBMP-4 as osteoinductive agent in non-decorticated spinal fusion model. Collagen as a major component of the extracellular matrix has been proved to be able to store various growth factors. The objective of the present study is to evaluate the effect of rhBMP-4 incorporated collagen hydrogel on osteogenesis of posterior spinal fusion without decortication.

Method: Collagen hydrogel was loaded with rhBMP-4 overnight at 4 ? to obtain the rhBMP-4 incorporated collagen hydrogel delivery system. The release characters of rhBMP-4 were evaluated by ELISA in vitro. In vivo, five rabbits underwent single level non-decorticated bilateral posterior intertransverse process spinal fusion at L5–L6, one side with the graft of rhBMP-4 (0.5mg) incorporated hydrogel and the other side with the graft of PBS incorporated hydrogel. New bone formation was evaluated with plain x-ray, microradiography and histomorphology.

Results: The rhBMP-4 incorporated collagen hydrogel delivery system showed a triphasic release profile in vitro, consisting of an initial burst of 27.59% at 2h, a second cumulative release of 39.79% at day 1 and a third cumulative release of 100% at day 7. In vivo, the percentage changes in both gap distance and bone volume showed significant difference when compared the side of rhBMP-4 incorporated hydrogel with the other side at week 7 post operation.

Conclusion: RhBMP-4 incorporated collagen hydrogel is a controlled release system and can effectively induce new bone formation in the non-decorticated spinal fusion model.

Conflict of Interest: None declared

P081-M

THE EFFECT OF LOW INTENSITY PULSED ULTRASOUND ON THE HEALING OF RHBMP-4 INCORPORATED HYDROGEL GRAFT IN SPINAL FUSION

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Background: It has been proved that daily therapy with low intensity pulsed ultrasound (LIPU) treatment for 20 minutes is safe and beneficial in the treatment of fractures, bone lengthening, and fracture nonunion. The objective of our study is to evaluate the effect of LIPU on the healing of rhBMP-4 incorporated hydrogel graft in spinal fusion model.

Method: 10 rabbits underwent single level non-decorticated bilateral posterior intertransverse process spinal fusion at L5–L6, both sides with the graft of rhBMP-4 incorporated hydrogel. All animals were randomly allocated into two groups with 0.125 μg (low dose) and 0.5 μg (high dose) of rhBMP-4 respectively. Each rabbit was treated with LIPU only on the one side and the other side served as control. New bone formation was evaluated with plain x-ray, microradiography and histomorphology.

Results: The percentage changes in both gap distance and bone volume showed a significant difference when compared LIPU treated side with the control side at week 7 post operation in both groups. Such difference also can be observed between the high-dose and low-dose groups.

Conclusion: LIPU can effectively enhance the healing of rhBMP-4 incorporated hydrogel graft in spinal fusion model. Moreover, the effect of rhBMP-4 is dose dependent.

Conflict of Interest: None declared

P082-T

APPLIED BIOMECHANICS IN MOTORIC THERAPY FOR OSTEOPOROTIC PATIENTS

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Aim: To set up a methodology of motoric therapy based on biomechanical laws of strength and elasticity of osteoporotic skeleton.

Methods: We have established the methodology of motoric therapy for osteoporotic patients on the basis of biomechanical analysis of the spinal load by compressive and tensile forces and bending moments. It is based upon: 1) mechanical characteristics of the resistance of biomaterial – bone – against its disruption and permanent deformation; 2) action and reaction forces, the stress intensity developed in the bone during the load and local concentrated stress intensity in the area of osteoporotic vertebral microfractures. Results: The principal axiom in the methodology of motoric therapy: During the exercise, whether lying down, standing or sitting, the position of the spine is stable, and retains its physiological curvature. In the set of exercises we exclude: strengthening exercises with weights, dynamic strengthening of one UL with rubber resistance band, jumps and vaults (exercises with transferring compressive force to long bones, vertebrae and joints), stretching dynamic and rapid swinging movements, strengthening dynamic rapid exercises, twisting spinal exercises, forward, side, and back bending. In the set of exercises we prefer: slow relaxing, stretching and strengthening exercises, strengthening exercises, symmetrical exercises with both UL, isometric exercises to reinforce the muscular corset of the spine, exercises activating coordination movements in simulated balance disruption. Conclusions: The aim of motoric therapy is as follows:

1) to prevent further bone mass loss and in the combination with medicamentous therapy to improve the bone quality (biomechanical properties: strength and elasticity)

2) by removing the dysbalance of torso musculature to alleviate or abolish pain and so to reduce the costs of medicamentous pain therapy, to achieve a proper posture that eliminates the incorrect mechanical load of osteoporotic vertebrae, preventing their deformations

3) to improve the coordination of movements in balance disruption (preventing falls).

Conflict of Interest: Anyone

P083-S

BONE TENDON JUNCTION REGENERATION WITH ARTICULAR CARTILAGE

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Background: Bone tendon junction often heals without fibrocartilage transition zone regeneration. It was postulated that autologous articular cartilage interposition would improve healing and regeneration of bone tendon junction.

Methodology: Goat partial patellectomy repair model was used. Articular cartilage harvested from the excised distal third patella was interposed between patella and patellar tendon during the repair. Control group received no cartilage interposition. The patella–patellar tendon complexes were harvested at 6, 12, and 24 weeks ($n = 6$) for histological examination. Bone formation and fibrocartilage zone regeneration were examined. The histological images were digitized and analyzed using an image analysis system. The length and area of new bone formation, length of basophilic line formed, fibrocartilage regeneration, and Safranin O staining were measured. Quantitative data were analyzed using SPSS version 14.0. Statistical significance level was set at $p < 0.05$.

Results: New bone formation was demonstrated at the healing bone tendon junction during partial patellectomy healing. Maximum new bone length increased from 1470 ± 326 μm at 6 weeks, to 3147 ± 323 μm at 12 weeks, and 3502 ± 358 μm at 24 weeks (p = 0.011, Kruskal–Wallis test), and the area of new bone formed increased from 7.55 ± 1.97 mm2 at 6 weeks to 20.15 ± 2.70 mm2 at 24 weeks (p = 0.015, Kruskal–Wallis test) the articular cartilage interposition group. There was no significant difference between the cartilage interposition group and control group at the same time point. The length of fibrocartilage formed in control group amounted to less than 10% of the healing junction length. Safranin O uptake was rarely seen. Articular cartilage interposition resulted in significantly more fibrocartilage regeneration with high proteoglycan content at all time points. The length of fibrocartilage formed measured 7760 ± 629 μm at 24 weeks with articular cartilage interposition, compared with 787 ± 274 μm in control group (p = 0.002, Mann–Whitney test). Safranin O length measured 3301 ± 1236 μm at 24 weeks with articular cartilage interposition, compared with 277 ± 187 μm in control (p = 0.03, Mann–Whitney test). Basophilic line formation, on the other hand, was comparable.

Conclusion: New bone formation occurs during bone tendon junction healing. Autologous articular cartilage interposition improved fibrocartilage transition zone regeneration. Bone formation was not affected.
Conflict of Interest: None declared

P084-M

UTILITY OF ALPHA CTX AS AN INDEX OF VELOCITY OF SKELETAL GROWTH IN PREPUBERAL CHILDREN

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Degradation products of collagen type I can be measured by CrossLaps (CTX) immunoassays, providing an index of bone resorption. The CTX epitope EKAHDGGR comprises a DG-motif susceptible to post-translational modifications. In newly synthesized collagen this motif is in the α-CTX form, but during aging of bone it is isomerized to β-CTX form. Both CTX reflects metabolic activity and rate of bone remodelling but as α-CTX arises from new bone, it could be associated with bone modelling at childhood. In the present work α-CTX was measured in children attending public schools of Vicente Lopez to assess if there were differences in α-CTX levels related to the usual calcium intake. In 48 normal healthy children, aged 6.2 to 9.2 years, calcium (Ca) (atomic absorptiometry), α-CTX (ELISA, Nordic Bioscience Diag. A/S) and creatinine (C) (modified Jaffé method) levels were performed on second morning void samples. The calcaneous ultrasound (QUI) was also measured (Sahara–Olohi). Ca and α-CTX were expressed as ratio to C excretion. Results: In normal healthy children, a biochemical nutritional index of usual Ca intake is Ca/C ratio determined in the second morning void which ranged between 0.07 and 0.15 when Ca intake is adequate. Results: As expected, according to food intake records, usual Ca intake which is necessary for skeletal growth was low in 37 of 48 studied children (77%). Levels of α-CTX correlated with both Ca/C and QUI values (r = 0.52; p < 0.001 and r = -0.43; p < 0.003, respectively). When children were divided according to their range of Ca/C: lower than 0.07(0.036 ± 0.018a); 0.07–0.15(0.096 ± 0.024b)and higher than 0.150(0.222 ± 0.06c), the α-CTX levels (mean ± SD) were: 0.73 ± 0.21a (n = 37); 0.93 ± 0.26b (n = 11)and 1.19 ± 0.38b (n = 5), respectively (different letters: p < 0.04)

Conclusion: Bone metabolic activity during growth could be estimated by α-CTX since it correlated with both, nutritional and bone density parameters. Although future studies are necessary, α-CTX seems to be a promissory index of skeletal growth and bone modelling to be used in cross-sectional screening studies of growth velocity. Supported by UBACyT, B703. SU Project
Conflict of Interest: None declared

P085-S

HRT, POSTMENOPAUSAL OSTEOPOROSIS AND WEIGHT GAIN

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Background: Weight gained during menopause increase the risk of high blood pressure, the diabetes, heart disease, and has been strongly linked to increased incidence of breast and other hormone-related postmenopause cancers.

Aim: looking over weight gain and osteoporosis treatment in climacteric.

Material and methods: 20 women who were 45 to 57 years old have been recruited. BMI was increased for age. Moderate to severe vasomotor symptoms, vulvar and vaginal atrophy and risk of postmenopausal osteoporosis were associated. They were separated into equal two 10 women groups. One group was assigned to 2 mg drospirenone /1 mg 17-beta-estradiol hemihydrate. The other group treated with tibolone 2,5 mg .

Results: In the women on 2 mg drospirenone/1 mg 17-beta-estradiol hemihydrate medication decreased symptoms of vulvar, vaginal atrophy and vasomotor symptoms associated with the menopause in regard to the other group. They had weight main loss of 4 kg (P < 0.05).

Conclusions: Human HRT is in relation to decrease osteoporosis. Nobody noted 17-beta-estradiol is in relation to breast cancer. Drospirenone has the unique property of reducing water retention often associated with the use of oestrogen and other synthetic progestin hormones. The impact of obesity on hormone replacement therapy is due to many women associate hormones with weight gain. This formula can be beneficial in minimizing uncomfortable symptoms, such as weight gain, hot flashes, night sweats, and mood swings, and osteoporosis. So, we need to conduct one big try to clarify these points.
Conflict of Interest: None declared

P086-M

ONCOSTATIN M SENSITIZES RAT OSTEOSARCOMA CELLS TO THE ANTI-TUMOR EFFECT OF PKC412

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Oncostatin M (OSM), a cytokine of the IL-6 family, reduces the growth and induces differentiation of osteoblasts/osteosarcoma cells into osteocytic cells. Moreover we recently described that OSM, via STAT5 and p53, sensitizes osteosarcoma and proliferating osteoblasts to apoptosis induced by various death inducers such as the kinase inhibitor Staurosporine (Sts). Here we asked whether OSM could synergize in vitro and in vivo with PKC412, a derivative of Sts and anti-cancer agent currently used in clinical trials.

We used the rat osteosarcoma OSRGA cell line for which we have a corresponding tumor model in rats. In vitro, OSRGA cells were resistant to the pro-apoptotic effect of PKC412 at concentration up to 10 μM, whereas OSM-treated cells were effectively killed by PKC412 (IC50 = 1 μM). The cell death observed with OSM + PKC412 was associated with membrane blebbing, nuclear condensation and caspase 3 activation, indicating an induced apoptosis. Next we analyzed the potent anti-tumor effect of the combination OSM + PKC412 in our rat osteosarcoma model. First we observed that alone an adenovirus coding OSM (AdOSM; 1.10^9PFU injected i.m.) reduced serum ALP and TRAP5b in correlation with a 42% reduced trabecular bone volume (trabecular tibiae BV/TV determined by micro-CT scan). Moreover AdOSM induced an anarchic ectopic bone apposition in the contact of the tumor (increased ectopic bone surface BS/BV) even if the animals were treated with the bisphosphonate Zoledronate to inhibit osteoclast-induced osteolysis. Preliminary experiments suggested that the association AdOSM + PKC412 (30mg/kg/day oral) was able to prevent progression of the primary tumor and the pulmonary metastatic dissemination, whereas these agents alone had no anti-tumor effect. The only side effects were limited weight loss and cachexia.

In conclusion, in vitro and in vivo experiments indicate that OSM alone does not induce osteoblast or osteosarcoma cell death but reduces matrix mineralization driven by these cells. OSM does not seem to possess strong pro-resorptive effect in vivo and its potent role on osteoclastogenesis needs further investigations. Because OSM sensitizes wild-type p53 osteosarcomas to apoptosis, the use of kinase inhibitors such as PKC412 in association with OSM could represent new anti-cancer treatments. Future experiments will determine whether an adjuvant treatment with OSM would be also benefic in association with standard chemotherapy such as doxorubicin or cysplatin.
Conflict of Interest: None declared

P087-T

INFLUENCE OF CANCER CELLS ON BONE MICROENVIRONMENT IN RAT METASTASIS MODELS

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Skeleton is the most common organ to be affected by metastatic cancer but interactions of tumor cells with the bone marrow microenvironment have been poorly investigated. Bone metastases are commonly characterized as osteoblastic or osteolytic or mixed. The expression pattern of molecules implied in the bone remodelling was searched in the bone marrow and others organs microenvironment in contact with osteoblastic (MatLyLu MLL) and osteolytic (Walker W256) metastases by immunochemistry and histology.

Copenhagen rats received an intracardiac, intramuscular, intrahepatic, intrasplenic or subcutaneous injection with MLL cells. Fisher rats received an intracardiac, intramuscular or subcutaneous injection with W256 cells. Rats were sacrificed 15 days (Copenhagen) or 9 days (Fisher) after the intracardiac injection of tumor cells. Immunochemistry for DKK1, cathepsin K, RANKL, MCSF, c-Fms or IL6 expression in the microenvironment of the tumors was performed in bone and other metastatic sites. A histoenzymatic technique also allowed the detection of Tartrate Resistant Acid Phosphatase (TRAcP) + cells.

In bone, stromal cells of the bone marrow in contact with metastatic foci of both MLL and W256 tumors showed a huge membrane expression of MCSF. DKK1 was also expressed at high levels by stromal cells in the vicinity of metastatic foci (membrane and cytoplasmic expression). Many RANKL+ stromal cells were found in contact with metastatic foci. Cathepsin K and TRAcP+ cells were found in direct contact with trabeculae but mono or multinucleated cathepsin K and TRAcP+ cells were also encountered in bone marrow spaces, far from trabecular surfaces and near metastatic cells.

In extraosseous tumors, cells in contact with malignant cells did not expressed DKK1, MCSF, c-Fms, cathepsin K. Some RANKL+ cells were found in the periphery of subcutaneous tumors but may represent Langerhans cells. The physiologic IL6 expression was found in the liver cells. No TRAcP+ cells were observed in the vicinity of malignant cells in any extraosseous metastases. In invaded and non-invaded spleens, TRAcP+ cells corresponding to activated macrophages were encountered.

Interaction between stromal and cancer cells leads to osteoclastogenesis only in the bone microenvironment. The major role of osteoclasts and osteoblasts in bone metastasis formation has led to underestimate the critical function of microenvironment in metastasis growth.

Conflict of Interest: None declared

P088-S

INCREASED BONE REMODELING DUE TO DISUSE INCREASES TUMOR GROWTH

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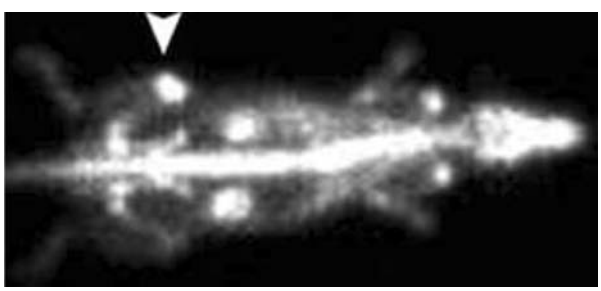
Bone microenvironment has an important role for survival and growth of tumoral cells. An IM injection of botulinum neurotoxin (BTX) in rat produces paralysis and induces a localized increase of bone remodeling leading to a bone loss. The effects of microenvironment on localization and growth of tumoral cells were evaluated in a bone metastasis rat model.

20 Copenhagen rats were randomized into 3 groups: injected with BTX in the right hindlimb; received an intracardiac injection of 3.5x104 MatLyLu cells (MLL); injected with BTX and MLL cells (MLL-BTX rats). BTX injection was done 2 weeks before MLL cells inoculation to obtain the highest bone remodeling. 2 weeks after the tumor cells inoculation, scintigraphs were done to evaluate bone metastases extensions (injection of technetium-99m MDP). After 24h, resident activity in femur and tibia were counted in a gamma counter. Ratio of right and left activities was determined. Bone volume and microarchitecture were evaluated by X-ray microCT on the femur (BV/TV, Tb.Sp, Tb.N, Tb.Th, Tb.Pf, SMI, fractal dimension).

Scintigraphic acquisitions showed a preferential uptake on the paralyzed hindlimb in BTX rats. The amount of uptake was furthermore increased on the right hindlimb in MLL-BTX compared to MLL or BTX rats. Ratio of ex-vivo counted activities in the femur and tibia of MLL-BTX rats were higher than in MLL or BTX rats, denoting higher activities in right femur and tibia. MicroCT showed a significant increase of bone loss in MLL-BTX rats compared to MLL (−13%) or BTX (−16%) rats. Tb.Pf, SMI, Tb.Th were also similarly altered. However, no significant difference of Tb.N, Tb.Sp and fractal dimension was obtained. Between MLL and BTX rats, no significant difference was found.

Our findings indicate that a localized increase of bone remodeling favours metastases growth. Bone cell activities should be reduced in patients having cancer with bone tropism to prevent bone metastases.



Conflict of Interest: None declared

P089-M

THE EXPRESSION OF BONE MORPHOGENETIC PROTEIN7 AND ITS RECEPTORS IN THE HUMAN NORMAL KIDNEY AND KIDNEY CANCER

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Bone morphogenetic protein-7 has an important role during fetal kidney development. In adult human kidney the expression was found in proximal tubule and glomerular podocytes. The function of BMP-7 in normal kidney is still unclear but the reviews revealed protective role of BMP-7 in acute and chronic kidney disease. BMP-7 is efficient as antifibrotic factor and is able to reverse epithelial–mesenchymal transformation.

The aim of the study was to explore the expression in human normal kidney and kidney cancer cells.

Immunohistochemical staining with anti BMP-7, anti BMPR-IA, anti BMPR-IB and anti BMPR-II was performed. Slides were incubated with the primary antibodies overnight at 4°C in PBS.

The expression of BMP-7 was found in epithelial cells of distal tubule. But the expression of related BMPs receptors was found in epithelial cells of distal and proximal tubules. Among receptors BMPR-IB expression was very strong. In the tissue of kidney cancer the expression of BMP-7 was negative. In the borderline tissue of the kidney cancer the expression of BMP-7 was still present. In the kidney cancer cells the expression of BMPs receptors was present especially BMPR-IA and BMPR-IB while BMPRII was less expressed.

Conflict of Interest: None declared

P090-T

BIPHASIC CHANGES IN SUBCHONDRAL BONE PLATE THICKNESS IN OSTEOARTHRITIS: AN IN VIVO MICRO-COMPUTED TOMOGRAPHY STUDY

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Background: During the osteoarthritis (OA) disease process the subchondral bone structure has been demonstrated to change. However, no information is available on the dynamics of these changes during the development of the disease process. The application of *in vivo* micro-computed tomography (micro-CT) enabled us to follow subchondral bone changes over time in a mouse model of osteoarthritis.

Methods: OA induction in male 16-week old C57Bl/6 mice (n = 8) was performed by intra-articular collagenase injection into the right knee joint, resulting in damage to the joint ligaments and creating instability. The left knee served as a saline-injected control. Mice were scanned in the 1076 *in vivo* micro-CT scanner (Skyscan, Belgium) at 0, 2, 4, 6, 10 and 14 weeks after OA induction. The thickness of the subchondral bone plate of the tibial epiphysis was measured. Parallel groups of mice were sacrificed at the in-between time points for histological analysis. Knee joints were embedded in methyl methacrylate and sectioned. TRAP staining was performed to visualize osteoclasts.

Results: Destabilization of the joint caused typical OA characteristics such as cartilage damage and osteophyte formation. Consecutive X-ray radiation at the indicated time points did not have any deleterious influence on body weight development or bone volume fraction in the proximal tibia of the saline-injected joints (9.0 ± 0.94% compared to 9.5 ± 0.81% in a group of mice which were scanned only once). The subchondral plate was thinner in the OA knee 4 weeks after OA induction: 144 ± 5.4 μm (control), 127 ± 4.6 μm (OA). However, at 14 weeks the subchondral bone plate in the OA knee had thickened again to control levels: 149 ± 7.7 μm (control), 141 ± 6.1 μm (OA). Histological examination at 4 weeks after OA induction demonstrated a marked invasion of osteoclasts directly underneath the subchondral bone plate in collagenase-injected joints, corroborating the observed decreased thickness.

Conclusion: *In vivo* micro CT analysis is a powerful tool to study subchondral bone changes over time in the same animal without deleterious effects. We demonstrated in a murine post-traumatic OA model a biphasic change in subchondral bone plate thickness, with early-phase thinning and late-phase thickening. This latter is in line with the clinical observations of subchondral sclerosis in the advanced stage of OA. Finally, the data demonstrate the interplay of cartilage and bone in the development of OA.

Conflict of Interest: None declared

P091-S

HEAVY METAL DEPOSITION IN PERIPROSTHETIC MINERALIZED BONE TISSUE: INVESTIGATION OF HIP ARTHROPLASTY WEAR DÉBRIS DEGRADATION UNDER DARKFIELD ILLUMINATION WITH PROTON INDUCED X-RAY EMISSION

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Background: Orthopaedic prostheses are subject to aging and resulting wear is dependent to application and endurance in patients. Abrasive joint replacement material that accumulates in the tissue induces reciprocal effects between prosthesis material and bone tissue.

Methods: A combination of microscopy and microanalysis was used to analyze wear particles in ground tissue specimens obtained from cadavers with firm hip replacements. The interface regions of five selected cases were light-microscopical investigated associated with image analysis. Accessory, conspicuous areas were ascertained by darkfield illumination and subsequent dissected with proton induced x-ray emission (PIXE).

Results: The wear particles were distributed unevenly in the tissue and the majority of particles in the samples from the cemented cases were degradation products of bone cement. There was hardly any evidence of metallic particles in the soft tissues. However, a considerable quantity of cobalt (Co) was found in mineralized bone tissue. The concentration of cobalt ranged from 20 ppm to almost 500 ppm. This finding indicates a correlation between cobalt concentration, time since implantation and distance from the implant. The volume of polyethylene wear debris generated by loose acetabular cups over a short period of time due to motion of the cup within its cement bearing is comparable to the volume of PE debris caused by motion of the metallic implant head within the cup over period of about ten years.

Conclusion: These findings suggest that in the effort to optimise implant design and implantation techniques, function unrelated mechanisms leading to contamination of bone tissue with degradation products from implant materials deserve more attention than the attempt to reduce wear primarily by improving bearing couples.

Conflict of Interest: None declared

P092-M

A GENE EXPRESSION SIGNATURE FROM BONE METASTASES AS PREDICTOR OF CLINICAL OUTCOME IN BREAST CANCER PATIENTS

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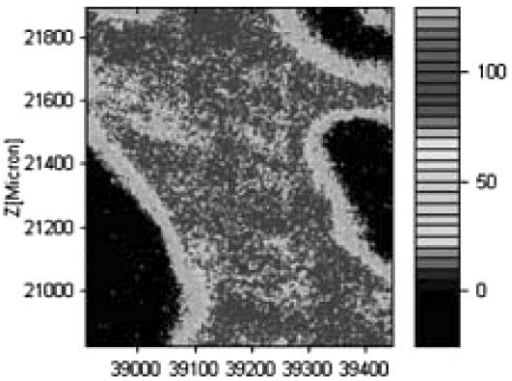
Bone is one of the preferential sites of distant metastasis in breast carcinoma (BrCa). While BrCa patients with bone-only metastases have frequently a good overall survival, the concomitant formation of metastases in other anatomical sites reduces drastically the clinical outcome. The identification of those genes selectively expressed in tumors able to generate metastases in multiple distant sites may help in establishing a more accurate prognosis prediction and in the development of effective therapies. In our study we compared the microarray profiles of bone metastasis specimens from patients with bone-only metastases with those of patients who developed secondary tumor growth in bone and other sites. Since sample contained also bone tissue, in order to exclude from subsequent analysis the genes in common with normal tissue, molecular profiles of metastases were matched with the microarray profile generated by a pool of normal bone. The resulting genes, about 10,000, were used to identify the 2-fold upregulated genes expressed in multiple metastatic samples relative to bone-only metastatic samples. This analysis evidenced a group of 98 genes. Classification according to GO ontology classes allows to divide the 50% of genes in 4 functional groups: response to stress (14 genes); nucleic acid binding (13 genes); signal transducer activity (12 genes); transporter activity (10 genes). In order to verify the expression of our metastatic signature in primary BrCas we analyzed public microarray datasets from comparable platform available in NCBI/ Genebank GEO database. We found that the 80% of genes found in our analysis had a high variation across primary tumor samples from metastatic patients ($SD > 100$) suggesting the possible involvement of these genes in the determination of primary tumor phenotypes. Moreover supervised hierarchical clustering using a 20 gene-restricted signature permitted to individuate with high sensitivity ($> 90\%$) those patients who developed distant metastases. Our gene expression analysis, if further validated, could result in an improvement in the choice for an effective mode of systemic therapy.



Conflict of Interest: None declared

P093-T

BONE QUALITY AND HISTOMORPHOMETRY IN 7 PATIENTS WITH FLUOROSIS

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Fluorosis is a disorder associated with excess fluoride (F)ingestion from drinking water and can be achieved from lifetime exposure at 2–4mg/L. Iatrogenic fluorosis has also been described when F was used in the treatment of osteoporosis. Fluorosis is characterized by new bone formation leading to osteosclerosis. Animal studies have provided supporting evidence that although F increases bone volume, bone strength declines at F concentrations of 6000 to 7000 mg/kg ash.

Seven patients with skeletal proven fluorosis were studied (due to NaF treatment or to drinking water). All had a bone biopsy studied by histomorphometry and microCT. Fourier transform infrared microscopic imaging (FTIRI) was used to investigate bone quality (mineral/matrix ratio, mineral crystallinity/maturity, and relative ratio of collagen cross-links) in 4 μm tissue sections. The mineral/matrix ratio based on the integrated area ratio of the PO4 (900–1200 cm^{-1}) and amide I (1585–1700cm^{-1}) peaks corresponds to ash-weight measurements. Mineral crystallinity (calculated based on the spectra absorbance ratio at 1030 and 1020 cm^{-1}.

Bone volume was increased in all patients. Osteoid volume and surfaces were significantly increased in all patients. Mineral apposition rate was decreased and a single band confirmed the marker intake in all patients. MicroCT measurement confirmed osteosclerosis with abnormal trabecular shapes. Linear mineralization defects and modifications of the perilacunar walls (mottled lacunae) were encountered in all patients. FTIRI illustrated mineralization heterogeneity within the trabeculae. Analysis of FTIR spectra confirmed the increase in crystallinity due to a better packing of the crystal lattice.

FTIRI appears a suitable technique to characterize bone quality in metabolic bone diseases due to altered mineralization.

Table: Normal Joint BMD values

Site of measurement	second finger (g/cm^2)	third finger (g/cm^2)	fourth finger (g/cm^2)	fifth finger (g/cm^2)
MCP-BMD Women	0.31 + 0.05	0.31 + 0.06	0.27 + 0.05	0.24 + 0.05
MCP-BMD Men	0.35 + 0.06	0.36 + 0.06	0.31 + 0.06	0.28 + 0.06
PIP-BMD Women	0.29 + 0.05	0.32 + 0.04	0.28 + 0.05	0.23 + 0.04
PIP-BMD Men	0.33 + 0.05	0.36 + 0.07	0.32 + 0.06	0.28 + 0.06

Conflict of Interest: None declared

P094-S

EVOLUTION OF PERI-ARTICULAR HAND BONE MINERAL DENSITY IN RHEUMATOID ARTHRITIS

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Between 2004 and 2005, 38 patients (30 women 8 men) mean aged 52,3 + 13,4 years, with RA (ACR 1987 modified criteria) with 67,1 + 46,2 months of evolution had prospectively a specific BMD measurement of metacarpo-pha-

langeal (MCP) and proximal inter-phalangeal joints (PIP) using a dedicated LUNAR prodigy software. Patients were reviewed after 13.2 + 3.1 months. The accuracy of the method assessed by paired repositioning and blind measurement at baseline was high with a coefficient of variation less than 1,3 % whatever the site studied , using the ROI copying file. Normal BMD values (g/cm^2) were measured on joints with normal erosion and normal joint space radiographic Sharp score. (Table 1). BMD joint values were significantly different according to sex ($p < 0.001$), and localisation ($p < 0.03$) The right and left side BMD values were similar . A significant decrease of BMD in percent of baseline value of either MCP joint ($r = -0.27$ $p < 0.01$) or PIP joint ($r = -0.22$ $p < 0.01$) with time was observed according to the activity of the disease assessed by the time average DAS28 score. The loss of bone was more pronounced in the dominant member ($p < 0.05$). No relationships were found neither with time average disability score HAQ, duration of the disease nor biological markers as urinary peptides of type 1 collagen for bone and type 2 collagen for cartilage degradations.

In conclusion , periarticular osteoporosis assessed by DEXA reflects local disease activity in RA and seems to be a useful tool for evaluation of disease modifying treatment.

Conflict of Interest: None declared

P095-M

IMMUNOHISTOCHEMISTRY OF BONE MORPHOGENETIC PROTEIN-9 IN HUMAN LIVER

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Background data. BMP-9, also referred to as growth/differentiation factor-2 (GDF-2) is a member of the transforming growth factor-β superfamily of cytokines that regulate cell growth and differentiation in both embryonic and adult tissues. It is demonstrated that rhBMP-9, like some other members of the TGF-β superfamily has the potential to initiate the osteoinductive cascade, but ability to induce osteogenesis is not developed like in some other heterodimers such are BMP-4/7 and BMP-2/7. BMP-9 is predominantly expressed in liver and in cholinergic neurons. RhBMP-9 binds to receptors expressed on tumor liver cell line and stimulates their proliferation. Liver non-parenchymal cells contain an abundance of immunoreactive BMP-9 and those cells change phenotype in liver cirrhosis. Even BMP-9 receptor has not been identified yet, BMP-9-pro-region complex binds to BMPRII in significant extent. BMP-9 is also involved in regulating glucose metabolism by lowering plasma glucose concentrations in normal and diabetic mice.

Aims. To explore BMP-9 and BMP's receptors expression in human liver tissue and to analyze the patterns of this morphogenetic protein activity in healthy and diseased organ.

Methods. Immunolocalisation was performed using BMP-9 goat polyclonal antibody (Santa Cruz, CA, USA).

Results. Positive activity of BMP-9 was found in: infant's liver, fatty liver and cirrhotic liver. Except for positive hepatocytes, infant's liver is mostly positive in bile duct epithelial tissue cells located in fibrous tissue between lobes. Infant's hepatocytes as well as bile duct epithelial cells are positive to both BMPRI and BMPRII. Fatty liver is characterized with multiplying fibrous tissue in spaces between lobules and initial destruction of the bile ducts epithelium. Intense positive intracellular cytoplasmic staining is observed only in hepatocytes. Hepatocytes are positive for BMPRII and BMPRI, while bile ducts epithelium express BMRI. Hepatocytes in cirrhotic liver are positive to BMP-9 antibody with less activity than in fatty liver, but with more activity than in infant's liver. In liver cirrhosis hepatocytes as well as bile duct epithelium express BMPRI.

Conclusions. BMP-9 and BMPs receptors are expressed in the human liver. Activity of the BMP-9 antibody is more intense in fatty liver and cirrhotic liver than in healthy infant's liver. Therefore we presume that BMP-9 activity in human liver could be protective.

Conflict of Interest: None declared

P096-T

ARE OSTEOBLASTS INVOLVED IN HUMAN AUTOSOMAL RECESSIVE OSTEOPETROSIS?

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Infantile Autosomal Recessive Osteopetrosis (ARO) is a genetic bone disease caused by a defect of osteoclast function and/or differentiation, which results in severe abnormalities, including brittle bones, blindness, deafness, haematological failure and hepatosplenomegaly, eventually leading to a fatal outcome within the first years of life. Osteopetrosis is referred to as an osteoclast disease, and bone fragility is believed to depend on persistence of non-remodelled primary bone. However, patients may present with abnormal osteoblast markers, therefore we asked whether the disease might be associated also with osteoblast anomalies. We analysed iliac crest biopsies obtained from twelve ARO patients and subjected to histomorphometry, identifying obviously elevated values of Oc.S/BS in six cases, high but still normal Oc.S/BS in one patient, and no osteoclasts in five patients. Interestingly, Ob.S/BS was increased in patients with clearly elevated Oc.S/BS. Consistently, patients not showing high osteoclasts in bone biopsies were also characterised by few osteoblasts and much lower serum bone alkaline phosphatase isoenzyme levels (~2.3-fold vs. normal values) compared to patients with high osteoclasts (~13.5-fold). A statistically significant correlation was found between osteoclasts and osteoblasts ($r2 = 0.63$). Circulating PTH, arising from deficient bone resorption, did not correlate with altered osteoblast formation in ARO, as the hormone was found to be always elevated, regardless of diverse osteoblast numbers in our biopsies. Thus, mature osteoclasts seem to be somehow favouring the formation of osteoblasts in their microenvironment by means of mechanisms largely independent of their resorption activity. It must be noted that malfunctioning osteoblasts are likely to form low-quality bone tissue, which might partly underlie the pathogenesis of fractures. Consistently, the only patient of our cohort with available serum osteocalcin, showed low concentration for age. These data promise interest for assessment of mechanisms of osteoclast–osteoblast cross-talk and identification of osteoblast anabolic factors whose regulation could be useful not only for the therapy of osteopetrosis but also for enhancing bone formation and prevent osteoporosis.

Conflict of Interest: None declared

P097-S

DOES HISTORICAL FORMS OF HYPERPARATHYROIDISM STILL EXIST?

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Background. There are great differences in incidence and clinical picture of primary hyperparathyroidism between developed countries and Romania where this condition is rarely described and operated on, sometimes for "historical" forms.

In the last three decades in our clinic among 40 cases of hyperparathyroidism, the largest series from our country we were confronted with two such "old" observations.

Case 1. A 43-year-old woman presented from four years successive fractures of the right humerus, both femurs and pelvis with invalidant consolidation. At admission, bad general condition with severe muscular atrophy and weight loss was noted. Calcemia was 3.3 mmol/L, iPTH 135 pg/ml and a 1.5 cm "cold" nodule was palpated in the right thyroid lobe. At operation a cystic intrathyroidal parathyroid adenoma was discovered and excised. Postoperative recovery was good.

Case 2. A 52-year-old man began his surgical "odyssey" thirty years ago, being operated on successively for perforated peptic ulcer, necrosing acute pancreatitis, developing afterwards a multirecidivated urolithiasis which imposed 6 right and 4 pyelo- or ureterolithotomies and finally a right nephrectomy. When we took over this patient he presented with a big stone on the remaining kidney and associated chronic renal failure Calcemia was 3 mmol/L, iPTH 110 pg/ml and a left parathyroid adenoma was identified at US and excised. Postoperative course was favourable, with good renal function.

Conclusion. Behind the mask of long-standing history of repeated fractures provoked by trivial trauma or multirecidivated urinary stones, the diagnosis of primary hyperparathyroidism must be evoked even in the 21st century.

Conflict of Interest: None declared

P098-M

SYNDECAN-2 IS INVOLVED IN THE APOPTOTIC RESPONSE TO CHEMOTHERAPEUTIC DRUGS IN OSTEOSARCOMA IN VITRO AND IN VIVO

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Syndecans are transmembrane heparan sulfate proteoglycans controlling cell adhesion, migration and proliferation. We previously showed that syndecan-2 is involved in the control of apoptosis in cultured osteosarcoma cells. These data led us to hypothesis that syndecan-2 may play a role in the apoptotic signaling in bone tumors. We immunohistochemically analyzed tissue sections from biopsies from 21 patients with well characterized osteosarcoma. These tissues expressed low levels of syndecan-2 as compared to osteoblasts and osteocytes in normal bone. Cultured human osteosarcoma cells also produced lower mRNA levels of syndecan-2 than normal osteoblastic cells. Analysis of syndecan-2 expression both in biopsies and in corresponding post-chemotherapy resected tumors and in cells treated with methotrexate or doxorubicin, showed that the cytotoxic action of chemotherapy was associated with an increase in syndecan-2 level. Moreover,

overexpression of syndecan-2 sensitized human osteosarcoma cells to chemotherapy-induced apoptosis, increasing the response to methotrexate, doxorubicin and cisplatin. Consistently, knockdown of the proteoglycan using stable transfection with a plasmid coding siRNA resulted in the inhibition of chemotherapy-induced apoptosis. These results provide support for a tumor suppressor function for syndecan-2. This identifies syndecan-2 as a new factor mediating the anti-oncogenic effect of chemotherapeutic drugs. Moreover, our data suggest that syndecan-2 may be a marker of the chemotherapeutic treatment efficacy in patients with osteosarcoma.
Conflict of Interest: None declared

P099-T

TUMOUR-INDUCED HYPOPHOSPHATAEMIC OSTEOMALACIA

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Background: Tumour-induced osteomalacia is a rare syndrome characterized by urinary phosphate loss with consecutive hypophosphataemic osteomalacia. The proposed pathogenic mechanism is paraneoplastic secretion of phosphaturic factors (so-called phosphatonins).

Case report: We describe a 34-year-old male patient who presented with severe pain of the spine and ribs for at least two years. Bone scan using technetium-99m-hydroxymethylene diphosphonate (Tc-99m HDP) scintigraphy showed multiple lesions suggesting metastatic disease. Bone biopsy revealed osteomalacia. The patient had subnormal plasma phosphate levels (0.42 mmol/l; normal range, 0.87–1.45) and markedly increased phosphate clearance (82.8 ml/min; normal range, 5.4–16.2). Fractional phosphate reabsorption was extremely low (57%; normal range, 82–90). Serum value for 1,25-dihydroxyvitamin D3 was also remarkably low (12.2 ng/l; normal range, 20–67). The patient was treated with phosphate supplementation (up to 5 g daily) along with calcium (1000 mg daily) and calcitriol (1.5 mcg daily). While this therapy did not correct hypophosphataemia it resulted in complete relief of pain within several months. As hyperphosphaturic osteomalacia of adult onset is most often a paraneoplastic syndrome, the patient underwent extensive work-up for suspected malignant disease, which could not be identified. However, In-111-pentetreotide scintigraphy showed a tiny lesion of 1 cm diameter projecting to the left hip region which could be localized to the left femoral neck in close vicinity to the greater trochanter by MRI and image fusion analysis. This lesion had not been visualized by Tc-99m HDP bone scintigraphy. Intraoperatively, use of a hand-held gamma probe after administration of radiolabelled In-111-pentetreotide clearly identified the tumour which was completely removed and was shown to be a hemangiopericytoma. Tumour cells stained positive for vimentin but were negative for c-kit and MIB-1. After removal of the tumour, phosphate metabolism normalized within one week without phosphate supplementation.

Conclusion: We conclude that hypophosphataemic osteomalacia, although rare, is an important differential diagnosis in patients with multiple lesions on bone scintigraphy. The underlying tumour may be detected only by In-111-pentetreotide scintigraphy. Preoperative labelling with In-111-pentetreotide is a useful tool in detecting these tumours during surgery.
Conflict of Interest: None declared

P100-S

OPG, RANK AND RANK LIGAND EXPRESSION IN THYROID LESIONS

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Receptor activator of NFKB (RANK), RANK ligand (RANKL) and osteoprotegerin (OPG) play essential roles in bone metabolism. RANKL binds to RANK, which is expressed by osteoclasts whereas OPG acts as its decoy receptor and blocks the RANK–RANKL interaction. OPG/RANK/RANKL are produced by numerous cell types and variety of tissues including epithelial and mesenchymal cells. Recent data suggest that RANKL and OPG mRNA are produced in the thyroid gland by follicular cells and are regulated by cytokines and hormone such as TSH, but their involvement in thyroid pathology is unclear. To study the involvement of this molecular triad, most of the data are based on RANKL and OPG serum levels. Any study analyses the tissue expression of these molecules in pathological thyroid glands. The aim of this study was to analyse the RANK, RANKL and OPG expression in thyroid resection specimens of patients suffering from benign or malignant pathologies. Twenty-seven specimens from total thyroidectomy were selected and classified according the WHO classification: 9 papillary carcinomas (PC), 9 medullary carcinomas (MC), 9 benign macrovesicular adenomas (MA). RANK, RANKL and OPG expression (localization and quantification) were studied by immunohistochemistry. RANKL was expressed in 30% of MC both in the cytoplasm and in nucleus of epithelial cells (moderate to strong intensity), in 22% of MA only in the cytoplasm (moderate intensity), and never detected in PC. Similar RANK possess similar expression pattern. Thus, RANK positive staining was observed in 30% of MC (both in nucleus and cytoplasm, weak signal), in 22% of MA (nucleus and/or cytoplasm staining, weak signal). Furthermore, 30% of PC expressed RANK (cytoplasm expression of epithelial cells and macrophages). OPG expression was restricted to the cytoplasm of epithelial in 1 MA and 1 MC (weak and moderate staining). No correlation was noted with tumor size or node involvement. In contrast to pathological tissues, any expression of OPG/RANK/RANKL was detected in healthy thyroid tissue. This work reveals for the first time that RANK, RANKL and OPG are expressed in the pathological thyroid gland by thyroid follicular cells and also by malignant parafollicular cells. OPG/RANK/RANKL molecular triad thus might play a role during pathogenesis of follicular and parafollicular tumors.
Conflict of Interest: None declared

P101-M

PARATHYROID HORMONE LEVELS, RENAL OSTEODYSTROPHY AND ECTOPIC CALCIFICATION IN HEMODIALYSIS PATIENTS

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BACKGROUND: Renal Osteodystrophy (ROD) is a common complication in hemodialysis patients. Bone histomorphometry, which is the diagnostic method of choice, has a low acceptance rate and biochemical markers are used for the 'atraumatic' diagnosis of the disease. Serum intact PTH (i-PTH) levels are thought to represent a good predictor of ROD. I-PTH levels greater than 500 pg/dl suggest high bone turnover ('hyperparathyroidism') and levels lower than 100 pg/dl predict low bone turnover disease (aplastic bone).

AIM: In the present study we investigate bone metabolism in hemodialysis patients using i-PTH levels and correlate these findings with radiovisible ectopic calcifications.

MATERIALS/METHODS: We studied 125 patients (66 males and 59 females) with a mean age 62,8 years (37–83) receiving hemodialysis with a duration ranging from 8 to 73 weeks. In all patients i-PTH levels were measured and correlated to radiovisible ectopic calcifications.

RESULTS: Twenty-seven patients (21.6%) had an i-PTH level > 500 pg/dl (562–4618) (Group A) suggesting high bone turnover.

Forty-two patients (33.6%) had an i-PTH level < 100 pg/dl (Group B) suggesting low bone turnover whereof 14 patients had levels < 45 pg/dl (aplastic bone disease).

Radiovisible ectopic calcifications were detected in 29 patients (23.2%) and most of them (20 patients/69%) where from Group A (high bone turnover). This finding is different from findings in other previous studies where ectopic calcification is mostly noted in hemodialysis patients with adynamic bone disease.

CONCLUSION: In this study we noticed a decrease of the number of hemodialysis patients with high bone turnover , and a sever increase of the group with low bone turnover (adynamic bone disease).

Ectopic calcification was in this study a common complication of hemodialysis and was mainly observed in patients with high bone turnover.
Conflict of Interest: None declared

P102-T

EXTRACELLULAR CALCIUM MODULATES IBANDRONATE-INDUCED GROWTH INHIBITION OF BREAST CANCER CELLS

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Bisphosphonates (BPs) are standard therapy to reduce breast cancer-induced skeletal complications. In vitro data indicate that they may directly inhibit proliferation and induce apoptosis of breast cancer cells. We report here the effects of an increase in extracellular Ca++ levels on the cytotoxic activity of ibandronate (Iban) in MDA-MB-231 and MCF-7 breast cancer cell lines.

Cancer cells were cultured in RPMI 1640 containing 5% FCS and supplemented with CaCl2 to achieve Ca++ concentrations from 0.6 to 2.0mM. In presence of 0.6mM Ca++, 30 μM Iban had no effect on MDA-MB-231 cell growth, while it slightly inhibited MCF-7 cell growth by $13.6 \pm 6.6\%$. By contrast, in presence of 2.0mM Ca++, 30 μM Iban dramatically inhibited cancer cell survival by $55.5 \pm 7.8\%$ and $76.1 \pm 4.6\%$ ($p < 0.05$) in MDA-MB-231 and MCF-7 cells, respectively. An increase in Ca++ concentrations from 0.6 to 1.6 mM decreased the IC50 values of Iban from 100 to 30 μM in MDA-MB-231 cells and from 60 to 10 μM in MCF-7 cells. The cytotoxicity of zoledronic acid was similarly affected by Ca++ concentrations. Ca++ chelation by 0.5mM EGTA, a concentration which did not affect cell growth, significantly reduced the growth inhibitory effects induced by 30 μM Iban in culture medium con-

taining 1.6mM Ca++. Similarly, competition for Ca++ chelation of 30 μM Iban and 100 μM clodronate, a concentration which had no detectable effect on cell survival, significantly diminished the growth inhibition induced by the former BP. In addition, cells exposed to 1.6mM Ca++ bound and/or incorporated more [^{14}C]-Iban (about 1.5-fold after 2 hours) than cells incubated with 0.6mM Ca++. The modulation of Iban cell uptake by Ca++ was further demonstrated by showing that increasing Ca++ concentration enhanced Iban-induced inhibition of protein prenylation. Indeed, 10 μM Iban was sufficient to produce a detectable inhibition of Rap1A prenylation in presence of 2.0mM Ca++, while 100 μM Iban was required to achieve a similar effect in presence of 0.6mM Ca++.

Altogether, our data suggest that extracellular Ca++, at physiologically relevant concentrations, increases the intracellular inhibitory activities of BPs. Thus, Ca++ released during the process of bone destruction could enhance the antitumor effects of BPs and contribute to their therapeutic activity. Whether this enhancing effect of Ca++ on BP uptake and activity is also true for osteoclasts needs to be investigated.

Conflict of Interest: None declared

P103-S

THE PREVALENCE OF THE OSSIFICATION OF POSTERIOR LONGITUDINAL LIGAMENT IN KOREAN

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Background: The ossification of posterior longitudinal ligament (OPLL) is known as a common hyperostotic disorder causing myelopathy among Japanese and other Asian population. However, the prevalence of OPLL has not been systematically investigated in Korean.

Objectives: The objective of this study was to determine the prevalence of OPLL in cervical spines in Korean.

Methods: We reviewed the reports of the radiologic examination of cervical spines from Jan 2002 to Sep 2005 in Hanyang University Hospital, Seoul, Korea. They have been reported by experienced spine radiologists. A few radiographs without reporting were screened by a rheumatologist and confirmed, and classified by an experienced bone and joint radiologist. OPLL was indicated by the presence of heterotopic ossification in the posterior longitudinal ligament on lateral cervical radiograph.

Results: Among 12,130 adults aged 16 years or more, 72 cases of OPLL were found (0.59%). The male to female ratio was 43: 29 (1.48: 1). The highest incidence was in patients aged 50–59 years (47.2%). In terms of the types of OPLL, the continuous type was noted in 32.0% of the patients, the segmental type in 32.0%, the mixed type in 30.5%, and the localized type in 5.5%. C4, C5, and C3 were most commonly involved in the order of frequency. Diffuse idiopathic skeletal hyperostosis, which is another common hyperostotic disorder, was also found in 8 male patients.

Conclusion: This study showed that the prevalence of OPLL in Korean was 0.59%, which was slightly lower than those of Chinese (0.83%) and Japanese (~2.0%), but higher than that of the Whites.

Conflict of Interest: Jae-Bum Jun, Handok Pharmaceuticals Co., Ltd., consultant

P104-M

OSTEONECROSIS OF THE JAW UNDER BISPHOSPHONATE THERAPY – A GERMAN REGISTER FOR PATIENTS WITH OSTEONECROSIS OF THE JAW

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The objective of the German Register for Osteonecrosis of the Jaw (ONJ) was to explore the development of ONJ as a serious adverse event of bisphosphonate (BP) treatment. Focus was placed on the patient's primary disease, dental status, co-medication, BP single and cumulative dosage, and the diagnosis & treatment of ONJ.

Nearly 77% of the cases were reported anonymously to the BfArM (German FDA) and 23% to the register directly using a standardised questionnaire. Data were analyzed according to five categories: 1) demographics, 2) primary disease incl. therapy 3) BP intake 4) dental status 5) ONJ location, diagnosis and therapy. We Included cases with either osteomyelitis or ONJ and excluded those with ONJ due to radiotherapy.

So far, 352 cases were registered (mean age 66.5 + 9.8). Women were affected twice as often. Primary diseases were malignant disease (85.1%), a combination of malignant disease & osteoporosis (12.2%) and osteoporosis alone (2.7%). Common malignant diseases were breast cancer (45.3%), multiple myeloma (31.5%) and prostate cancer (13.5%). Primary disease was treated with chemotherapy (80.0%), radiotherapy (72.7%) and Glucocorticosteroids (50.4%). A single BP was given to 67.2% of patients: Zoledronate (79.7%) or Pamidronate (12.4%). Two BPs were administered consecutively to 30.7% of the patients: Pamidronate/Zoledronate (53.5%). A previous tooth extraction (67.2%) was the most common pathological dental status. ONJ was found twice as often in the mandible as in the maxilla. ONJ extended over > 4 dental areas in 26.7 % of the patients. Commonly reported clinical signs were infection (33.3%), mucosal changes (21.4%), wound healing disturbance (21.4%) and exposed bone (11.9%). Frequent histological signs were infection (51.9%) and osteonecrosis (34.2%). Radiologists often diagnosed osteolysis (19.5%) and osteomyelitis (14.3%). ONJ was treated by surgical (45.3%), conventional (13.2%), or a combination of surgical & conventional interventions (41.6%). Success rate was reported highest (17.0%) for the combination therapy. Data could not be collected for every patient in each of the five categories.

ONJ under bisphosphonate therapy is an unexpected serious adverse event that almost exclusively affects patients with malignant disease and pathological dental status. The etiopathogenesis remains unclear but is likely to be complex. ONJ presents a variety of clinical, histological & radiological findings.

Conflict of Interest: None declared

P105-T

RESVERATROL ANALOGS AS POTENTIAL TREATMENT OF MULTIPLE MYELOMA AND BONE RELATED DISEASE. EFFECT ON MYELOMA CELLS, OSTEOCLASTS AND OSTEOBLASTS IN VITRO

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Background: Multiple myeloma (MM) is a fatal B-cell neoplasia nearly always associated with a destruction of surrounding bone. The bone lesions result from increased osteoclastic bone resorption and impaired osteoblastic bone formation in the vicinity of myeloma cells. Currently available treatments allow a median survival of about 5 to 7 years, thus there is an urgent need for more efficient treatment. Resveratrol (RSV, trans-3,4,5-trihydroxystilbene) is a polyphenolic, antifungal natural phytoalexin found in various food products. A recent in vitro study showed that RSV may be a potential drug candidate of MM, however the RSV effect is mediated at rather high concentrations. Therefore it is of great interest to investigate its structurally modified analogues in respect to MM.

Methods and results: RSV analogues displaying selective affinity for different molecular targets were screened for their potency towards myeloma cells, osteoclasts and osteoblasts in vitro. Two of five tested analogues inhibited osteoclast differentiation at doses around 5,000 times lower than the natural compound. However, there was no direct effect on bone resorptive activity of mature osteoclasts indicating that analogues interfere with early events of osteoclast differentiation.

As impaired bone formation in MM patients is highly correlated with diminished osteoblastic activity, RSV analogues were screened for induction of the expression of osteoblastic markers in bone marrow mesenchymal stem cells differentiating into mature osteoblasts. In the preliminary study three screened compounds induced alkaline phosphatase (ALP) and osteocalcin expression at nanomolar doses synergistically with vitamin D3 treatment. Malignant B cells are the driving force of MM, not only stimulating their own proliferation and survival, but also factors promoting recruitment of osteoclasts and impairing osteoblasts function. Therefore, RSV analogues were tested for their ability to suppress myeloma cell proliferation and survival. In contrast to RSV, none of the RSV analogues affected cell proliferation or could induce apoptosis in tested myeloma cell lines, U266 and OPM-2.

Conclusions: In summary, our preliminary data suggest that RSV analogues may be of therapeutic use in respect to inhibiting osteoclast differentiation and promoting osteoblastic activity. Future studies may be needed to define the molecular target of the analogues.

Conflict of Interest: None declared

P106-S

CALCIUM PYROPHOSPHATE DIHYDRATE (CPPD) CRYSTALLINE DEPOSITS IN THE KNEE: ARTHROSCOPIC, RADIOGRAPHIC AND THE SYNOVIAL FLUID STUDY

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Background/aims: CPPD crystal deposition disease is the most common crystalline arthropathy. Intraarticular crystals were first described as weakly positive birefringent nonurate crystals at polarized light microscopy in joint synovial fluid (SF) in patients with gout-like arthritis. Radiographic manifesta-

tion of chondrocalcinosis is seen more frequent like meniscal calcifications than hyaline cartilage calcifications. The arthroscopy (AS) demonstrates and determines the frequency and localization of calcifications within the knee joint without discriminates type of crystals. The purpose of our study was to determine whether the presence of crystals of CPPD in synovial fluid identified arthroscopically have an effect on standard knee radiographs.

Methods: Between 1995 and 2005 were identified 47 patients with SF knee joints specimens positive for CPPD crystals and age range 55–76 years. Knee pain and effusion were the most common indication for clinical examination. SF was aspirated immediately prior AS. SF was analyzed for CPPD crystals on the day of aspiration using Leitz compensated polarized light microscope. Visualization of at least 5 such crystals was necessary to confirm the presence of CPPD crystals. Knee preoperative radiographs were obtained by methods standard for the practice and read by a skeletal radiographer. All the patients undergone low pressure knee arthroscopy using 4.5 mm arthroscope. AS were performed with local anesthesia. Methodological visual evaluation of the cartilage and synovium was made.

Results: At arthroscopy evident chondrocalcinosis was seen only in twelve patients (42.5%) with CPPD crystals in SF and active knee synovitis clinically. In 9 joints with severe, grade 4 radiographic degenerations, crystal deposits were arthroscopically established in 8 (88.9%). In the patients with arthroscopic evidence of chondrocalcinosis our results show decreased sensitivity, specificity, and accuracy of knee standard radiographs to find out of crystal induced arthropathy (35%, 44% and 37% respectively).

Conclusion: Our results show the unsatisfied effect of the standard radiographs on the knee imaging diagnosis of chondrocalcinosis. Diagnostic sensitivity and specificity of the standard X-ray examination is very low. Only joints with CPPD crystals in SF and with severe, grade 4 radiographic degeneration were X-ray positive for chondrocalcinosis.

Conflict of Interest: None declared

P107-M

THERAPEUTIC RELEVANCE OF ZOLEDRONIC ACID IN PROSTATE CARCINOMA USING A NEW RAT SYNGENIC MODEL OF OSTEOBLASTIC METASTASES

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Prostate adenocarcinoma is associated with the formation of osteoblastic metastases in bone. Animal models that mimic human prostate carcinoma skeletal metastasis are therefore required to improve the therapeutic options. As there is a paucity of good preclinical models to study osteoblastic lesions, we developed a new model in Sprague–Dawley rats by the direct injection of rat AT6.1 prostate cancer cells into the bone marrow of the femur. In vitro, AT6.1 cells were characterized at the phenotypic level by the expression of both osteoblastic (alkaline phosphatase, Cbfa1, type I collagen, osteocalcin and bone sialoprotein) and osteoclastic (TRAP, cathepsin K, calcitonin receptor) markers. However, the induction of nodule mineralization and phosphatase alkaline activity could not be demonstrated in vitro. When AT6.1 cells are injected in the medullar cavity of the distal right femora in rats, disorganization of the trabecula at the level of the growth zone is observed as well as cortical erosion, whose intensity is correlated with the number of cells injected. Moreover, the tumor itself is associated with bone formation as revealed by SEM analysis. Altogether, these bone modifications revealed by radiography and micro-scanner analysis are comparable to the bone lesions observed in prostate patients.

As it has been hypothesized that osteoclastic bone resorption is a critical component before the development of these osteoblastic lesions in bone, the AT6.1 model was used to evaluate the efficacy of zoledronate (ZOL), a N-bisphosphonate inhibiting osteoclast activity that may prevent or halt the formation of metastatic prostate cancer lesions in bone. The rats were treated with 100 microg/kg doses twice a week for 6 weeks, beginning three days post-tumor cell inoculation. Treated rats showed a high bone protection that prevented bone lesions induced by prostate carcinoma cells associated with inhibition of tumor growth. In vitro, ZOL directly inhibits AT6.1 cell proliferation by a mechanism independent of caspase 3 activation, inducing cell cycle arrest. These results demonstrate the relevance of using ZOL in bone lesions associated with prostate carcinoma, as this compound shows bi-functional effects both on bone remodelling and tumor cell proliferation.

Conflict of Interest: None declared

P108-T

EXPRESSION OF BONE MORPHOGENETIC PROTEIN-2 AND -7 DURING EXPERIMENTAL INFLAMMATORY BOWEL DISEASE

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Osteoinductive bone morphogenetic proteins such as BMP-2 and BMP-7, along with their osteogenic function, play important role in development and healing of different organs. The recent findings of BMP expression in developing and adult intestine as well as BMP receptor IA and Smad4 mutations in colonic diseases suggest on importance of BMPs in the proliferation and differentiation of colonic cells.

The aim of our study is to reveal expression of BMP-2 and BMP-7 during experimental inflammatory bowel disease (IBD) in rats. Although BMP-2 and BMP-7 are different molecules, as the member of BMP family, they showed homology in structure and similarity in activity after clinical applications.

We investigated the expression of BMP-2 and BMP-7 in colon tissue during acute and chronic phases of experimental inflammatory disease as well as after 14th day of BMP-7 treatment. Colonic damage was induced by the intracolonic administration of the hapten 2,4,6-trinitrobenzensulfonic acid (TNBS) in 30% ethanol. Rats were treated systemically by recombinant human BMP-7 for 2 weeks following injury. Treatment was initiated 24 hours after inducing colitis and then on day 2, 3, 5, 7, and 10 thereafter. Immunolocalisation of BMP-2 and -7 was performed using BMP-2 and -7 goat polyclonal antibody (Santa Cruz, CA, USA).

Our immunohistochemical studies in rat colon showed strong staining with BMP-2 and -7 antibodies in rat colon samples. In acute phase of colitis (2nd and 5th day after colitis induction) the pattern of BMP-2 and -7 expressions was similar. The positive BMP staining was predominately located in damaged mucosa of ulcer region. Conversely, during chronic phase (14th and 30th day after colitis induction) BMP-2 and BMP-7 showed different pattern of expression. The BMP-7 expression was mostly placed in the colonocytes along the crypt while BMP-2 is expressed in the colonocytes at the intercrypt table. BMP-7 treatment significantly reduced the expression of both BMP-2 and -7.

Different pattern of BMP-2 and -7 expressions during experimental IBD suggest on the important role of BMPs in control the damage progression of colon cells during disease.

Conflict of Interest: None declared

P109-S

EFFECTIVE USE OF PAMIDRONATE IN PEDIATRIC PATIENTS WITH AVASCULAR NECROSIS FOLLOWING GLUCOCORTICOID THERAPY: 3 CASE REPORTS

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Background: Osteonecrosis (ON) of bone is a complication of glucocorticoid therapy for malignant and non-malignant disease. Two previous studies have shown that bisphosphonates can delay collapse of the femoral head. We now report beneficial effects of pamidronate in 3 pediatric patients with multi-site ON.

Case 1. Stage IVc ON of right shoulder and stage II ON of left shoulder were diagnosed in a 16-year-old girl with acute lymphocytic leukemia (ALL).

Case 2. Stage II ON of multiple sites (shoulders, hips, knees) was diagnosed in a 15-year old girl following dexamethasone therapy for meningitis.

Case 3. Stage II ON of multiple sites (elbows, shoulders, knees, hips) was diagnosed in a 14-year old boy with ALL.

X-rays, bone-scan and MRI were compatible with ON in all cases. Bone pain was unresponsive to anti-inflammatory agents, codeine or gabapentin.

Aims: To assess outcome of young patients with ON treated with pamidronate for pain and prevention of bony collapse.

Methods: IV Pamidronate was administered as 1–3 day cycles: 1 mg/kg/day. Frequency of dosing was once every 1–3 months. The total yearly dose was ≤ 12mg/kg. Visual analog scale for pain (VAS), serum calcium and alkaline phosphatase were measured at base line and at monthly intervals during pamidronate treatment. Plain x-rays and MRI of the affected site(s) were obtained at base line and every 6 months.

Results: Case 1 received 4 3-day cycles of pamidronate. VAS for pain changed from 10/10 to 4/10 at12 months. MRI was unchanged with no new bony collapse.

Case 2 received 4 3-day cycles of pamidronate. VAS for pain changed from 10/10 to 1/10 at 4 months. MRI normalized by 12 months, with no bony collapse.

Case 3 has received 4 1-day cycles of pamidronate. VAS for pain changed from 8/10 to 3/10 by 4th treatment. X-rays show no bony collapse, but because of persistently abnormal MRI, treatment will be continued.

All laboratory tests were normal. No osteonecrosis of the jaw was observed.

Conclusions: 1. IV pamidronate was safe and effective in decreasing pain, improving function, and preventing bony collapse in 3 pediatric patients with ON of multiple sites.

2. These limited but encouraging results warrant further study of pamidronate in steroid induced ON.

Conflict of Interest: None declared

P110-M

RANK LIGAND INHIBITION WITH OPG-FC ENHANCES THE INHIBITORY EFFECT OF DOCETAXEL ON THE GROWTH OF PROSTATE CANCER PC-3 TUMORS IN THE MOUSE SKELETON

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Bone metastases are a frequent complication of breast and prostate cancer. Tumor cells interact with the bone microenvironment to induce osteoclastogenesis, leading to bone destruction. RANKL is essential for osteoclast formation, function, and survival. RANKL inhibition in metastatic models of breast and prostate cancer inhibits tumor-induced osteolysis and reduces the progression of skeletal tumor burden. The chemotherapeutic agent docetaxel has been shown to increase survival, reduce pain, and improve quality of life for patients with advanced prostate cancer. Therapy that combines chemotherapies to control tumor growth and RANKL inhibition to suppress tumor-induced osteolysis may provide greater benefit than chemotherapy alone.

We examined the in vivo efficacy of RANKL inhibition by OPG-Fc in combination with docetaxel on prevention of tumor-induced osteolysis and reduction in skeletal tumor burden in a mouse model of prostate carcinoma metastasis. Human prostate carcinoma (PC-3) cells were engineered to express firefly luciferase to allow a non-invasive means of assessing tumor burden by bioluminescence. PC-3 cells were injected into the left cardiac ventricle. Beginning 10 days after tumor challenge, mice were treated therapeutically with OPG-Fc (SC, 3mg/kg, 3x/week) or docetaxel (SC, 5 and 10 mg/kg 1x/week) alone, in combination, or with PBS. Tumor progression was monitored twice weekly by bioluminescence, and tumor volume and area were assessed by histology.

OPG-Fc treatment had little effect on the growth rate of PC-3 cells in hind limbs, but it prevented the development of osteolytic bone lesions and reduced tumor volume in bone. Docetaxel significantly reduced the growth rate of PC-3 cells in vivo resulting in a significant decrease in tumor burden by end of study (day 27, $P < 0.0001$). This reduction in tumor burden delayed the development of osteolytic bone lesions. The combination of OPG-Fc and docetaxel resulted in a significantly greater suppression of skeletal tumor growth than docetaxel alone (day 27, $P = 0.0005$). Treatment with either OPG-Fc or docetaxel alone resulted in ~80% reduction in the tumor volume in hind limb bones, whereas combination treatment resulted in tumor reduction of 97.5%.

In summary RANKL inhibition effectively inhibited pathologic osteolysis induced by human prostate carcinoma PC-3 cells in animals with established tumors, and enhanced the effectiveness of docetaxel to reduce skeletal tumor burden in vivo.

Conflict of Interest: R Miller, Amgen Inc., Employee M Roudier, Amgen Inc., Employee J Jones, Amgen Inc., Employee M Tometsko, Amgen Inc., Employee A Armstrong, Amgen Inc., Employee J Canon, Amgen Inc., Employee W Dougall, Amgen Inc., Employee

P111-T

MOUSE OSTEOSARCOMA CELLS PRODUCE INSULIN-LIKE GROWTH FACTORS THAT INDUCE PRE-OSTEOBLAST CELL PROLIFERATION; INTERACTION BETWEEN BONE CELLS AND OSTEOSARCOMA CELLS

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Background: Osteosarcoma, one of the most frequent primary malignant bone tumors, typically affects children and young adults. Important pathogenetic roles of *p53*, *RB* and *mdm2* were reported; however the pathogenesis of osteosarcoma, especially the biology of cancer cell in bone microenvironment remains obscure. Thus, to elucidate the interactions between bone cells and osteosarcoma cells might lead to a more rational strategy for devising novel therapies. The present study was therefore designed to better characterize the interactions between bone cells (MC3T3-E1, mouse calvaria-derived pre-osteoblast cells) and mouse osteosarcoma cells (MOS-J and POS-1). Methods: cell proliferation and signal transduction analyses were performed. Results: When MOS-J/POS-1 conditioned medium (CM) were added to MC3T3-E1 cells, they induced a significant up-regulation of MC3T3-E1 cell proliferation in a dose-dependent manner ($p < 0.001$). In addition, MOS-J/POS-1 CMs clearly induced phosphorylation of ERK 1/2, STAT3 and Akt in a time- and dose-dependent manner in MC3T3-E1 cells. LY294002, a PI3K inhibitor or rapamycin, an mTOR/p70S6K inhibitor abolished the effects of osteosarcoma CMs on osteoblast proliferation. Among several specific neutralizing inhibitors used, anti-mouse insulin-like growth factor (IGF)-I and IGF-II antibodies significantly abrogated MOS-J cell-induced MC3T3-E1 cells proliferation ($p < 0.05$), whereas only anti-mouse IGF-I antibody significantly abrogated POS-1 cell-induced MC3T3-E1 cells proliferation ($p < 0.05$). Taken together, IGF-I, II and IGF-I produced by MOS-J or POS-1 play a pivotal role in respective cells-induced markedly up-regulated MC3T3-E1 cells proliferation *via* PI3K/mTOR/p70S6K pathway. IGFs have been reported as an osteoclast activator as well as a key osteoblast growth factor. Osteoclasts release tumor-supportive growth factors stocked in bone matrix that stimulate the vicious cycle reside between pathologic bone remodelling and osteosarcoma development by bone degradation. Furthermore, IGFs directly induce osteosarcoma development. The local production of IGF-I and IGF-II from osteosarcoma cells may modulate both osteoblast–osteoclast and bone cell–osteosarcoma cell interaction and play an important role in osteosarcoma development. Moreover, mTOR pathway has been recently involved in osteosarcoma metastasis. Conclusion: Thus, targeting IGFs and mTOR pathway may provide novel appropriate therapeutic approaches for osteosarcoma treatment.
Conflict of Interest: None declared

P112-S

THE ASSOCIATION OF RADIOGRAPHIC OSTEOARTHRITIS OF KNEE AND LUMBAR SPINE WITH PAIN: THE RESEARCH ON OSTEOARTHRITIS AGAINST DISABILITY (ROAD) STUDY

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Backgrounds/aims: Although osteoarthritis (OA) of 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 clinical study called ROAD (research on osteoarthritis against disability) in 2005 to clarify the environmental and genetic backgrounds. We began the study by creating a comprehensive and systemic database including clinical and genomic information in two cohorts of urban and rural areas. From their baseline data, we investigated the association of radiographic OA of knee and lumbar spine with the respective local pain.

Methods: We recruited 1,885 inhabitants older than 50 years (mean age = 73.5 yrs.): 1,030 (355 men & 675 women; mean age = 76.9 yrs.) from the urban and 855 (317 men & 538 women; 69.3 yrs.) from the rural areas. The radiographic severity of OA was determined according to the 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 by a blinded orthopaedic surgeon. Logistic regression analysis was performed after adjustment for age and BMI to determine the association.

Results: Prevalence of radiographic knee OA was significantly higher in female and rural residence, whereas that of lumbar OA was significantly higher in male sex and urban residence. The radiographic severity of knee OA was positively associated with pain in the urban (OR = 10.1, 95% CI = 2.2–46.0 in men; OR = 3.5, 95% CI = 1.67–7.18 in women; for KL3/4 compared to KL0/1), and more strongly in the rural (OR = 2.9 and 11.6, 95% CI = 1.44–5.75 and 4.55–29.4 in men; OR = 2.9 and 12.3, 95% CI = 1.71–5.06 and 6.47–23.4 in women; for KL2 and KL3/4, respectively, compared to KL0/1). For the lumbar spine, the radiographic OA at all intervertebral spaces except for L1/2 (OR = 1.8–2.8 for KL3/4 compared to KL0/1) in women and at only L3/4 (OR: 2.1) in men was significantly associated with the low back pain in the urban area. Similarly in the rural area, the radiographic OA at all interspaces except for L3/4 and L4/5 (OR: 2.0–4.3) in women and at no interspace in men was associated with this pain.

Conclusion: We found that gender and community differences were distinctly associated with the knee and lumbar OA. The radiographic knee OA showed a strong association with the knee pain in all genders and communities. Contrarily, the radiographic lumbar OA had a moderate association with low back pain in women, but not in men.
Conflict of Interest: None declared

P113-M

BREAST CANCER CELLS INDUCE SPONTANEOUS OSTEOCLASTOGENESIS IN HUMAN CO-CULTURE SYSTEM

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Aims: We want to demonstrate that breast cancer could support osteoclastogenesis regulating RANKL/RANK/OPG pathway. Inhibition of RANKL–RANK (by bisphosphonates i.e. Neridronic Acid) interactions may offer a promising therapeutic target for interfering with tumor metastasis.

Methods: As a model system of osteoclastogenesis, we used MCF7 human breast cancer cells co-cultured with human osteoblasts. MCF7 and human osteoblasts were cultured at a density of 10exp5 cells/mL in RPMI-1640 complete medium. Cells were grown for 7 days and fixed in a solution of 4% paraformaldehyde. TRAP assay: For cytochemical TRAP analysis, cells were stained using a leukocyte acid phosphatase kit. After TRAP reaction slides are count-

erstained with hematoxylin solution, dried on air and evaluated microscopically. Immunocytochemistry. Specimens were incubated overnight at 4°C with mouse anti Actin monoclonal antibody diluted 1: 50 in blocking buffer then they were incubated with an antimouse FITC conjugated secondary antibody diluted 1: 100. Slides were reacted with 0.1 mg/mL 4–6-diamidino-2 phenylindole diluted to detect cell nuclei. Results. In order to elucidate a mechanism for cancer-induced osteoclastogenesis we postulate that bone destruction by breast cancer is mediated directly by cancer cells. We want to demonstrate that breast cancer could support osteoclastogenesis regulating RANKL/RANK/OPG pathway. Inhibition of RANKL–RANK (by bisphosphonates i.e. Neridronic Acid) interactions may offer a promising therapeutic target for interfering with tumor metastasis and progression in bones and giving to patients with cancer much better quality of life. Conclusion: This work provides conclusive evidence that breast cancer are capable of stimulating osteoclast formation as a result of direct interaction with osteoblast cells without the interference of hematopoietic cells.
Conflict of Interest: None declared

P114-T

CLINICAL BENEFITS OF MIGLUSTAT ON BONE DISEASE IN ADULT TYPE 1 GAUCHER DISEASE (GD1): A META-ANALYSIS

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Background: Gaucher disease (GD) is the most common of the glycosphingolipid storage diseases. Clinical features of GD1 include bone disease, hepatosplenomegaly and blood dyscrasia. Bone manifestations in GD1 are among the most debilitating aspects of the disease and remain a major issue in clinical management of the disease. Enzyme replacement therapy (ERT) is proven at reducing organomegaly and improving haematological abnormalities, however, skeletal response to ERT is limited.

Miglustat is a small molecule that reversibly inhibits glucosylceramide synthase, which catalyses the first committed step of glycosphingolipid synthesis. Its physico-chemical properties ensure wide tissue distribution. The efficacy of miglustat on blood parameters, organomegaly, and chitotriosidase activity in GD1 is proven. This meta-analysis aims to evaluate the effect of miglustat on GD1 bone disease.

Methods: All skeletal complications during a 2 yr observation period were noted. Bone mineral density (BMD) was assessed by dual-energy absorptiometry (DEXA) at the lumbar spine (LS) and/or the femoral neck (FN).

Results: 72 GD1 patients were included in the analysis. Mean ± SD age was 41 ± 13 yrs. Miglustat (100 mg tid) was given to 31 (43%) therapy-naïve patients and 41 (57%) patients switched to miglustat after a minimum of 2 yrs on ERT. Twenty (28%) patients had a history of prior splenectomy. Bone pain and osteoporosis were the most common baseline bone-related manifestations. Osteoporosis (z-score <1) was reported by 71% of patients. Bone pain was present in 63% of all patients, and was statistically more frequent in switched vs naïve patients (76% vs 46%, $p=0.01$).

BMD z-scores significantly improved at both lumbar spine and femoral neck at each time-point vs baseline. A significant increase in z-score was observed both at LS ($+0.15$ $p=0.022$) and FN ($+0.147$ $p<0.0001$) at 6 mo, which remained significant at 12 mo (LS $p=0.012$; FN $p=0.017$) and 24 mo (LS $p=0.015$; FN $p=0.038$). BMD increases were also seen in high-risk patients (i.e. prior splenectomy and/or with marked osteoporosis). 78% patients with baseline bone pain reported improvement after 2 yrs of miglustat; no cases of bone crises, avascular necrosis or pathologic fractures occurred.

Conclusion: These data suggest that miglustat can provide significant clinical benefit such as reducing the occurrence of bone pain and improving bone density in patients with GD1 bone disease, including those in a high-risk category.
Conflict of Interest: None declared

P115-S

RELEVANCE OF USING 99mTc-NTP 15-5 IN CHONDROSARCOMA IN VIVO IMAGING TO DETECT EARLY TUMOR RECURRENCE: DIAGNOSTIC APPLICATIONS TO ZOLEDRONATE-TREATED RATS

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Chondrosarcoma is defined as a malignant cartilage tumor difficult to treat, as it is chemo- and radio-resistant. Surgical treatment leads to severe disability, with high rates of local recurrence that are life-threatening. No adjuvant therapy is effective in differentiated chondrosarcomas. The aim of the present study was to determine the effects of the N-bisphosphonate zoledronic acid (ZOL) which exerts a high anti-bone resorption activity on chondrosarcoma tumor progression.

A rat model of intralesional curettage that reproduces the surgical intervention in patients was used. An innovative imaging approach named "cartilage targeting imaging strategy" was developed using a specific tracer of cartilage (NTP 15-5) containing a quaternary ammonium that selectively binds with high affinity to the polyanionic functions of the cartilage proteoglycans. This quaternary ammonium function was used as a carrier to selectively deliver a gamma-emitting radioisotope (99mTechnetium) to the cartilage tissue. In a pilot experiment, 99mTc in vivo imaging was performed on rats bearing Swarm chondrosarcoma: 99mTc-NTP 15-5 scintigraphy was performed from 10 to 50 days after orthotopic implantation.

The results showed an intense radioactivity uptake associated with the chondrosarcoma development, demonstrating the relevance of using 99mTc-NTP 15-5 in chondrosarcoma imaging as an in vivo tracer of cartilage proteoglycans. This sensitive approach was then used to quantify the effect of ZOL (100 microg/kg s.c. twice a week) on early tumor recurrence after intralesional curettage in the rat Swarm chondrosarcoma model. Our ongoing results revealed that 99mTc-NTP 15-5 scintigraphic imaging is a relevant in vivo method for the follow-up of ZOL treatment that significantly inhibits tumor progression after intralesional curettage and thereby increasing overall survival.

The present study allows the validation of molecules possessing quaternary ammonium moiety that exhibit a high affinity for cartilage in chondrosarcoma imaging. Using this imaging strategy, we demonstrate that in addition to surgery, the therapy of chondrosarcoma with bisphosphonates might be beneficial in preventing tumor recurrence.
Conflict of Interest: None declared

P116-M

TERIPARATIDE IN OSTEGENESIS IMPERFECTA: TREATMENT RESULTS OF AN 18 MONTHS PROSPECTIVE OBSERVATIONAL STUDY

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Osteogenesis imperfecta (OI), also known as brittle bone disease, is a genetic disorder of connective tissue characterized by fragile bones and a susceptibility to fracture from mild trauma or normal impacts on bone of daily living. While it was shown that an antiresorptive therapy with bisphosphonates has beneficial effects on the clinical outcome of OI there is a lack of data on the use of osteoanabolic drugs in this disorder.

The aim of the ongoing "Teriparatide in Osteogenesis Imperfecta" trial (TOI-trial) is to study the efficacy of an anabolic treatment of teriparatide (rhPTH-1–34) in adults with clinically symptomatic disease. In this prospective, observational, single-center, 18 months trial we included 7 patients (4 men, 3 women) above the age of 30 years (mean age 45.7). All received daily subcutaneous injections of 20 μg teriparatide plus 1200mg calcium and 800 IU vitamin D per day. Inclusion criteria were BMD values at the lumbar spine $<$ -3.0 and at the total hip <-2.0, and at least each one prevalent vertebral and non-vertebral fracture. As previous treatments fluoride, bisphosphonates and alfacalcidol had been adopted over different intervals, but during the last year only calcium and plain vitamin D.

Initial characteristics of patients included a mean height of 153cm, mean weight 67kg, average lumbar spine BMD −4.3 and total hip −3.4 T-score, and a mean number of 1.4 new vertebral and 0.7 non-vertebral fractures per patient during the last year before intervention. BMD measured at 6 months intervals showed highly significant increases at both sites and amounted to an average gain of 12.4% at the lumbar spine and 9.4% at the total hip after 18 months. During the 18 months we observed no new vertebral fracture and only one non-vertebral fracture. 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 and moderate non-persisting adverse events occurred in 3 out of 7 patients.

Based on these encouraging preliminary results we have included further patients. All patients will be followed during a subsequent antiresorptive therapy up to month 36.
Conflict of Interest: None declared

P117-T

A HIGH THROUGHPUT METHOD OF MEASURING BONE ARCHITECTURAL DISTURBANCE IN A MURINE CIA MODEL BY MICRO-CT MORPHOMETRY

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Bone architectural disturbance is an important part of the pathology of rheumatoid arthritis. This study evaluated high-speed micro-CT analysis as a

screening method to detect and quantify these bone changes from CIA and their prevention by treatment with methotrexate. Thirty mice were divided into three groups of 10. One group was an untreated control, the second and third groups were injected with collagen type II/CFA adjuvant to cause arthritis (CIA). After 21 days a boost injection with collagen Type II/IFA was given. Simultaneously with the beginning of CIA adjuvant injection, the third group received daily treatment of methotrexate while the second group received only vehicle in daily gavages. 42 days following initiation of CIA and treatment administration, all mice were euthanased and their hind paws harvested for analysis. The ankle region including the whole calcaneous of each mouse was scanned by micro-CT (Skyscan 1172) in short scans of less than 10 minutes (10 micron pixel), and a range of morphometric parameters were calculated for the three groups. Analysis was performed on a volume of interests delineating the whole calcaneous only. Significant changes in morphometric parameters were measured reflecting the bone changes induced by CIA. New low density bone formation increased by three times in the CIA vehicle group compared to controls ($p < 0.05$), while methotrexate treatment reduced the low density formation to below the control level ($p < 0.01$). Corresponding significant changes in measured parameters reflecting bone disturbance by CIA and its prevention by methotrexate, were recorded for bone surface, surface to volume ratio, fractal dimension and mean number and area of cross-sectional objects (indices of structural dissociation). In summary, high throughput micro-CT proved to be a sensitive test for bone architectural disturbance caused by CIA and its prevention by drug treatment.
Conflict of Interest: L. Oste, L. van Rompuay, G. Dixon: employees of Galapagos Ltd. P. Salmon: employee of Skyscan N.V.

P118-S

A PROSPECTIVE TWO YEARS FOLLOW-UP OF THORACIC AND LUMBAR OSTEOLYTIC VERTEBRAL FRACTURES CAUSED BY MULTIPLE MYELOMA TREATED WITH BALLOON KYPHOPLASTY

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Purpose: Balloon kyphoplasty is a minimally invasive procedure for the stabilization of osteoporotic and osteolytic vertebral fractures. The purpose of this prospective study was to evaluate this operative procedure in the treatment of osteolytic vertebral fractures in reduction of pain and functional improvement of the patients and further to evaluate the restoration of vertebral height postoperatively.

Materials and methods: In this study 45 patients (35 male, 10 female) with osteolytic vertebral fractures were treated with Balloon kyphoplasty. 95 vertebral fractures were treated with Balloon kyphoplasty, 38 patients (30 male, 8 female) with 83 vertebral fractures could be followed up over the period of 24 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 and 24 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 24 months. A radiation therapy and / or chemotherapy could be performed without loss of time.

Conclusion: In the treatment of osteolytic vertebral fractures Balloon kyphoplasty led to a quick and sustained reduction of pain and functional improvement of the patients. A restoration of the vertebral height and reduction of the kyphotic angle was especially 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. Balloon kyphoplasty is an outstanding alternative in comparison to the established therapeutic concepts in the treatment of osteolytic vertebral fractures.
Conflict of Interest: Pflugmacher, Robert, Kyphon, Research support

P119-M

BONE LOSS IN THE COURSE OF THREE YEARS AFTER KIDNEY TRANSPLANTATION

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Chronic kidney failure is deleterious for the skeletal integrity. Successful kidney transplantation corrects most metabolic disorders, but hyperparathyroidism might persist for two or more years. Bone turnover may be increased in this period and the rate of bone loss variable. The aim of this investigation was follow-up of bone mass after kidney transplantation. This study comprised 86 kidney transplant recipients (52 men, 34 women, $43.9 +/- 10.4$ years) with stable kidney function monitored by densitometry of the lumbar spine, left hip and forearm. Measurements were performed 2 – 4 times in various post-transplant intervals between 1 and 40 months (13.03 ± 11.15). No difference between sexes existed for age, hemodialysis duration, creatinine and initial T-scores in the first six post-transplant months. Densitometry data was analysed for the entire patient group, according to post-transplant periods: < 6 months (first), 7–12 months (second), 13–24 months (third) and > 25 months (fourth). Osteopenia (T-score < 1) in the lumbar and hip region was present in 50% and for the forearm in 74% of patients in the first six months after transplantation. In the second post-transplant year osteopenia of the lumbar spine and hip was greater than 65%, but did not change for the forearm. Forearm T-scores were significantly lower than in the spine and hip, except for the femur in the second period. Spine and hip T-scores did not differ, except in the fourth period ($p < 0.04$) with greater bone loss in the spine. No correlation of T-scores existed with post-transplant period. Paired T-test showed statistically significant decrease of initial T-scores in the second (< 0.0005), third ($p < 0.0005$) and fourth post-transplant period ($p < 0.001$) for the lumbar spine; the second ($p < 0.001$) and third ($p < 0.002$) period for the hip; and for the third ($p < 0.002$) and fourth ($p < 0.0005$) period for the forearm. Only for the forearm, a statistically significant decrement was also found between the third and fourth period ($p < 0.003$). These results indicate that bone loss proceeds after kidney transplantation within the follow-up period of three years. Decrease of bone mass was more pronounced in the first year and continues at a slower rate in the later period. The degree of bone loss is similar for the spine and hip in the first two post-transplant years, but significantly greater for the forearm. This study stresses the increased risk of osteoporosis in kidney transplant recipients irrespective of sex.
Conflict of Interest: None declared

P120-T

FEMORAL HEAD OSTEOARTHRITIS – ANALYSIS OF BONE STRUCTURE AND TURNOVER

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Increased bone remodeling of the subchondral bone occurs in the course of osteoarthritic destruction of bone and cartilage, also resulting in osteophytic growth. The aim of this study was investigation of static and dynamic histomorphometric parameters in bone specimens from the osteoarthritic femoral head obtained during hip replacement. Bone was analysed from areas of preserved articular cartilage, exposed subchondral bone and osteophyte from 23 patients (10 men, 13 women, 55–65 years) after double tetracycline labelling. Histomorphometric analysis was performed on undecalcified bone tissue sections stained with toluidine blue. Areas with bone cysts were excluded from analysis. Bone volume (BV/TV) was greater ($p < 0.04$–0.001) in areas of preserved and damaged cartilage, trabecular thickness (TbTh) was greater ($p < 0.02$) in preserved cartilage as compared to osteophytic bone. In women additionally, trabecular separation (TbS) was less ($p < 0.008$–0.003) in areas of preserved and damaged cartilage, and osteoblast surface (ObS/BS) was greater ($p < 0.02$) in preserved cartilage as compared to osteophytic bone. No statistically significant difference existed for histomorphometric parameters of bone resorption and dynamic parameters. Osteophytic bone was characterised by reduced bone structure as compared to other investigated areas of subchondral bone. Additionally in women the osteophyte trabecular network was reduced and less osteoblasts were found. Bone turnover did not differ with regard to destruction degree of cartilage and bone in advanced osteoarthritis of the femoral head. The observed reduced bone structure in the osteophyte was probably related to its biomechanical traits, and more pronounced in women possibly due to postmenopausal osteoporosis. Bone turnover variations presumably occurring in the course of osteoarthritis are either absent in advanced stage or masked by variance due to histomorphometry and sample size.
Conflict of Interest: None declared

P121-S

DEVELOPMENT OF AN IN VITRO MODEL TO INVESTIGATE JOINT OCHRONOSIS IN ALKAPTONURIA

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Alkaptonuria is an autosomal recessive disorder caused by the lack of the enzyme homogentisic acid oxidase (HGO). This enzyme is responsible for the breakdown of homogentisic acid (HGA), a key intermediate in the metabolism

of tyrosine and phenylalanine. In the absence of the enzyme a polymer of HGA, termed ochronotic pigment, is deposited in collagenous tissues, including perichondrium and periosteum. This eventually leads to connective tissue disorders including severe arthropathies, and in some cases increased bone resorption and reduced bone mass.

In order to investigate the mechanism of formation of ochronotic pigment and to determine how the deposition of pigment in collagenous matrix leads to arthropathy, we have developed an in vitro model of ochronosis. Osteosarcoma cell lines, MG63, SaOS-2 and TE85, representing different stages of osteoblastic differentiation, were cultured for extended periods in medium containing HGA. At 10^{-3} M HGA there was a toxic effect on cells, but between 10^{-7} M and 10^{-5} M HGA there was no effect on cell viability or activity and no effect on the synthesis of type I collagen, assessed by measurement of P1NP, or on secretion of osteoprotegerin. However, microscopic examination revealed a dose dependent formation of pigmented granules in the cell layers and associated matrix. The deposition of pigment was greatest in MG63 cells and least in Te85 cells. The deposited pigment was closely associated with matrix and /or cell proteins and could be extracted into SDS buffers and resolved as specific bands on PAGE. Characterisation of the protein/pigment complex is currently underway.

In summary, we have developed an in vitro model for ochronosis to study the mechanism of pigment deposition and joint destruction in alkaptonuria that may contribute to a general understanding of the aetiology of osteoarthritis and abnormalities of bone metabolism.

Conflict of Interest: None declared

P122-M

LONG-TERM NORMALIZATION OF BONE REMODELLING WITH A SINGLE INFUSION OF ZOLEDRONIC ACID IN A SEVERE, MULTIFOCAL, BIOLOGICALLY ACTIVE PAGET'S DISEASE OF BONE, RESISTANT TO TREATMENTS: A CASE REPORT

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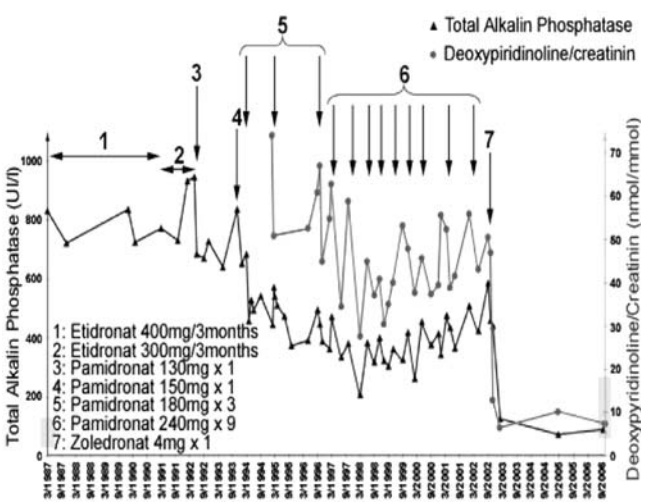
Bisphosphonates are the first choice in the treatment of Paget's disease of bone. Their efficacy is well established, but the issue of resistance to this therapy has been repeatedly raised. We report the case of a white Caucasian woman with Paget's disease of bone, diagnosed in 1977, affecting skull, pelvis, sacrum, right proximal femur and 3 lumbar vertebrae. At that time, s-tot-ALP was 7-fold above upper limit of normal range and u-OH-proline/creat, 5-fold. Etidronate then clodronate, were administered without significant biochemical response. Since 1992, pamidronate was administered iv at a dose of 180 mg then 240 mg every 6 months. Despite that, the decrease in bone remodelling markers was minute, and the effect was rapidly lost. None of these treatments was able to normalize bone remodelling. In October 2002, the patient received a single 4mg iv of zoledronate . One month later, u-d-pyr and total ALP were normalized and remained so for more than 4 years. Thus this patient with severe, multifocal and biologically active Paget's disease of bone, did not show significant responses to various bisphosphonates, but her bone remodelling was fully and steadily normalized by a single injection of zoledronate.



Conflict of Interest: None declared

P123-T

IMPROVE BONE METASTASIS IMAGING AND THERAPY

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Background : More than 60% of women with breast cancer develop skeletal metastasis. The most specific study for detection of bone metastasis is still scintigraphy. Unfortunately some times bone scintigraphy could provide controversial results. The aim of this study was to investigate how dual-energy x-ray absorptiometry (DEXA) can improve the imaging of bone metastasis.

Methods: 45 patients (all women) with breast cancer underwent bone scintigraphy with Tc-99m for possible detection of bone metastasis. All 45 patients were reported bone metastatic. Bone mass density (BMD) of all regions of interest was performed with DEXA.

Results: All 45 patients performed an abnormal BMD. 31 patients were diagnosed osteoporotic. 14 patients were diagnosed with a high BMD. Elevated BMD was determined as osteoblastic type of metastasis.

Conclusion: We concluded that DEXA can provide specific information about bone condition and could probably give some information about histopathology of the investigated region as a noninvasive method. The diagnosis of different types of bone metastasis could be very important in long term management of bone metastasis.

Conflict of Interest: None declared

P124-S

EFFECT OF ANTI-TUMOUR NECROSIS FACTOR ALPHA (INFLIXIMAB) ON HUMAN OSTEOBLAST CELL VIABILITY IN PATIENTS WITH ACTIVE INFLAMMATORY BOWEL DISEASE: AN IN-VITRO STUDY

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Background: Bone mineral density (BMD) loss in patients with inflammatory bowel disease (IBD) is common. Circulating inflammatory cytokines have been implicated in this process. Recently, infliximab (anti-TNFα) has been shown to have beneficial effect on bone metabolism markers in patients treated with this drug, although the mechanisms of action are not known.

Aims: To try to unravel the mechanisms involved in IBD associated bone metabolism derangement, and the beneficial effects of infliximab thereon, we developed a combined clinical and osteoblast in vitro model.

Methods: BMD was measured using dual energy X-ray absorptiometry (DEXA). Sera from 7 IBD patients (5 ulcerative colitis, 2 Crohn's disease) and 6 controls, routine bloods, C-reactive protein, bone formation markers (PN1P-pro collagen type 1 N propeptide and OC-osteocalcin), and bone resorption marker (sCTx-serum carboxyterminal cross linking telopeptide of bone collagen) were measured pre and six weeks post infusion. Parathyroid hormone (PTH) and vitamin D were also analyzed. Human fetal osteoblasts were treated with serum from IBD patient's pre, post infliximab and control. Cell viability was then measured using Alamar Blue assay.

Results: Median age [38 years – IBD, 45 years – control], bone mass index were normal, vitamin D [55.7 – IBD, 81.3 – control] and PTH [28.4 – IBD, 22.8 – control]. Bone formation markers [P1NP (ng/ml) – 21.58(pre); 44.17(post); 45.8(control)], [OC (ng/ml) – 13.46(pre); 25.3(post); 22.7(control)]. Bone resorption marker [sCTx (ng/ml) – 0.61(pre); 0.47(post); 0.44 (control)]. Osteoblast cell viability at 1 day sera exposure at 10% serum was 10.13%(pre), 6.52% (post); $p = 0.041$, 5.39% (control); $p = 0.007$ and at 1% serum exposure was 21.13% (pre), 15.62% (post); $p = 0.002$, 16.20% (control); $p < 0.001$. Osteoblast cell viability at 4 day sera exposure at 10% serum was 23.51% (pre), 19.30% (post); $p = 0.004$, 18.10% (control); $p = 0.006$ and at 1% sera exposure 34.75% (pre), 31.62% (post); $p = 0.001$, 30.70% (control); $p < 0.001$.

Conclusion: Infliximab treatment has a beneficial effect on bone metabolism markers in patients with both Crohn's and ulcerative colitis. Interestingly, osteoblast cell viability in vitro is increased when co-cultured with pre treatment IBD sera whereas post treatment sera causes viability to approximate that of controls. This requires further study and bone growth factors will be measured in future study.

Conflict of Interest: None declared

P125-M

EFFECT OF ALENDRONATE ON CYTOSKELETAL ORGANIZATION AND DYNAMICS OF ACTIN IN PC-3 PROSTATE CANCER CELLS

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Aminobisphosphonate alendronate is an analogue of pyrophosphate and a potent inhibitor of osteoclastic bone resorption. It also inhibits invasion and migration of PC-3 human prostate cancer cells. The effects of alendronate have been associated with its ability to suppress the mevalonate pathway which produces intermediates for isoprenylation of small GTPases that are essential for various cell functions.

In the present study, we investigated the role of mevalonate pathway in invasion and F-actin organization of PC-3 cells. We also studied the effect of alendronate on GFP-paxillin, GFP-cofilin and actin cytoskeleton and on co-localization of these proteins. Phalloidin/Hoechst staining was used to study F-actin organization. The role of prenylation reactions in regulation of PC-3 cell invasion and F-actin organization was studied using specific inhibitors of Ras, Rho and Rab prenyltransferases (FTI-277, GGTI-298 and NE-10790, respectively) and of Rho kinase (Y-27632). The effect of alendronate and prenylation inhibitors on the dynamics of actin, paxillin and cofilin was studied using fluorescence recovery after photobleaching (FRAP)

The results demonstrate that an 8-hour pre-treatment with alendronate was required to obtain inhibition of PC-3 cell invasion. The F-actin organization was also destroyed after 8 hours of alendronate treatment. The Rho and Ras prenyltransferase inhibitors (GGTI-298 and FTI-277) effectively decreased invasion of PC-3 cells, but the Rab prenyltransferase inhibitor NE-10790 did not have any effect. The prenyltransferase inhibitors (except NE-10790) also caused changes in the F-actin organization of PC-3 cells. The treatment of PC-3 cells for 2 hours with the Rho kinase inhibitor (Y-27632) alendronate totally disrupted F-actin organization and blocked invasion of PC-3 cells. The FRAP analyses showed that alendronate and inhibitors of Rho and Ras prenylation inhibited the recovery of F-actin, but the Rab prenylation inhibitor NE-10790 had no effect.

As a conclusion, our results suggest that the prenylation dependent functions of Rho and Ras are important in alendronate inhibition of F-actin organization and dynamics as well as in inhibition of invasion of PC-3 cells.

Conflict of Interest: None declared

P126-T

EVALUATION OF A NEW TWO SITE-ELISA FOR SERUM DKK-1 IN PATIENTS WITH BREAST CANCER AND BONE METASTASES

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Background: The Wnt signaling pathway plays a major role in osteoblastic differentiation. Wnt signaling is regulated by different factors including Dickkopf-1 (Dkk-1), a soluble inhibitor of Wnt. Increased production of Dkk-1 by myeloma cells has been shown to play a major role in the decreased bone formation found in patients with multiple myeloma. Recently we found that human breast cancer cell lines (MDA-MB-B02) that produce bone metastases when injected in mice expressed Dkk-1. The aim of our study was to evaluate the technical performance of a new ELISA for serum Dkk-1 and investigate circulating Dkk-1 in patients with breast cancer with or without bone metastases.

Methods: Polyclonal antibodies raised against synthetic peptides of human Dkk-1 were used in a sandwich ELISA (Dkk-1 ELISA, Biomedica). The sequences of the peptides used as immunogen were identified by bioinformatics selection utilizing a combined evaluation of secondary structure, accessibility, and predicted antigenic determinants. Serum Dkk-1 was measured in women with breast cancer and bone metastases (n = 17) (mean age 63 years), women with breast cancer without bone metastases (n = 12) (mean age 56 years) and women with breast cancer and metastases at sites other than bone (n = 15) (mean age 54 years).

Results: Intra and inter assay coefficients of ELISA for serum Dkk-1 were below 7% and 12%, respectively. The dilution recovery range was from 93 to 109%. The detection limit was determined to be 18.2 pg/ml. Serum Dkk-1 levels were significantly higher in women with breast cancer and bone metastases (7663 +/−6198 pg/ml) compared to women with breast cancer without bone metastases (4171 +/−2065 pg/ml; +27.1%, $p = 0.045$) and to women with breast cancer and metastases in other sites (3730 +/−1128 pg/ml; +35.1%, $p = 0.0087$).

Conclusion: The new ELISA for serum Dkk-1 demonstrated adequate technical performances. Women with breast cancer and bone metastases are characterized by increased serum Dkk-1, suggesting that alterations of the Wnt signaling pathway could play a role in abnormalities of bone turnover in these patients. The assessment of Dkk-1 with this new ELISA should be useful for the clinical investigation of malignant bone diseases.

Conflict of Interest: None declared

P127-S

REGULATION OF PARATHYROID HORMONE-RELATED PROTEIN RELEASE AND EXPRESSION BY EXTRACELLULAR CALCIUM AND PHOSPHATE IN OSTEOBLAST PROGENITOR CELLS

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BACKGROUND. Parathyroid Hormone-Related Protein (PTHrp) has been shown to have anabolic effects in women with postmenopausal osteoporosis (Horwitz et al. 2003). On the cellular level PTHrp promotes the recruitment of osteogenic cells and prevents apoptotic death of osteoblasts and osteocytes (Miao et al. 2005). Cells sense changes in the extracellular concentration of calcium through the calcium receptors (CaR). In plasma the calcium concentration is normally around 1–2 mM, but in the vicinity of resorbing osteoclasts the calcium concentration can reach levels of up to 40 mM (Silver 1988). As the inorganic part of bone largely consists of basic calcium phosphate, phosphate is also liberated from bone during resorption. The aim of this work was to study the effects of calcium and phosphate on the expression of PTHrp in osteoblastic progenitor cells.

METHODS. Adult human mesenchymal stem cells (hMSC) were cultured and differentiated by addition of beta-glycerophosphate, ascorbic acid and dexamethasone. The expression of PTHrp mRNA was assayed by real-time PCR. The PTHrp release into the culture media was measured by a commercial immunoradiometric assay.

RESULTS. Increasing the extracellular calcium from 1 mM to 5 mM resulted in up to 8-fold increase in PTHrp release ($p < 0.01$). The effect of calcium could be seen within 60 min of treatment. The CaR agonist neomycin mimicked the effects of calcium. The effect of calcium was more pronounced in osteoblast progenitors than in fully differentiated osteoblasts. PTHrp mRNA was increased by elevated calcium levels. Phosphate alone (7 mM) and in combination with 5 mM calcium decreased both the PTHrp mRNA and the release of PTHrp.

CONCLUSIONS. Our studies using mesenchymal stem cells, pre-osteoblasts and differentiated osteoblasts showed that an increase in extracellular calcium levels increases, whereas phosphate decreases the release and expression of PTHrp. The effects of calcium and phosphate on PTHrp-release could act as a cellular mechanism by which bone formation is locally regulated in areas of bone resorption.

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Conflict of Interest: None declared

P128-M

STRONTIUM RANELATE PROMOTES AN OSTEOCYTE-LIKE PHENOTYPE FROM HUMAN PRIMARY OSTEOBLASTS EX VIVO

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Strontium ranelate (SR) is a new treatment for osteoporosis that reduces the risk of hip and vertebral fractures in post-menopausal women. Clinical studies, and studies in animal models of bone loss, have demonstrated that SR acts by increasing bone formation, while decreasing bone resorption. There is increasing evidence of an important role of osteocytes in bone metabolism, with decreased osteocyte density in osteoporosis and prolonged survival of osteocytes in case of glucocorticoid treatment with estrogen supplementation, or bone loading. SR has a number of effects on primary osteoblasts (human and rodents) and osteoblast cell line, but the ability to modulate osteocyte differentiation and activity has not been reported yet. The aim of our study was therefore to investigate the effect of SR in adult primary human osteoblasts (NHBC) in culture, in terms of differentiation up to the osteocyte-like stage.

NHBC were cultured for up to 6 weeks in medium alone or medium containing 1, 5 or 10 mM SR. Cells were assayed weekly for cell-surface expression of alkaline phosphatase (AP) and STRO-1 (FACS analysis): the level of expression of these markers reflects the osteoblast differentiation status. In addition, RNA was collected for real-time reverse transcription PCR analysis for expression of dentin matrix protein (DMP)-1 mRNA, which appears to be expressed exclusively by osteocytes in human bone.

Over a 6-week treatment period, SR time- and dose-dependently increased the percentage of cells that were negative for AP and STRO-1 expression, a phenotype consistent with that of osteocyte-like cells. Concomitantly, the mRNA level DMP-1 increased time- and dose-dependently, to relatively high levels in response to SR (up to 4-fold with 10 mM), also suggestive of the presence of osteocytes in the cultures.

This study provides new insight into the mechanism of action of SR on cells of the osteoblast lineage, and suggests the possibility that SR can promote either the differentiation of osteocytes, and/or osteocyte survival. A positive effect on

osteocytes by SR could, at least in part, explain its ability in vivo to correct the balance between bone resorption and formation in osteoporosis.
Conflict of Interest: G Atkins, Servier, Grant Research Support, D Findlay, Servier, Grant Research Support

P129-T

VISCOELASTICITY AND FORCE TRACTION OF BONE CELLS IN 3-DIMENSIONAL MORPHOLOGY

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To understand how the mechanosensing by bone cells relate to cellular metabolism, a physical portrait of cell viscoelasticity is required. Thus, we developed a novel application of two-particle microrheology to characterize the viscoelasticity, and probe the mechano-activity of single MLO-Y4 osteocytes in comparison with other bone cell types under round suspended morphology. The cells were suspended in cell culture medium and assumed a round morphology. These cells were then attached with fibronectin-coated spheres at opposite ends using optical tweezers. The viscoelastic properties of the cells were measured by actively moving one of the attached spheres to deform the cell (active mode). Using two-particle microrheology, we monitored the thermal fluctuations of the beads to determine cellular viscoelastic properties (passive mode of measurement). We found that the elastic modulus of round suspended MLO-Y4 osteocytes was below 1kPa, as well as for MC3T3-E1 osteoblasts, and primary osteocytes and osteoblasts. MLO-Y4 cells' complex compliance showed an elastic plateau below 15 Hz, viscoelastic response between 15–100 Hz, and a viscoelastic stiffening response above 100 Hz. The nitric oxide released by MLO-Y4 osteocytes increased after stimulation with 5pN, using integrin-bound probes. This shows that bone cells respond to forces at a similar range for deforming integrins. MLO-Y4 cells interacted with the integrin-bound probes by changing their shape from spherical to being polar at 37°C. However, the same shape change at 22°C required stimulation with 5–20pN forces. This suggests that temperature has a significant effect on cell morphology. To probe the mechano-activity of bone cells, we measured the fluctuation of force induced by the cells on the probes. We found that the force fluctuation magnitude was proportional negatively quadratic for all cell types. Compared to MLO-Y4 cells, CCL-224 fibroblasts had a higher force fluctuation magnitude, as might be expected considering the motility of fibroblastic cells. These results demonstrate that microrheology is a useful tool for understanding the varied observations on mechanosensing by cells. By quantifying the mechanical properties of single bone cells, our results provide novel implications on the osteogenic response of bone to mechanical loading.
Conflict of Interest: None declared

P130-S

MECHANOSENSITIVITY AND ELASTICITY OF BONE CELLS UNDER ROUND AND FLAT MORPHOLOGIES

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There is increasing evidence that cell function and mechanical properties are closely related to morphology. Thus, cell morphology plays an important role for the design of engineered artificial tissue constructs. However, most in vitro studies investigate flat adherent cells, which might not reflect physiological geometries in vivo. Osteocytes, the mechanosensors in bone, reside within ellipsoid containment, while osteoblasts adhere to flatter bone surfaces. It is unknown whether morphology difference, dictated by the geometry of attachment is important for cell rheology and mechanosensing. Thus, we studied the rheology and mechanosensitivity of bone cells under different morphologies using atomic force microscopy and our two-particle assay for optical tweezers. Primary osteoblasts were isolated from avian bone using a sequential collagenase treatment. These cells were cultured in 2% chicken serum overnight prior to experiments. MLO-Y4 cells were used to model osteocytes and MC3T3-E1 cells for osteoblasts. These cell lines were cultured in 10% fetal bovine serum until near confluency prior to experiments. All cells were incubated at room temperature in CO_2-independent medium without serum immediately before measurements. Elasticity was determined using a Hertzian contact model for both flat and round morphologies. We found that the elastic modulus of MLO-Y4 osteocytes when flat and adherent ($>$ 1kPa) largely differed when round but partially adherent ($<$ 1kPa). The elasticities of round suspended MLO-Y4 osteocytes, MC3T3-E1 osteoblasts, and primary osteoblasts were similarly $<$ 1kPa. The mechanosensitivity of round suspended MLO-Y4 osteocytes was investigated by monitoring nitric oxide (NO) release, an essential signaling molecule in bone. These cells were stimulated by oscillatory undulations of the integrin-bound spheres up to 30pN force. Interestingly, the NO released increased in response to 5pN force stimulation, in contrast with flat cells, which required 20nN force stimulation while releasing lesser NO. Our results suggest that a round cellular morphology supports a less stiff cytoskeleton configuration compared with flat cellular morphology. This implies that osteocytes take advantage of their ellipsoid morphology in vivo to sense small strains benefiting bone health. Our assay provides novel opportunities for in vitro studies under a controlled suspended morphology versus commonly studied adherent morphologies.
Conflict of Interest: None declared

P131-M

ENHANCED RESPONSE OF BONE CELLS TO NOISY FLUID SHEAR STRESS

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Stochastic resonance is exhibited by non-linear systems, where the response to a small signal is enhanced by noise at an optimum level. It is possible that bone cell mechanosensitivity is enhanced by noise as a mechanism for an amplified response to small stresses. $\epsilon\mu$Daily normal loading on bone is expected to occur at strains as low as 10 Furthermore, microdamage might impair normal fluid shear stress levels for stimulating bone cells. Since bone formation correlates with mechanical loading, understanding how bone cells might perceive low stresses under normal or special conditions of loading is imperative. Previously, we found that bone cells required an initial stress-kick to respond to fluid shear stress in a rate-dependent manner. This provides a basis for stochastic resonance to occur in bone as a non-linear biological system. Thus, we studied whether noise of varying intensities (0.03–1.4Pa) enhanced the mechanosensitivity of MLO-Y4 osteocytes in comparison with MC3T3-E1 osteoblasts. Nitric oxide (NO) and prostaglandin E2 (PGE2) production were measured as parameters for bone cell activation. We found that the NO response of MLO-Y4 osteocytes to a small periodic fluid shear stress was acutely enhanced by noise at intensity 0.25Pa. The NO response of MC3T3-E1 osteoblasts to noisy stress was optimum at noise intensity 0.7Pa. MLO-Y4 osteocytes showed an increase in PGE2 release at noise intensity 0.7Pa, and MC3T3-E1 osteoblasts showed a peak response at noise intensity 0.42Pa. Our in vitro results also implied differences in stress-thresholds for NO and PGE2 production for MLO-Y4 and MC3T3-E1 bone cells. Since NO and PGE2 regulate bone formation as well as resorption, our results explain how bone cells might cooperate in vivo in driving the mechanical adaptation of bone. The noise-amplified response by bone cells provides a novel paradigm for understanding the osteogenic benefits of low amplitude dynamic loading, and an innovative therapeutic option for preventing bone loss.
Conflict of Interest: None declared

P132-T

STRONTIUM RANELATE EFFECTS IN HUMAN OSTEOBLASTS SUPPORT ITS UNCOUPLING EFFECT ON BONE FORMATION AND BONE RESORPTION

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Strontium ranelate reduces the risk of vertebral and hip fractures in postmenopausal women. Previous studies have shown that strontium ranelate increases bone formation and decreases bone resorption. In the current study, we investigated the uncoupling effect of strontium ranelate in primary human osteoblasts (HOB). For this, we assessed the strontium ranelate effects on indirect markers of bone formation (HOB proliferation, alkaline phosphatase (ALP) activity), on the regulation of osteoclastogenic signals by osteoblasts (OPG mRNA level and RANKL mRNA and protein level) and on HOB lifespan. HOB were cultured in Dulbecco's modified Eagles medium with 10% fetal bovine serum, adapted to serum-free and calcium-free medium for 24h, and then treated with strontium ranelate in physiological Ca^{2+} (1mM). After a 48h-treatment, strontium ranelate increased HOB proliferation, assessed by thymidine incorporation, in a dose-dependent manner, up to 3.8-fold with 2 mM Sr^{2+} ($p < 0.01$). ALP activity was increased after 72 h with strontium ranelate by almost 2 fold (1 and 2 mM, $p < 0.01$). After only 24 h, strontium ranelate dose-dependently increased OPG mRNA expression, by qRT-PCR, up to 1.9-fold with 2 mM Sr^{2+} ($p < 0.001$). Under the same conditions, RANKL mRNA expression was dramatically decreased compared with RANKL expression observed in vehicle: remaining expression was below 25% with strontium ranelate concentrations $\geq$ 0.1mM ($p < 0.001$). These results were confirmed at the protein level by RANKL-specific western blotting. Finally, strontium ranelate increased

HOB survival under oxidative stress conditions induced by peroxide (0.1 mM, $p < 0.05$, 1 and 2 mM, $p < 0.01$), and decreased serum deprivation-induced apoptosis as measured by caspase 3 and caspase 7 activities (0.1 and 1.0 mM, $p < 0.05$). In conclusion, strontium ranelate, at strontium concentrations close to those observed in patients treated with the therapeutic dose of 2g/day, increases human osteoblast replication, differentiation and the ability to withstand stress, parameters associated with promotion of bone formation. In parallel, human osteoblasts stimulated by strontium ranelate express more OPG and less RANKL, thereby decreasing their capability to stimulate osteoclastogenesis. Overall, these results strongly support the dissociation effect of strontium ranelate on bone formation and bone resorption in human osteoblasts.
Conflict of Interest: TC Brennan, Servier, Project Funding MS Rybchyn, Servier, Project Funding P Halbout, Servier, Staff member AD Conigrave, Servier, Project Funding RS Mason, Servier, Project Funding

P133-S

NEW INSIGHTS INTO PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA ACTION: STIMULATION OF HUMAN OSTEOBLAST DIFFERENTIATION

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Peroxisome proliferator-activated receptor γ (PPARG) is a member of the nuclear receptor superfamily, which orchestrates responses to environmental factors by sensing diverse nutrients and xenobiotics. Imbalances in nuclear receptor signalling have been associated with various aging-related diseases, e.g. osteoporosis, metabolic syndromes and cancer. Two PPARG proteins exist derived from one single gene by alternative promoter usage and splicing: PPARγ1, which is translated from three alternative transcripts PPARG1, 3 and 4, and PPARγ2, which is translated from PPARG2 transcript. PPARG2 is known to control adipogenesis. Here we report our recent findings concerning the presence, regulation and functional significance of PPARG signalling during differentiation and mineralization of our human osteoblast cell model SV-HFO. The latter can be induced by dexamethasone (dex) to differentiate and to mineralize the extracellular matrix formed in a three-week period. Realtime-PCR analysis of the four PPARG transcripts revealed a at least 250, 30 and 150-fold higher expression of PPARG1, 3 and 4, respectively, compared to PPARG2 at all time points during culture. This markedly contrasts the expression pattern in adipocytes in which PPARG2 is strongly expressed. Expression of the four PPARG transcripts increased during differentiation, with PPARG3 showing strongest induction. Short-term dex-treatment for three hours increased expression of all four PPARG transcripts suggesting direct regulation by dex. We furthermore studied the effect of PPARG ligands on human osteoblast differentiation and mineralization. Treatment with rosiglitazone, a PPARG-specific agonist, resulted in significant increases in alkaline phosphatase (ALP; a specific osteoblast differentiation marker) activity and mineralization at all three time points during culture. The PPARG antagonist GW9662, however, revealed a significant decrease in ALP activity and did not show any effect on mineralization. In conclusion, PPARG expression is controlled by cell-type specific promoter usage and splicing, leading to PPARG1, 3 and 4 (= PPARγ1) expression in osteoblasts and PPARG2 (= PPARγ2) expression in adipocytes. The PPARG ligand rosiglitazone does not exclusively control adipocyte differentiation but also stimulates human osteoblast differentiation. Our data implicate an alternative view on PPARG in the balance between adipocyte and osteoblast differentiation in relation to the development of osteoporosis.
Conflict of Interest: None declared

P134-M

THE EFFECTS OF SURAMIN ON LYSYL OXIDASE (LOX), TELOMERASE ACTIVITY AND THE DIFFERENTIATION OF OSTEOSARCOMA CELLS

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LOX is a copper-containing mono-amine oxidase and plays a critical role in the stabilization of the extracellular matrix by cross-linking of collagen and elastin. It also has a function in the morphogenesis and repair of connective tissues. LOX is secreted by osteoblasts and fibroblasts as a precursor, which is processed by BMP1 to the active enzyme and to a protein functioning as a tumor suppressor(1). Suramin, a polysulphated naphthylurea, is nowadays used for the treatment of different tumors, especially prostatic cancer, where it inhibits the binding of growth factors to their receptors. Whether it acts via interruption of the autocrine growth factor pathways or via upregulation of LOX is still unknown. Telomeres, nucleoprotein structures, protect cells from chromosomal loss and recombination by capping chromosome ends. When telomeres become critically short, senescence or apoptosis is induced(2). To analyse whether and how Suramin effects osteosarcoma (OS) cells we studied growth, differentiation, telomerase-activity (TA) and expression of LOX in the human MG63 and U2OS, and the rat UMR106 OS cell lines.

Human OS cells were seeded in amend, UMR 106 cells in DMEM and treated with Suramin (150 μM). Thereafter, cell number, alkaline phosphatase activity, telomerase activity and LOX-mRNA expression was analysed.

Data show that Suramin inhibited cell number in all cell lines tested. While in UMR 106 the decrease was low, in both human OS-cells the decrease was significant. According to these results TA was less inhibited in UMR 106 and strong in U2OS. Surprisingly, TA was not regulated in MG63. Alkaline phosphatase activity was upregulated by Suramin in the human but not in the rat OS cells after 3 days. LOX was expressed in all cell lines tested and upregulated in the human OS cells after 3 days of culture but not in confluent cells (6 days).

In conclusion Suramin inhibits cell multiplication of OS cells and increases osteoblastic differentiation. These effects could be mediated by the tumor suppressor part of LOX, which was upregulated by this drug.
1. Kagan H. et al. 2003 J Cell Biochem. 88; 660
2. Harley CB 1991 Mutat Res 256; 271
Conflict of Interest: None declared

P135-T

GENE MICROARRAY ANALYSIS IN MOUSE OSTEOBLASTS REVEALS SETS OF RELEVANT MRNAS REGULATED UNDER MODELED MICROGRAVITY

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Mechanical unloading is detrimental for the skeleton, but the underlying molecular mechanisms are not fully elucidated. To identify new pathways involved in the regulation of bone homeostasis in unloading conditions, we performed transcriptome analysis of mouse calvarial osteoblasts grown for 5 days at unit gravity (1xg) or under modelled microgravity (0.008xg) in the NASA-developed Rotating Wall Vessel bioreactor. Osteoblasts were then collected and RNA extracted, reverse transcribed and labelled with cy3-dCTP and cy5-dCTP fluorochromes. Labelled probes were hybridised onto a 22K mouse Oligomicroarray chip (Agilent), and the slides scanned and acquired using the Jaguar 2.0 Software. Elaboration of gene profiling results (three experiments, each run in double to invert cy3/cy5 labelling, cut off 2, $P < 0.05$) evidenced that among the 20,000 gene probes evaluated N. 30 genes were up-regulated and N.120 were down-regulated in microgravity vs. unit gravity. Interestingly, among the latter group we found mRNAs of genes involved in osteoblast differentiation, including alkaline phosphatase and Runx2, and bone matrix proteins, including osteocalcin, periostin, osteomodulin, fibronectin and osteoglycin, while osteopontin and collagen 1A2 were not modulated. Validation by real time RT-PCR confirmed significant decrease of alkaline phosphatase, Runx2 and osteocalcin, whereas osteopontin and collagen 1A2 remained unaffected. In agreement with our previous results, osteoblasts cultured under microgravity also showed a significant increase of the RankL/OPG ratio, both in the gene transcriptome and in the real time RT-PCR validation assays, consistent with the ability of conditioned media from microgravity-exposed osteoblasts to induce osteoclastogenesis in mouse bone marrow cultures. We next clustered the significantly modulated genes by using the GOTM (gene ontology tree machine) software which allowed to identify the related biological processes. This analysis evidenced up-regulation of apoptosis-inducing genes and of genes involved in the response to oxidative stress. Other pathways, related to Wnt signals, extracellular matrix and cell growth, were down-regulated. In conclusion, our global analysis under microgravity could contribute to a better understanding of the mechanisms underlying bone mass regulation and to identify new targetable molecules in order to prevent and cure bone loss in individuals subjected to unloading conditions or affected by bone pathologies.
Conflict of Interest: None declared

P136-S

OLEUROPEIN INDUCES OSTEOBLASTOGENESIS AND INHIBITS ADIPOGENESIS: EFFECT ON DIFFERENTIATION FROM OF BONE MARROW-DERIVED STEM CELLS

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Osteoblasts and bone marrow adipocytes share a common progenitor: multipotential mesenchymal stem cells. It has been proposed that the plasticity among these lineages may explain the reduced bone formation observed during aging. In fact, age-related bone loss is associated with an insufficiency in osteoblasts needed to replace the bone removed by osteoclasts during remodelling. This is evidenced by a thinning of the trabeculae and a loss of microarchitectural connectivity, accompanied by an increase in bone marrow adipose tissue. It has been hypothesized that the oxidative stress could play a role in the differentiation

switching between these two cell lineages. On the other hand, it has been found that the oleuropein, polyphenol extracted of the leaves and unprocessed olive drupes of the olive tree (Olea europaea L.) shows a strong anti-inflammatory and antioxidant activity in vitro and in vivo. Besides, it reduces the bone loss in estrogen-deficient rats when supplied in the diet.

We have studied the effects of the different concentrations of oleuropein (10^{-4} M up to 10^{-7} M) in the differentiation process of marrow-derived stem cells after induction to either osteoblasts (10^{-8}M dexamethasone, 0.2 mM ascorbic acid, 10 mM β-glycerolphosphate) or to adipocytes ($5{\cdot}10^{-7}$M dexamethasone, 0.5 mM isobutylmethylxanthine, 50 μM indometacine). The expression profile of different genes has been quantified: *osteoprotegerin*, *alkaline phosphatase*, *runx2*, *rankl*, *osteocalcin* and *collagen* (osteoblasts) and *ppar-*γ2 y *lpl* (adipocytes). Furthermore, the cells were monitored by analyzing the enzymatic activities of protein markers like the alkaline phosphatase and by staining as oil-red. The osteoblast-induced cells showed a dose-response of the gene expression of osteoblastic genes like the osteoprotegerin (opg) and alkaline phosphatase (alp) with the oleuropein concentration used (the highest gene expression was found at 10 μM). Conversely, the adipocyte-induced cells showed a marked reduction of the adipogenesis when exposed to 10^{-4} M oleuropein. Therefore, the oleuropein can both induce the osteoblastogenesis, and also inhibit the adipogenesis, therefore enhancing the bone formation. These results support the hypothesis that the oxidative stress could be an important contributor to the age-related decrease of bone formation.

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Conflict of Interest: None declared

P137-M

IN VITRO AND EX VIVO ANALYSIS OF CHROMOSOME BREAKS IN MURINE OSTEOBLAST CELLS TREATED WITH INTERMITTENT PTH

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Intermittent PTH (iPTH) has been approved as anabolic treatment for osteoporosis. Toxicological studies have demonstrated that iPTH is associated with osteosarcoma in rats. However, there is no solid evidence on the potential mutagenic effect of iPTH and its safety remains controversial. In the present study we investigate the genotoxic potential of iPTH on murine osteoblasts. Micronuclei (MN) assay was used to detect chromosome breaks in murine cells exposed to iPTH. MC3T3 cells were grown in aMEM, supplemented with 10% fetal bovine serum (FBS) and antibiotics. Cells were treated with PTH at 50 and 100 mM for 21 days, 6 hours a day. MN assays were performed at different time points (6 hours, 7, 14 and 21 days). Non-treated cells and cultures exposed for 6 hours to ethyl methanesulfonate (EMS)were used as negative and positive controls, respectively. At the time-points, cytochalasin B (4.5 μg/ml) was added to the cultures to inhibit cytokinesis at the end of mitosis and 36 hours later, cells were fixed in methanol, stained with acridine orange and chromosome breaks were visualized by fluorescence microscopy. We observed a 2–3-fold increase in the prevalence of MN in PTH treated cells as compared to the controls. The effect was detectable 6 hours after PTH treatment, was more significant with the highest PTH concentration and increased with time in culture. Moreover, nucleoplasmic bridges were detected, suggesting that iPTH induces genomic instability and can be considered clastogenic in vitro. For ex vivo studies, 12 month-old C57/BL mice were divided in 4 groups (10 animals per group): PTH1-34 20mg/kg and 40 mg/kg body weight, saline (negative control) and EMS 200 mg/kg (positive control). PTH was given daily by subcutaneous injections during 10 weeks. EMS was administered as a single dose at the end of 10 weeks. Animals were sacrificed, long bones were taken and osteoblasts were extracted. After adhesion of the cells to the culture dish, cytochalasin B was added and cells were analyzed for MN 36 hours later as described above. Analyzes of bone marrow cells extracted from mice treated with iPTH showed an 1.8 fold increase in MN rate as compared to the negative controls, lower than the 3-fold increase required for considering a drug clastogenic in the assay. Our results demonstrate that iPTH interact with DNA and induces chromosome breaks in vitro but not in vivo, at least in the concentrations and conditions used here.

Conflict of Interest: None declared

P138-T

STRONTIUM RANELATE INCREASES OSTEOBLAST REPLICATION THROUGH ACTIVATION OF AN ORIGINAL CELLULAR MECHANISM

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Strontium ranelate (SR) is a new treatment for osteoporosis both increasing bone formation, while decreasing bone resorption with a demonstrated vertebral and hip antifracture efficacy. In vitro studies have shown that SR enhances preosteoblastic cell replication, perhaps through activation of the calcium sensing receptor. In this study, we analysed the signaling pathways involved in SR-induced osteoblastic cell replication. Preosteoblastic MC3T3-E1 cells were selected among 8 different cell lines has having the best mitogenic response to SR. In these cells, SR dose-dependently (1–10 mM) enhanced DNA synthesis measured by 3H-thymidine incorporation (1.8x – 8.3x) and cell number (1.4x – 1.8x). As expected, CaCl2 increased DNA synthesis and induced a rapid (5 min) activation of ERK and PKCs. In contrast, activation of signaling pathways by SR, including ERK, PKC and PKD, was only detected after one hour incubation and lasted about 3–5 hours. Enhanced signaling and DNA synthesis induced by SR and CaCl2 were associated with increased expression of cyclin D1 after 24 h, a critical cell cycle regulator. Functional analysis of the role of signaling pathways activated by SR was performed using specific inhibitors. Inhibitors of PKC (G06983, 5 microM) and PKD (G06976, 0.2 microM) markedly reduced cell replication induced by SR. An inhibitor of the ERK signaling pathway (U0126, 5 microM) mainly reduced basal cell number but not the SR response, indicating that ERK is not involved in SR-induced osteoblastic replication. In conclusion, SR dose-dependently enhanced cell replication in osteoblastic cells. Both SR and CaCl2 induced activation of MAP kinases and PKC pathways. The effect of SR was however delayed compared with that of CaCl2 suggesting an original mode of activation, possibly through the synthesis of an autocrine growth factor. Finally, functional analysis indicate a predominant role of PKC but not of MAP kinases in mediating SR-induced cell replication in osteoblastic cells. These results provide first evidence for a different cellular mechanism by which strontium ranelate enhances osteoblastic cell replication compared with CaCl2.

Conflict of Interest: J. Caverzasio, Servier, Grant Research Support

P139-S

(−)-EPIGALLOCATECHIN-3-GALLATE (EGCG) MODULATE OPG/RANKL/RANK IN MURINE BONE MARROW MESENCHYMAL STEM CELL AND OSTEOBLAST

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Background: Green tea is one of the most popular beverages. Among catechins, (−)-epigallon-catechin-3-gallate (EGCG) receives most attention. Recent studies showed higher bone mineral density with reduced risk of hip fracture in habitual tea drinkers. However, the effective components and the action mechanisms of tea on bone remodeling remain unclear. Osteoprotegerin (OPG)/receptor activator of nuclear factor-kB ligand (RANKL)/RANK is the current model for preosteoblastic/stromal cell regulation on osteoclastogenesis. The effects of EGCG on OPG in murine bone marrow mesenchymal cells (D1) and osteoblasts (MC3T3-E1) were tested in this study.

Methods: D1, cloned from mouse bone marrow cells were maintained in DMEM. MC3T3-E1, cloned from newborn mouse calvaria, were maintained in MEM-α;. The cells were plated at a density of 10000 cells per cm^2 with medium changed every 48 hours with or without EGCG, 1 and 10 μmol/L. Gene expression was analyzed by RT-PCT after treatment for 2 days. Protein secretion was analyzed by ELISA after treatment for 4 and 7 days.

Results: EGCG increase the mRNA expression and protein secretion of OPG both in D1 and MC3T3-E1 cells. After EGCG, 1 and 10 μmol/L, treatments, mRNA expression increased by 103% and 125% respectively in D1 cells and 65% and 110%respectively in MC3T3-E1 cells. In protein secretion in D1 cells, EGCG, 1 and 10 μmol/L, increased 30% and 88% after treatment for 4 days respectively. In the 7th day, no significant increase was noted after treated with EGCG. In MC3T3-E1 cells, EGCG, 1 and 10 μmol/L, increased 119% and 176% after treatment for 7 days respectively. However, no significant increased was found after treatment for 4 days.

Conclusion: EGCG has been reported to induce apoptotic cell death of osteoclast-like multinucleated cells. Our results illustrated that the effective concentration of EGCG is at the range of 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 via increasing the gene expression and protein secretion of OPG. Osteoclastogenesis may be decreased through the intensified OPG by EGCG which is a novel modulation between EGCG and OPG/RANKL/RANK both in a bone marrow mesenchymal cell line and an osteoblast cell line. Therefore EGCG may inhibit osteoclastogenesis via OPG/RANKL/RANK system.

Conflict of Interest: None declared

P140-M

THE INFLUENCE OF GLUCOSE AND INSULIN TO HUMAN OSTEOSARCOMA CELL LINE MG63

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[Background] Osteoporosis (OP) and diabetes mellitus (DM) are two common diseases of endocrinology. As the complex mechanism of OP and DM, different manifestations of osteopathy are observed in DM. But the relationship between the two diseases is still uncertain. [Object] In this research, we took an insight into the influence of glucose and insulin to human osteosarcoma cell line MG63, hoping to give an explanation to the mechanism of the OP in DM.

[Method] We set 5 .5mmol/L, 10mmol/L, 20mmol/L, 40mmol/L 4 levels of glucose concentration and 0mol/L, 10^{-7}mol/L, 10^{-6}mol/L, 10^{-5}mol/L 4 levels of insulin concentration, and then combined them into 16 different culture media. Human osteosarcoma cell line MG63 was cultured in these media successively for 21 days. The proliferation rate of cells, secretion of ALP and BGP, percentage of apoptosis cells and the formation of calcified nods were observed.

[Result] We found only the simulated normal glucose concentration, which was 5.5mmol/L, was optimal for the secretion of ALP and BGP and the mineralization of MG63 cells with the lowest percentage of apoptosis. Although the elevated glucose concentration would prompt the proliferation of MG63 cells, the secretions of ALP and BGP and the mineralization of MG63 cells were inhibited, with the increase of the percentage of apoptosis cells. But the influence of insulin to the bone formation was not clear. Low dose of insulin would inhibit the secretion of ALP, and prompt the apoptosis, but hyperinsulinemia would prompt the secretion of ALP, and inhibit the apoptosis. Insulin would increase the proliferation rate, but couldn't influence the BGP secretion and the mineralization of the MG63 cells.

[Conclusion] In our study, we confirmed the toxicity of hyperglycemia to MG63 cells. It suggested that tight control of the serum glucose could be a good method to prevent the occurrence of osteoporosis in diabetes mellitus.

Conflict of Interest: None declared

P141-T

IDENTIFICATION OF NOVEL DIFFERENTIALLY EXPRESSED MEMBRANE PROTEINS BY QUANTITATIVE PROTEOMICS IN HUMAN MESENCHYMAL STEM CELLS UNDERGOING OSTEOBLAST DIFFERENTIATION

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One of the major limitations for understanding the biology of human mesenchymal stem cell (hMSC) is the absence of prospective markers needed for their isolation and monitoring of their differentiation. We have previously employed mass spectrometry (MS)-based proteomics in order to profile membrane proteins of hMSC. In this study, we employed Stable Isotope Labelling with Amino acids in Culture (SILAC), a method that allows sensitive quantification of large numbers of proteins simultaneously during osteoblast (OB) differentiation. We have furthermore employed Fourier Transform LC-ESI-MS-MS-MS that provides very high resolution quantitative data, including MS3 level fragmentation with extremely high confidence. SILAC, membrane protein enrichment and subsequently mass spectrometry was performed in the hMSC-TERT cell line at day 0, 1 and 4 during osteogenic induction by a mixture of dexamethasone, calcitriol, ascorbic acid and beta-glycerophosphate. We have identified a total number of 1144 different proteins of which 992 are membrane, membrane associated or GPI anchored proteins. A large number of different CD markers (n = 34) and low abundance GPI anchored proteins (n = 17) were identified demonstrating the high resolution of the identifications (e.g. CD71, Cd105, Cd166, CD44, Thy1, alkaline phosphatase). Upon osteogenic differentiation, one third of all identified proteins were quantified as upregulated (more than 2 fold) at day 4, one third down regulated (less than 50 percent of day 0 level) at day1 and one third exhibited no change. Some of these proteins are known osteogenic makers and are expected to increase e.g. alkaline phosphatase and collagen type I while others are new. We are currently verifying these new proteins. The nature and potential biological role will be presented.

Conflict of Interest: None declared

P142-S

EXPOSURE OF MC3T3-E1 OSTEOBLAST-LIKE CELLS TO ARACHIDONIC ACID, DOCOSAHEXAENOIC ACID AND PARATHYROID HORMONE MODULATES OSTEOPROTEGERIN AND RANKL SECRETION

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Bone is continuously remodeled through resorption by osteoclasts and the subsequent synthesis of bone matrix by osteoblasts. Cell-to-cell contact between osteoblasts and osteoclast precursors is required for osteoclastogenesis. RANKL expressed on osteoblastic cell membranes stimulates osteoclastogenesis, while osteoprotegerin (OPG) secreted by osteoblasts inhibits osteoclastogenesis. Although polyunsaturated fatty acids (PUFAs) have been implicated in bone homeostasis, the effects thereof on OPG and RANKL secretion have not been investigated.

In this study, MC3T3-E1 osteoblasts were exposed to the n-6 PUFA arachidonic acid (AA) and the n-3 PUFA docosahexaenoic acid (DHA) as well as the bone active hormone parathyroid hormone (PTH) and the effects thereof tested on OPG and sRANKL secretion. PGE2, a product of AA metabolism and previously implicated in bone homeostasis, was included in the study. For measurement of OPG and sRANKL, cells were precultured for 24h, exposed to vehicle (0.1% ethanol), PGE2 (10nM), PTH (10nM) AA or DHA at 2.5 to 20 μg/mL for 24h and the conditioned media harvested. In some cases 1 μM indomethacin, a cyclooxygenase blocker was added to growth media 45 minutes prior to addition of test substances. Three separate experiments were conducted (n = 4).

AA (5.0 to 20 μg/mL) decreased OPG secretion with 25% to 30%, possibly via PGE2 formation, as PGE2 alone also significantly reduced OPG secretion. Abolishment of the effect of AA by indomethacin, confirmed this observation. DHA at higher concentrations reduced OPG secretion, but concomitant exposure to indomethacin had a less prominent effect. The slight reduction of OPG by DHA could be due to endogenous PGE2 production, as DHA itself is not a substrate for PGE2 synthesis. The MC3T3-E1 cells secreted very low basal levels of sRANKL but AA stimulated sRANKL secretion thereby decreasing the OPG/RANKL ratio. No sRANKL could be detected after exposing the MC3T3-E1 cells to DHA. PTH did not affect OPG secretion but stimulated sRANKL secretion. This study demonstrates that AA and PTH reduce the OPG/sRANKL ratio and thereby may increase osteoclastogenesis. DHA, however, had no significant effect on OPG or sRANKL. To research this effect further, it is necessary to extend the study to include less differentiated cell lines such as primary rat/human osteoblasts.

This work was supported by the National Research Foundation (South Africa).

Conflict of Interest: None declared

P143-M

CONSEQUENCES OF ALTERED RHO/RHO KINASE SIGNALLING IN OSTEOBLAST DIFFERENTIATION

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We have previously reported that the unique bacterial protein toxin, Pasteurella Multocida Toxin (PMT), is a potent inhibitor of osteoblast differentiation and bone nodule formation of primary mouse calvarial cells. PMT is a potent mitogen and activates several intracellular signalling pathways, most notably the heterotrimeric G-proteins, Gq/11 and G12/13, leading to stimulation of the small GTPase Rho and its downstream effector Rho Kinase (ROCK). PMT also activates other pathways downstream of Gq such as PLC and PKC, in addition to the Ras/MAP kinase pathway. The mechanism of PMT inhibition of osteoblast differentiation is due to the activation of the Rho pathway since the block in bone nodule formation is rescued by Rho and ROCK inhibitors. Moreover, treatment of primary calvarial osteoblasts with Rho/ROCK inhibitors alone stimulated bone nodule formation, demonstrating that Rho/ROCK signalling is a critical regulator of osteoblast differentiation and bone formation.

We have analyzed the role of the Rho-ROCK pathway in osteoblast differentiation and bone formation using ex vivo organ culture experiments and transgenic mice overexpressing either constitutively active or dominant negative RhoA in osteoblasts using the 2.3-CollI promoter. Treatment of embryonic metatarsals and calvariae with the ROCK inhibitor, Y-27632, stimulated bone formation, consistent with the increase in bone nodule formation seen previously in vitro. In contrast, PMT treatment caused marked mitogenic effects in mesenchymal cells, resulting in an apparent reduction in calvarial osteogenesis. Interestingly, PMT-treated calvariae showed ectopic chondrocyte differentiation in the suture area, suggesting a possible effect of PMT/Rho signaling on mesenchymal stem cell commitment. Preliminary observations using Coll1-RhoV14 (constitutively active) and Coll1-RhoN19 (dominant negative) transgenic mice have indicated significant effects on in vitro CFU-OB formation. Paradoxically, whereas bone marrow stromal cells from RhoV14 mice exhibited a greater number of CFU-OB, stromal cells from RhoN19 mice showed a marked inhibition in CFU-OB formation compared to their respective non-transgenic littermates. The alterations in osteoblast differentiation were confirmed by RT-PCR analysis of osteoblast marker gene expression. Taken together, these results provide evidence of an important role of the Rho/ROCK pathway in osteoblast differentiation and bone formation.

Conflict of Interest: None declared

P144-T

ABSTRACT WITHDRAWN

P145-S

DECREASED OXIDATIVE CAPACITY AND INCREASED EXPRESSION OF PROAPOPTOTIC GENES IN MSC DURING AGEING IN VITRO

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Mesenchymal stem cells (MSC) are developed as targets for cell based therapeutic strategies in tissue engineering procedures for bone and cartilage regeneration. During ageing in vivo and in vitro MSC stop proliferating, lose their differentiation capacity, change their morphology and show signs of cellular senescence. We analyzed the expression pattern of proapoptotic genes and antioxidative selenoproteins in long-time cultures of MSC and investigated the influence of selenite (Se) supplementation on MSC ageing.

MSC obtained from five different subjects were cultured with and without 100 nM sodium selenite supplementation until they stopped proliferation. The expression profile of selenoproteins on mRNA and enzymatic levels during ageing was investigated. The expression of apoptotic markers (e.g. transglutaminase (TGM) and the activities of caspases 3 and 7 were also determined and the influence of Se supplementation on MSC proliferation was analyzed.

Expression of thioredoxin reductase (TrxR) was elevated while expression of glutathione peroxidase (GPx) decreased during ageing at mRNA and enzymatic levels. The response of enzyme activities to Se supplementation was enhanced during ageing. The expression of selenoprotein P (SeP), which is a selenite storage protein, decreased in Se-deficient cultures providing a putative explanation for this enhancement. SeP expression could be restored by Se supplementation. Analyses of apoptotic markers like TGM revealed that in Se deficiency the disposition for apoptosis is elevated although we could not find an influence of Se supplementation on caspase activities. Se supplementation had no influence on cell proliferation and population doubling rates of MSC.

We have previously shown that the antioxidative capacity of MSC is altered in vitro under conditions of Se deficiency (e.g. conventional media), which leads to DNA and cell damage. Se supplementation of MSC cultures restored the antioxidative capacity and prevented the formation of micronuclei. TrxR and GPx are antioxidative Se dependent enzymes, while the former is also reported to facilitate apoptosis. We show here that during long-time cultures of MSC cells get Se deficient. GPx expression decreases, while in contrast TrxR activity increases even in Se deficient cultures. TGM is enhanced in Se deficient cultures only. This would indicate that a proapoptotic condition in ageing "presenescent" MSC is facilitated under conditions of Se deficiency.

Conflict of Interest: None declared

P146-M

DOWN REGULATION OF BETA-CATENIN AND TRANSDIFFERENTIATION OF HUMAN OSTEOBLASTS TO ADIPOCYTES UNDER ESTROGEN DEFICIENCY

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Postmenopausal osteoporosis, caused by estrogen deficiency, is characterized by the structural deterioration of bone accompanied by an increase in bone marrow adipocytes. Transgenic animal models have shown that there is a reciprocal relationship between osteoblastogenesis and adipogenesis in vivo. This study investigated whether the estrogen and the canonical Wnt signaling pathways are linked together and regulate the phenotype and function, differentiation and proliferation of human osteoblasts using an in vitro estrogen deficiency model. Human osteoblasts (hFOB 1.19) and fulvestrant, an estrogen receptor blocker, were used to mimic estrogen deficiency in vitro. Osteogenic and adipogenic differentiation was measured by using specific stains and microscopy, as well as by measuring expression of bone cell specific markers with rtPCR. Expression of estrogen receptor alpha and beta-catenin was detected in Western blots, and by immunoprecipitation. The cells expressed the 46 kDa and the 77 kDa estrogen receptor alpha isoforms and beta-catenin. Fulvestrant reduced expression of estrogen receptor alpha and beta-catenin. Beta-catenin was co-immuno precipitated with estrogen receptor alpha, indicating that these two proteins form a new signalling complex and transcription factor. In addition, it induced intracellular lipid droplet accumulation and down-regulation of bone cell markers, indicating adipocyte differentiation.

In conclusion, we can say that estrogen receptor alpha may interact with beta-catenin and form a new transcription factor complex supporting osteoblast differentiation.

Conflict of Interest: None declared

P147-T

RANKL ISOFORMS INFLUENCE ON THE OPG AVAILABILITY

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The OPG/RANK/RANKL (Osteoprotegerin/Receptor Activator of Nuclear Factor kB/RANK Ligand) triad is involved in various osteolytic pathologies. While many studies described the use of OPG during the treatment of bone diseases, its bio availability and the mechanism by which the cells control the extracellular OPG remain uncertain. The present work uses cellular models to assess the role of RANKL isoforms in the bio availability of exogenous OPG. The human kidney cell line 293 which initially express neither OPG nor RANKL was stably transfected with different isoforms of mouse RANKL – full length (293RL) – with a truncated intracytoplasmic domain (293RL 2.0) – without intracytoplasmic domain (293RL 2.1). Flow cytometry studies showed that an intracytoplasmic domain is necessary for the membranous localization of RANKL (293RL and 293RL 2.0). Osteoclastogenesis is obtained when 293RL and 293RL 2.0 cells are co-cultured with RAW 264.7 cells in contrast to 293 and 293RL 2.1 cells, thus pointing out that the membranous localization of RANKL is essential for its biological activity. When OPG is incubated with 293RL or 293RL 2.0 cells, the extracellular concentration of OPG was strongly decreased in a time dependent manner. The OPG disappearance, which was not inhibited by the addition of several protease inhibitors thus excluding any extracellular protease degradation, was abolished by an antibody against RANKL. Confocal microscopy demonstrated an internalization of OPG mediated by membranous RANKL. Western blotting analysis revealed that the half life of RANKL was greatly reduced in the presence of OPG, demonstrating that OPG binding to RANKL induces an enhancement of the ligand internalization. In light of these results, the inhibitory effect of OPG on bone resorption can be explained not only by a decoy receptor function, competitor inhibitor of the RANK/RANKL binding, but also by the modulation of the RANKL half-life induced by OPG. Reciprocally, this modulation contributes to reduce the bio availability of OPG.

Conflict of Interest: None declared

P148-S

CAMKIBETA MRNA IS EXPRESSED IN THE GROWTH PLATE AND INHIBITS PROLIFERATION OF PRIMARY OSTEOBLASTS IN CULTURE

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Background/Aims: Calcium is a universal second messenger and is necessary for cell growth and division, and for gene expression. The Ca^{2+}/calmodulin dependent protein kinases (CaMKs) mediate many of the effect of Ca^{2+}. Our group has previously cloned the CaMKIβ from a hypothalamus subtraction cDNA library (Gautvik et al., PNAS, 1996). The purpose of this project was to study the expression pattern of CaMKIβ mRNA in bone and cartilage and to assess its possible function.

Methods/Results: By using *in situ* hybridization, CaMKIβ mRNA was expressed in mouse skeletal tissue especially in the growth plate of long bones and in hypertrophic chondrocytes. In mouse hind limbs the expression of CaMKIβ was first observed from 14.5 embryonic day and showed a high sustained expression until day 10 after birth. Electron microscopy and immunogold labelling showed the same distribution of the protein. CaMKIβ was also expressed in chondrocytes and osteoblasts *in vitro*, as shown by RT real-time PCR analysis. The knock-down of the CaMKIβ mRNA by siRNA (24h of treatment) significantly decreased the proliferation of primary osteoblasts, as demonstrated by more than 50% reduction of [3H]-thymidine incorporation compared to untreated cell cultures ($p < 0.05$). Preliminary RT real-time PCR data indicated a significant lower expression of c-fos gene in the CaMKIβ siRNA treated osteoblasts compared to untreated controls ($p < 0.05$), but Runx-2, Osterix, Osteocalcin, ALP and Sox-4 mRNAs were not affected.

Conclusions: CaMKIβ mRNA is highly expressed in the growth plate and in hypertrophic chondrocytes both pre- and postnatally, and can be demonstrated in primary mouse osteoblasts and human chondrocytes cultures. The knock-down of CaMKIβ gene expression inhibits proliferation of primary osteoblasts

and decrease c-fos mRNA levels. The present data, showing high expression of CaMKIβ in skeletal tissue, suggest a role in chondrocyte maturation and in osteoblast development.
Conflict of Interest: None declared

P149-M

IDENTIFICATION OF A FUNCTIONAL GENE INVOLVED IN MURINE BONE MARROW MESENCHYMAL CELL DIFFERENTIATION INTO OSTEOBLASTS

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Bone marrow-derived mesenchymal stem cells (MSCs) are adherent cells that differentiate into chondroblasts, osteoblasts or adipocytes. One important challenge is to identify factors and pathways that can promote the osteogenic commitment of MSCs in order to use MSCs for optimal bone repair. In this context, genomic analyses may help to identify molecules that could be used to promote osteogenic differentiation of MSCs. To this goal, we used microarray analysis to identify genes that are upregulated during osteoblast differentiation of an established murine bone marrow stroma-derived cell line (BMC10) which exhibits osteogenic characteristics in vitro and in vivo (Dennis et al., 1999). Cells were cultured in DMEM supplemented with 10% heat inactivated FCS and treated with dexamethasone (Dex, 10^{-7}M) for up to 3 days. Osteoblast differentiation was checked by histochemical staining for alkaline phosphatase. Total RNAs were collected and used for microarray hybridizations using cDNA arrays consisting of 20,000 clones. The results of 2 separate experiments showed that 92 genes were upregulated during osteoblast differentiation induced by Dex. Using the Ingenuity software, we found that several networks can connect the identified upregulated genes. Some of the genes that were upregulated by Dex in BMC10 cells include genes such as FHL2 that was recently found to regulate directly or indirectly bone formation. Quantitative RT-PCR analysis confirmed that Dex promoted the expression of FHL2 together with the expression of the osteoblast markers Runx2 and alkaline phosphatase (ALP) in BMC10 cells, thus validating the microarray analysis. Consistent with the Dex-induced upregulation of these genes, glucocorticoid sequences were found in this gene. Transfection with a FHL2 vector (kindly provided by Prof. R. Schuele, University of Freiburg, Germany) increased ALP staining and Runx2 expression in BMC10 cells. Consistent with these data, overexpression of FHL2 in clonal tripotent human bone marrow stromal F/STRO1 + A cells upregulated Runx2, type I collagen and ALP gene expression, showing that FHL2 promotes osteoblast differentiation in both murine and human MSCs. Altogether, these results identify a functional role for FHL2 gene in osteoblast differentiation of bone marrow stromal mesenchymal cells induced by dexamethasone. This may result in the development of novel therapeutic strategies to promote the differentiation of human MSCs towards the osteoblast lineage.
Conflict of Interest: None declared

P150-T

THE EXPRESSION OF TWO-PORE DOMAIN POTASSIUM (K2P) CHANNELS IN OSTEOBLASTIC CELLS

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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 to pH changes, and in mechanotransduction (1). Recently, one member of the K2P family, TREK-1, has been reported to be present in human osteoblasts (2). This channel may be involved in setting the resting membrane potential in these cells, and possibly also in mechanotransduction. However, the presence and role of other K2P channels in bone cells remains unknown.

In order to better understand K2P channel expression in bone cells, we have systematically screened for the presence of these channels in the rat-derived UMR-106 osteoblast-like cell line using RT-PCR. Primers were designed to identify all known K2P channels. We have detected mRNA for TASK-1, TRAAK and TWIK-2 channels in UMR-106 cells. Although most of the work done on K2P channels so far has been conducted using neuronal cells, their properties have been fairly well documented. TASK-1 was found 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 (3).

Possible roles for these channels in vivo include involvement in pH-sensitive and mechanosensitive bone remodelling. Further work aimed at clarifying the significance of the RT-PCR results using immunocytochemistry and electrophysiology is on-going. Functional examination of this family of channels is awkward due to their insensitivity to classical K + channel blockers. Our work will also include identifying possible splice variants of these channels.

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P151-S

MECHANICAL STIMULATION OF A SINGLE OSTEOCYTE INDUCE INTERCELLULAR CALCIUM WAVES THROUGH THE OSTEOCYTE NETWORK

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Osteocytes are the most abundant bone cells and are ideally located in the mineralized bone matrix where they form a gap-junctions linked cellular network. Osteocytes are thought to sense and transmit mechanical signals throughout bone, thus regulating especially loading induced bone remodeling. However, we still lack direct evidence to demonstrate what kind of molecular signals can be transmitted within osteocyte network. We have previously shown that isolated primary osteocytes could form a cellular network in vitro, and that they express connexin 43 and form functional gap junctions. In order to test the hypothesis that mechanical loading of a single cell can induce cellular response throughout the osteocyte network, we followed a spread of calcium wave induced by local mechanical stimulation. Primary osteocytes were isolated from rat bones and were cultured on the bottom of glass dishes or glass coverslips for 2 days as described earlier (Gu et al. 2006). Cells were loaded with Fura 2. Imaging was performed with the Olympus MT 20 imaging system. After a single cell was stimulated mechanically with a glass microinjector tip, which was fixed to a micromanipulator system, appearance and spread of calcium waves were followed and recorded at the wavelength of 340 nm and 380 nm respectively. In order to study the role of gap junction in the regulation of intercellular calcium waves through a cellular network, gap junction specific inhibitor (AGA) was added 2 hours before stimulating the cells and 3 μM of AGA was present all the time during the following experiments. Our data clearly demonstrate that the calcium wave can be stimulated by mechanical loading of a single cell and transmitted to the adjacent cells in the cellular network of cultured primary osteocytes. Whenever gap junctions were blocked, calcium signaling was also inhibited in the cellular network. These results indicate that intercellular calcium waves can be initiated by mechanical stimulation of a single osteocyte, thus suggesting that even very small and local loading of cellular network could induce various cellular responses through a large number of cells.
Conflict of Interest: None declared

P152-M

MORPHOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF BONE PHENOTYPE IN A CHONDRODYSPLASIA MOUSE MODEL

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Mutations in the *diastrophic dysplasia sulfate transporter (DTDST)* gene cause a family of recessively inherited chondrodysplasias that includes diastrophic dysplasia (DTD). This gene encodes for a sulfate/chloride antiporter expressed on the surface of many cell types (including osteoblasts), whose function is crucial for the uptake of inorganic sulfate that is needed for proteoglycan sulfation. We have recently generated a mouse model for diastrophic dysplasia (dtd mouse); dtd mice show a partial loss of function of the DTDST, causing a chondrodysplastic phenotype that recapitulates essential aspects of DTD phenotype in man.

We analysed long bones from 1 (n = 12) and 2 (n = 6) month old dtd and wild-type mice in order to better characterize bone phenotype, which has never been studied in DTD patients. Older animals were not studied since survival is reduced in dtd mice.

Mice received two fluorochromes by intraperitoneal injection at set intervals of time and they were killed the day after the last injection. Tibiae and femora were dissected, cleaned from soft tissues and stored in 70% ethanol. Femora were then dehydrated, defatted and embedded in methyl methacrylate. We performed DXA (PIXimus) and X-ray analyses on tibiae and histomorphometric analysis on femora.

Alterations in the bone phenotype of dtd mice were observed and confirmed by statistical analysis.

DXA and X-ray analyses showed that, at all the age points considered, long bones of dtd mice are shortened ($p < 0,0001$) and more bowed when compared to wild-type animals, with significantly decreased BMC ($p < 0,0001$) and BMD ($p < 0,05$).

Static histomorphometry showed that dtd mutation has a significant effect on trabecular architecture with reduced trabecular bone volume ($p < 0,05$) and increased trabecular spacing ($p < 0,005$) at all the age point considered. Trabecular thickness and cortical thickness were significantly decreased only in one month old mice ($p < 0,05$). Dynamic histomorphometry indicated that mineralizing surfaces as well as mineral apposition rate and bone formation rate were not affected. Furthermore, the osteoclast number per trabecular bone surface in our mouse model was normal.

These data indicate that there is no primary osteoblastic defect in our mouse model and suggest that the observed bone phenotype might be a consequence of increased osteoclastic activity.

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Conflict of Interest: None declared

P153-T

ISOLATION OF OSTEOPROGENITOR CELLS FROM HUMAN BONE MARROW-DERIVED MESENCHYMAL STEM CELLS AND DEFINING THEIR MOLECULAR SIGNATURE

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Human mesenchymal stem cells (hMSC) are a heterogeneous group of cells with respect to their differentiation potential in vitro and in vivo. Currently, the nature of subpopulations of MSC with in vivo bone forming capacity (termed here osteoprogenitor cells) is not known. Thus, in order to identify osteoprogenitor cells among MSC, we studied proliferation and differentiation heterogeneity of hMSC at a single cell level. By using the limiting dilution cloning procedure, we obtained 91 single cell clones from our previously established telomerized hMSC cells (hMSC-TERT) and among these 12 clones were selected for extensive characterization. Substantial differences in morphology, growth rate and differentiation ability were observed among various clones. Furthermore, clones exhibited significant differences in their capacity to differentiate into adipocytes and osteoblasts in vitro and to form ectopic bone in vivo when transplanted subcutaneously mixed with hydroxyapatite/tricalcium phosphate (HA/TCP) ceramics into immune-deficient mice. Interestingly, we found that in vitro growth rate (negatively correlated) but not in vitro mineralization ability to be predictive for in vivo bone formation capacity. In addition, based on DNA microarray analysis, we identified several gene clusters that were predictive for in vivo bone formation ability of the cells including several genes involved in skeletal development, extracellular matrix genes and immune response genes. Our results corroborate that MSC represent a heterogeneous populations of cells with various differentiation potentials. The presence of clusters of genes predictive for in vivo bone formation represent a molecular signature to be used in identifying and isolation of osteoprogenitor cells from the heterogeneous MSC and also to monitor the osteoblastic nature of MSC to be used in clinical settings.

Conflict of Interest: None declared

P154-S

MODULATION OF THE RESORPTION SURFACE BY INHIBITORS CHANGES OSTEOBLAST ADHESION

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Previous studies have demonstrated that osteoblasts preferentially attach in resorption lacunae. Patients with osteopetrosis due to defective acidification of the resorption have low resorption, but normal or increased bone formation. In contrast, patients with pycnodysostosis, due to defective cathepsin K, have low resorption and disorganized bone formation leading to poor bone quality. A major difference between these phenotypes is the modulation of the pit surface.

To investigate whether the differences in bone formation, observed in osteopetrotic and pycnodysostotic patients, were due to differences in the resorption surface we investigated osteoblast adhesion to resorbed bone surfaces and expression of proteins likely to be involved in signaling from osteoclasts to osteoblasts, such as TRACP and osteopontin.

Mature human osteoclasts were cultured on bone slices in the presence or absence of Bafilomycin A1 and E64, which inhibit the V-ATPase and cysteine proteinases (cathepsin K) respectively. The osteoclasts were then either fixed in formaldehyde for immunocytochemistry, or removed by washing for the osteoblast adhesion experiments. Osteoblast adhesion was investigated by seeding MC3T3-E1 preosteoblastic cells on the bone slices for 8 hours, and then by scoring the number of cells attached inside and outside the resorption pit.

Treatment with both inhibitors reduced CTX-I release. We found that treating the osteoclasts with bafilomycin, but not E64, led to accumulation of TRACP in the resorption pits. In addition, we found that treatment with E64 led to relocalization of osteopontin from the pits to the edges of the resorption pits, whereas bafilomycin treatment did not change the localization.

We also found that the bafilomycin reduced the attachment of osteoblasts to the resorbed surface by 75%, and that E64 reduced attachment to the resorbed surface by 40%, however in the bafilomycin case this was likely due to a dramatic reduction in the pit area, whereas for E64 the pit area was increased.

In conclusion, we found evidence supporting the findings that different forms of modulation of resorption have different effects on the subsequent resorption, potentially explaining the reduction in bone quality observed in the pycnodysostosis patients.

Conflict of Interest: None declared

P155-M

P2X7 RECEPTORS ARE IMPORTANT IN CELL SIGNALING AND PORE FORMATION IN MATURE PRIMARY HUMAN OSTEOBLASTS

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The ionotropic ATP-gated P2X7 receptor is primarily expressed in hematopoietic cells, but also in primary human osteoclasts (OC), where it is involved in signaling to OC from osteoblasts and among OC. Thus the receptor is involved in the regulation of osteoclast function, and subsequently in the regulation of bone metabolism. However, several studies have addressed the function and expression of the receptor in osteoblastic cells but with conflicting results. The aim of this study was to assess the expression of the P2X7 receptor in primary osteoblasts and to determine the function in relation to cell signaling and cell function in these cells. Bone marrow derived stromal cells (BMSC) (not fully differentiated osteoblasts) were derived from human bone marrow by gradient centrifugation, and culturing the adherent cell population. Osteoblasts (HOB) derived from human trabecular bone are the most differentiated osteoblasts and were obtained from orthopedic surgical procedures. Bone chips were washed, digested with collagenase and cultured. P2X7 expression was determined on the RNA level (RT-PCR) and on the protein level (immunostaining). Receptor function was assessed as measuring calcium transients upon ligand stimulation (using the calcium indicator dye Fura-2 and stimulating with the P2X7 agonist benzoylbenzoyl-ATP (BzATP)), mechanically induced intercellular calcium waves (ICW), and dye uptake upon agonist stimulation, using YO-PRO as a large-molecule dye. RT-PCR showed expression of P2X7 mRNA in BMSC and HOB, but the receptor was only detected on the cell membrane in the HOB. Agonist stimulation revealed no response on calcium concentrations in BMSC, while HOB responded with an increase indicating the presence of functional P2X7 receptors. ICW were elicited by mechanical stimulation in HOB and P2X7 receptors were involved in ICW propagation in addition to gap junctional communication and P2Y receptors. Finally, stimulation with BzATP induced uptake of YO-PRO in the HOB indicating that P2X7 receptor activation induces conformational changes in the receptor leading to pore formation which has been shown to be related to initiation of apoptosis in other cell types. In conclusion, we have demonstrated that functional P2X7 receptors are expressed only in the most mature osteoblasts and that these receptors are involved in cell signaling and in pore formation, an event that might be related to regulation of cell survival.

Conflict of Interest: None declared

P156-T

OXYGEN TENSION IS AN IMPORTANT MEDIATOR OF THE TRANSFORMATION OF OSTEOBLASTS TO OSTEOCYTES

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Osteocytes are derived from osteoblasts, but reside in the mineralized bone matrix without direct blood flow. It has been reported that MLO-Y4, osteocyte-like cells show higher expression of ORP150, which is induced by hypoxia, than osteoblast-like cells. Accordingly, we hypothesized that the oxygen tension may regulate the transformation of osteoblasts to osteocytes. MC3T3-E1 cells and calvariae from 4-day-old mice were cultured under normoxic (20% O2) or hypoxic conditions (5% O2). To investigate osteoblastic differentiation and transformation to osteocytes, Alkaline phosphatase (ALP) staining and alizarin red staining were done and expression of various factors (osteocalcin, DMP1, MEPE, FGF23, Cx43, ORP150) was assessed by real time RT-PCR, ELISA

analysis, western blot analysis, immunocytochemical analysis, and immunohistochemical analysis. Hypoxic culture promoted the increased synthesis of mineralized matrix by MC3T3-E1 cells. ALP activity was increased initially during hypoxic culture, but decreased during osteogenesis. Osteocalcin production was also increased by hypoxic culture, but decreased after mineralization. Furthermore, expression of DMP1, MEPE, FGF23, and Cx43, which are osteocyte-specific or osteocyte-predominant proteins, by MC3T3-E1 cells was greater under hypoxic than normoxic conditions. In mouse calvarial organ cultures, the number of cells in the bone matrix and cells expressing DMP1 and MEPE were increased by hypoxia. In MC3T3-E1 cell cultures, ORP150 expression was only detected in the mineralizing nodules under normoxic conditions, while its expression was diffuse under hypoxic conditions, suggesting that the nodules were hypoxic zones even in normoxic cultures. These findings suggest that low oxygen tension promotes osteoblast maturation and subsequent transformation to osteocytes.
Conflict of Interest: None declared

P157-S

SEPARATE EFFECTS OF PRESSURE AND FLUID FLOW ON BONE CELLS IN VITRO

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Bone is subjected to repetitive loads, which cause deformation of the matrix and fluid flow through the lacunar–canalicular system. It has been hypothesised that, instead of the deformation of the matrix itself, fluid flow through the bone canaliculi could produce enough shear stress to stimulate bone cells and induce bone remodelling. The existing commercial models to study the effects of fluid flow on cells have been reported to apply pressures from 4 kPa up to 6 kPa. Considering the elastic modulus of a bone cell is reported to be $\sim$ 1 kPa, this pressure inside the chamber could be partly responsible for the responses observed in these fluid flow systems. We measured pressure in three types of flow chambers we routinely use in our laboratory. These are two parallel plate flow chambers and an Ibidi(R) microSlide chamber. Pressures were measured with a manometer and a pressure transducer, at different flow speeds that generate shear stresses of 1, 1.5, 2 and 2.5 Pa. In a traditional parallel plate system we measured pressure at the inlet and outlet and found, depending on the shear stress produced, an initial increase in pressure of 3 to 9 kPa, as well as a pressure drop across the chamber of 0.06 to 4 kPa respectively. Since these pressures are significantly higher than the cell modulus, we developed a new fluid flow system setting, that results in reduced pressure and microstrain compared to most fluid flow systems currently in use. In our system the initial increase in pressure is between 0.05 and 0.38 kPa. We have also designed a system in which we can apply pressure only to cells. To test the response of bone cells in both systems, we use immunofluorescent staining for β-catenin, which we and others have found translocates reliably to the nucleus in response to mechanical stimulation using the earlier flow systems. This translocation is an early indication that mechanical stimulation may directly influence gene expression. Our data now demonstrates flow alone can induce this translocation, but studies are underway to see if pressure induces a similar response. This research is supported by the Oliver Bird Rheumatism Programme of the Nuffield Foundation.
Conflict of Interest: None declared

P158-M

BIOACTIVE PEPTIDES POSSESS BONE POTENTIAL BY IMPROVING OSTEOBLAST FUNCTION

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Biomaterial tissue engineering has used bioactive molecules, such as Arg-Gly-Asp (RGD), for surface coating to direct tissue formation. RGD promotes osteoblast cell attachment, proliferation, differentiation, and mineralized matrix formation and arrests osteoclast bone resorption enabling efficient bone formation. Similar short amino acid chains are produced when dietary protein is hydrolysed in the GI-tract. Such bioactive peptides have various in vivo functions. Our aim was to study the effects of common food derived bioactive peptides, IPP, VPP, and LKP on osteoblast gene expression and function in vitro. We used UMR-106 cells, human mesenchymal stem cells (hMSC), and osteoblasts differentiated from hMSC. Microarray analysed short-term effects of IPP, VPP, and LKP showed increased osteoblast gene-expression, also cell proliferation was increased. Principal component analysis of the data showed especially IPP to increase the expression of genes related to osteoblast differentiation, cell growth, and viability. The results were confirmed with qPCR. Long-term treatment with IPP increased the expression of genes related to matrix production and decreased apoptosis related caspase-8. Furthermore RANKL/OPG ratio was reduced and bone mineralization was increased by IPP. Our results show that bioactive peptides stimulate pathways resulting to increased osteoblast function and bone formation. Agents that increase the number and function of osteoblasts can improve bone mass and structure and decrease fracture risk. Bioactive peptides such as IPP may well conduct some of the positive effects dietary protein has on bone.
Conflict of Interest: None declared

P159-T

NITROGEN-CONTAINING BISPHOSPHONATES INHIBIT BONE MINERALIZATION IN VITRO VIA A MECHANISM INDEPENDENT OF INHIBITION OF PROTEIN PRENYLATION

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Bisphosphonates (BP) are the most widely used therapeutic agents for the management of skeletal disorders such as Paget's disease of bone, postmenopausal osteoporosis and metastatic bone disease. Although BP are very effective anti-resorptive drugs, they are also known to blunt bone formation and interfere with the anabolic effect of parathyroid hormone. Here we examined the effects of BP on osteoblast growth and function in vitro. The nitrogen-containing Bisphosphonates (N-BP) Alendronate (ALN), Pamidronate (PAM) and Risedronate (RIS) inhibited survival and induced apoptosis of osteoclasts and osteoblasts with equal potency in a dose dependent manner at concentrations of 20–100 μM, whereas no effects on either cell type were observed at BP concentrations below 10 μM. The order of potency in stimulating apoptosis was: PAM > RIS > ALN in both cell types. All three BP caused accumulation of unprenylated Rap1A, stimulated sustained p38 and JNK phosphorylation, and activated Caspase-3 in osteoblasts and osteoclasts at 50 μM. Neither the p38 inhibitor SB203580 (10 μM) nor the JNK inhibitor SP600125 (10 μM) was capable of preventing BP-induced Caspase-3/7 activation or apoptosis. Further studies showed that ALN, PAM and RIS strongly inhibited bone nodule formation at 0.5 μM. The order of potency of the BP tested was as follows; PAM = RIS > ALN, different from their inhibitory effect on protein prenylation and their well-documented binding affinity for bone minerals. Although we found that N-BP inhibited protein prenylation in vitro (1 μM) and in vivo (0.1mg/kg), inhibition of nodule formation was not prevented following rescue with Farnesyl pyrophosphate (10 μM). Furthermore, other inhibitors of protein prenylation such as Mevastatin (1–2 μM), FTI (0.5 μM) and GGTI (1 μM) had no effect on bone nodule formation, indicating a mechanism independent of inhibition of protein prenylation. We conclude that the nitrogen containing bisphosphonates inhibit osteoblast growth and promote osteoblast apoptosis at similar concentrations as those required to inhibit osteoclast activity. These effects appear to be mediated by targeting the mevalonate pathway. However, the inhibitory effects of the N-BP on bone nodule formation seemed independent of inhibition of protein prenylation and were not obviously related to the affinity of the compounds for hydroxyapatite binding suggesting that bisphosphonates may exert inhibitory effects on osteoblast differentiation by a novel mechanism.
Conflict of Interest: None declared

P160-S

PHOSPHATE STIMULATES MATRIX GLA PROTEIN EXPRESSION IN OSTEOBLASTS: ROLE OF ERK1/2 AND FRA-1

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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 cells and primary calvaria-derived osteoblasts (OB) were used. MGP and Fra-1 expression was examined by real-time PCR and Western Blot. The activation and role of signaling pathways were respectively determined by Western Blot and the use of specific inhibitors. Chromatin Immunoprecipitation (ChIP) analysis was used to examine the in vivo binding of Fra-1 to the MGP promoter.

In MC3T3-E1 cells, we first evaluated the expression of MGP mRNA as a function of osteoblast differentiation. 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 MC3T3-E1 cells. Pi was also found to trigger a significant increase in MGP expression in

OB. The expression pattern of Fra-1 in MC3T3-E1 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 MC3T3-E1 cells and OB. UO126, a specific inhibitor of the ERK pathway, blocked Pi-stimulated Fra-1 and MGP expression, indicating that ERK1/2 mediates Pi effects. Finally, to identify a possible relationship between Pi, Fra-1, and MGP, we questioned whether Pi could stimulate the binding of Fra-1 to the MGP promoter in MC3T3-E1 cells. Our ChIP results indicate that Pi significantly increased the binding of Fra-1, suggesting that this transcription factor could regulate MGP expression in response to Pi.

These data established for the first time that Pi may regulate MGP expression in osteoblasts via the ERK1/2-Fra-1 pathway. To further demonstrate the role of Fra-1 in Pi-stimulated MGP expression, we are currently using osteoblasts from Fra-1 knockout mice.

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P161-M

ULTRASTRUCTURAL STUDIES ON THE CENTRAL DARK LINE AND OCTACALCIUM PHOSPHATE

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It is important to establish the mechanism of crystal nucleation process for assessing the relationship between harmful chemical substances such as fluoride ions and the bone disease accompanying the crystal defects. However, many researchers still consider the central dark line (CDL) as a central planar defect based on their belief that this line might consist of a single unit cell thickness of octacalcium phosphate (OCP) embedded in an apatite lattice in mature crystals. Recently we have demonstrated that the thermal properties of CDL are different from those of OCP and we would like to provide further evidence for differentiating CDL from OCP. In this study, we have tried to demonstrate morphologically the presence of OCP in the hard tissues other than the vertebrates. Also, we compared the CDL-bearing pathway in the bone with the OCP-converting pathway for apatite crystal formation. By electron microscopy, the presence of lattice images compatible with that of OCP were confirmed in both the invertebrate hard tissues and the dental calculus. Furthermore, the conversion of OCP into its apatite form was observed. In the case of the CDL-bearing pathway, crystal growth took place directly, not via OCP conversion. Our findings suggest that the mechanism for crystal formation in the vertebrate hard tissues is absolutely different from the OCP pathway in the invertebrates. Also, we expect that our calcification model may shed light on the relationship between the harmful chemical substances and the endemic bone disease including osteoporosis. This work was supported by the Frontier Science Promotion Program at Nihon University.

Conflict of Interest: None declared

P162-T

REDUCED PLASMA MEMBRANE ACCESS OF ANK CAUSES CRANIOMETAPHYSEAL DYSPLASIA

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Heterozygous mutations in the ANKH gene, the ortholog of the murine ankylosis (Ank) gene, cause craniometaphyseal dysplasia (CMD). The CMD phenotype is characterised by a striking sclerosis of the skull and reduced modelling of the long bones. ANK, the gene product of ANKH, is a transmembrane protein assumed to shuttle the mineralisation inhibitor pyrophosphate (PPi) from the cytosol to the extracellular space. It is currently unclear how the hyperostosis observed in CMD might be caused by altered extracellular PPi concentrations.

To get insight into the molecular basis of the CMD phenotype we established a transgenic mouse line (TgC1AnkF377Del) which expresses Ank harbouring the CMD mutation F377Del in osteoblasts under the control of the 2.3 kb ColI promoter. Histomorphometry and μCT analysis revealed a two-fold increase of trabecular BV/TV and trabecular number in femora of 4 month old female mutant mice. Dynamic histomorphometry of femoral cortical bone showed no difference in bone formation rates.

Complementary in vitro experiments using RCAS retroviral expression of WT and mutant Ank in primary chick osteoblasts revealed no effect on alkaline phosphatase activity. In contrast, the inhibitory effect of Ank expression on matrix mineralisation was absent in all three CMD mutations tested (C331R, F377Del, G389R). In line with this finding, cell surface biotinylation of RCAS-infected chick osteoblasts revealed diminished plasma membrane accessibility of the G389R mutant, while mutants C331R and F337Del were practically absent from the cell surface.

Taken together, our data implies a dominant negative loss of function effect of CMD mutations. On the other hand, we could show that expression of a CMD mutation in osteoblasts causes increased bone mass in vivo without elevated bone formation rates, most likely by indirectly influencing osteoclast resorptive activity.

Conflict of Interest: None declared

P163-S

GROWTH DEFECT, REDUCED MINERALIZATION AND TIBIAL BOWING IN A MOUSE MODEL FOR NEUROFIBROMATOSIS TYPE 1

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Besides the typical skin manifestation neurofibromatosis type 1 (NF1; von Recklinghausen disease) comprises skeletal symptoms in app. 50% of cases. These include sphenoid wing dysplasia, kyphoscoliosis, reduced bone mineral density, mild short stature, tibial bowing and pseudarthrosis. The mouse models for this disorder available so far do hardly reproduce this skeletal phenotype. In order to investigate the role of Nf1 in long bone development we inactivated the Nf1flox conditional allele in the limb bud mesenchyme and in the calvarial bone by crossing with the Prx1cre mouse line.

The long bones of the resulting Nf1Prx mutants were shorter, which correlated with a diminished growth plate width and a reduced chondrocyte proliferation. As in many NF1 patients tibial bowing was observed in Nf1Prx mutants. This was most likely due to a reduced calcium content of the cortical bone, measured by back scattered electron microscopy, and an increased porosity. A dysbalance of bone matrix formation and mineralization was also indicated by an increased cortical osteoid thickness and elevated osteoblast numbers. Increased osteoclast numbers at the chondro-osseous junction and caused a 70% reduction of the trabecular bone. Moreover, osteoclast resorption most likely contributed to the decreased growth plate width and resulted in complete erosion of the growth plate at 6 weeks of age. Additionally, variable joint fusions were observed highlighting a hitherto unknown role of Nf1 in joint formation.

In summary, Nf1Prx mice recapitulate different aspects of the human NF1 bone phenotype, which will help to unravel the underlying molecular mechanisms that might be influenced by novel therapeutic strategies.

Conflict of Interest: None declared

P164-M

SCIATIC NERVE SECTION DECREASES CALLUS RESPONSE TO LOW INTENSITY PULSED ULTRASOUND

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Background: Low Intensity Pulsed Ultrasound (LIPU) has been shown to accelerate fracture healing, but the precise mechanism is still unknown. Innervation in bone has been found to play an important role in fracture healing. The relationship between callus innervation and LIPU on fracture healing was investigated in this study.

Methods: A diaphyseal transverse fracture was created on the right tibia in 80 matured female Sprague-Dawley rats. They were assigned randomly into 4 groups: the sham LIPU neural intact group, the LIPU neural intact group, the sham LIPU neurectomy group and the LIPU neurectomy group. Sham LIPU/LIPU was given daily to the fracture site starting on day 1 post-fracture. Resection of the sciatic nerve/patella tendon of the fractured limb was performed on the animals in the neurectomy/neural intact groups. Rats were sacrificed for pQCT and tissue morphometrical analyses on days 7, 14 and 21 post-fracture. Two-way ANOVA was used for statistical comparisons among the four groups ($\alpha = 0.05$).

Results: Significant interaction ($p < 0.05$) was found on total bone mineral density (BMD) between the two factors (innervation and LIPU). Results on the Bonferroni's multiple comparison test suggested that the introduction of LIPU could significantly increase the total BMD in the fractured callus ($p < 0.05$) in the neural intact limbs. However, such an increase in total BMD, caused by the introduction of LIPU, was not found in the limbs with sciatic neurectomy. Results on morphological studies indicated that groups with intact nerves healed better and faster than those without, and that a more matured callus was found in the LIPU-treated group when compared with the sham treated group in the

groups with intact nerves. The rate of maturation was similar in the two groups with nerve resection.

Conclusion: Results of the present study imply the important role of the callus innervation in sensing and responding to the mechanical stimulus generated by LIPU on fracture healing.

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Conflict of Interest: None declared

P165-T

EFFECTS OF IONIZING RADIATION ON MURINE OSTEOBLASTIC CELL DIFFERENTIATION AND GENE-EXPRESSION

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Radiation-induced premature differentiation of murine bone cells was investigated regarding the biosynthesis of specific osteogenic marker molecules and osteoblast specific transcription factors (TF). Osteoblastic cells play a crucial role in bone matrix synthesis and differentiate either into bone-lining cells or into osteocytes. Premature terminal differentiation has been reported to be induced by a number of DNA damaging or cell stress inducing agents including ionizing and ultraviolet radiation as well as treatment with mitomycin C. Changes in the expression of osteocalcin (OC), osterix (OSX), RUNX2 (cbfa1/AML3/Pebp2αA) and Transforming growth factor β (TGF-β) after exposure to ionizing radiation was investigated using quantitative real-time reverse transcription PCR (qRT-PCR) assay and immunofluorescent techniques. In the present study, we compared the effects of sequential differentiation by cell culture either in standard culture medium or in osteoinductive medium containing β-glycerophosphate and ascorbic acid. During the process of osteoblastic differentiation the expression of bone specific marker genes were investigated on day 3, 7, 14 and 21 after cell seeding. To study the effects of radiation on the differentiation process, the cells were exposed to ionizing radiation of different qualities. Gene expression alterations of the candidate genes cbfa1, OSX, OC and TGF-β were measured 2, 4, 8, 24, 48 and 72h after exposure. Cell morphological changes were detected in osteoblasts expressing OC and RUNX2. Fluorescence analysis of Osteocalcin reveals that OC was present intracellularly in all investigated cell lines after 14 days of culture time. The changes in gene expression pattern and morphology give rise to the question; whether the differentiation process affects the cellular response to DNA-damaging agents like ionizing radiation (IR). Immunofluorescent and immunohistochemical markers, combined with molecular analysis of mRNA expression levels, can provide an increasingly detailed understanding of radiation-induced differentiation and help to define health risks from exposure to low radiation.

Conflict of Interest: None declared

P166-S

BIM IS TRANSCRIPTIONALLY REGULATED DURING INDUCTION OF APOPTOSIS BY GROWTH FACTOR WITHDRAWAL IN OSTEOBLASTS

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Growth factor depletion contributes to cell death following vascular disruption and matrix degradation due to injury or disease in the skeleton. It has recently been reported that osteoclast apoptosis in response to M-CSF withdrawal is critically regulated in vivo and in vitro by the pro-apoptotic protein Bim. We report that osteoblasts also respond to mitogen withdrawal by upregulating Bim and undergoing apoptosis. Withdrawal of serum or reduction to 1% FCS triggered mass apoptosis within 24h in MBA-15.4 mouse osteoblasts, assessed by TUNEL, acridine orange and DAPI staining. Bim protein levels were very low in unstressed cells but accumulated from 2h after serum reduction, reaching a peak between 8 and 24h. Caspase 3 activity increased strongly from 4 to 16h subsequent to Bim induction. The upregulation of Bim at 8h was completely blocked by co-treatment with either actinomycin D or cycloheximide, indicating rapid, de novo protein synthesis. Real time RT-PCR revealed that Bim mRNA increased after serum depletion in a time-dependent manner and reached a peak between 6 and 24h, in agreement with protein data. Treatment with the MEK-ERK inhibitor, U0126, or the PI3K-PKB inhibitor, LY294002, induced strong expression of Bim protein and active, phosphorylated PKB and ERK levels also decreased within 4h of serum starvation, indicating regulation of Bim by survival kinases in osteoblasts. Bim protein markedly increased following treatment with the proteasome inhibitor MG132, indicating regulation by proteasomal degradation. Other cell stressors which reduced survival kinase signalling such as detachment or treatment with high-dose glucocorticoids also triggered upregulation of Bim in MBA 15.4 osteoblasts. Bak and Bax, the downstream effectors of Bim, are abundant in osteoblasts and levels remained unchanged during 24 h of serum reduction. However, immunoprecipitation data showed that serum withdrawal induced Bax activation. Knockdown of Bak or Bax protein levels alone by siRNA did not prevent apoptosis induced by growth factor withdrawal but knockdown of both Bak and Bax was partially protective. In conclusion, unlike osteoclasts, osteoblasts depend for their survival on multiple growth factors. However, withdrawal of serum depletes growth factors in culture and osteoblasts respond to this by upregulating Bim and undergoing apoptosis.

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Conflict of Interest: None declared

P167-M

INTRACELLULAR ACCUMULATION OF ABNORMAL COLLAGEN CAUSES ER STRESS AND OSTEOBLAST APOPTOSIS IN A NEW MOUSE MODEL OF OSTEOGENESIS IMPERFECTA

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The AGA2 is an autosomal dominant mutant mouse line characterized by reduced bone mass and multiple fractures that was isolated from the Munich N-ethyl-N-nitrosourea (ENU) mutagenesis program. Positional cloning studies mapped the causal gene for AGA2 to chr. 11, and a C-terminal mutation in the col1a1 gene was identified. Accordingly, AGA2 represents a new murine model for type II osteogenesis imperfecta (OI). Patients with OI exhibit increased bone fragility and high bone turnover, but the molecular mechanisms responsible are poorly understood. In this study we explored the mechanisms for increased bone fragility in AGA2 and provide evidence that the col1a1AGA2 mutation initiates an endoplasmic reticulum (ER) stress-specific cascade which activates caspases 12 and 3 and promotes osteoblast apoptosis, thereby impairing the mechanical integrity of the skeleton.

Apoptosis was studied using the TUNEL assay and by analyzing caspase expression in primary calvarial cultures. Pro-col1α1 was measured using a C-telopeptide antiserum. PDI and GOLPH4 antibodies were used to visualize the ER and Golgi. Activation of the unfolded protein response (UPR) was examined by studying BiP (a member of the hsp70 family of chaperones) expression using qRT/PCR. ER ultrastructure was assessed by transmission electron microscopy (TEM).

AGA2 fibroblasts showed increased intracellular aggregation of procollagen and decreased extracellular collagen compared to wild type. AGA2 procollagen was abnormally colocalized in the ER, rather than Golgi. TEM revealed an increased percentage of vacuoles and dilated ERs ($2.83\% \pm 0.6$ SD control vs. $21.44\% \pm 1.6$ SD AGA2/+; $p < 0.0001$). There was also a marked upregulation of AGA2 relative BiP transcript (4.01 ± 0.8 SD, $p < 0.001$), and an increase in activated caspases 12 (p20/10) and 3 in 14-day-old primary osteoblasts and in femoral periosteum. We also observed an increase in DNA fragmentation as detected by TUNEL-assay in cultured osteoblasts ($0.83\% \pm 0.4$ SD control vs. $7.51\% \pm 0.2$ SD AGA2/+; $p = 0.0002$) and in the periosteum in vivo.

Our data show that the AGA2 mutant develops intracellular accumulation of mutated type I collagen, and this causes ER stress-induced apoptosis preceded by the cytoprotective UPR cascade. The osteoblast apoptosis presumably contributes to the increased bone fragility that is characteristic of OI, thereby revealing a previously unidentified role of ER stress signaling in the pathogenesis of bone fragility in osteogenesis imperfecta.

Conflict of Interest: None declared

P168-T

MODULATION OF THE HUMAN ESTROGEN RECEPTOR ALPHA GENE BY A C-SRC/PKC-DEPENDENT MECHANISM IN CELLS OF THE OSTEOBLAST LINEAGE

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The transcription of the human Estrogen Receptor alpha (ERα) gene can be driven by several distinct promoters, of which the F promoter alone is found to be active in primary osteoblasts. We studied the response of this promoter to differentiation-related signals. For this purpose we created human osteoblastic cells, Saos-2, expressing a luciferase reporter gene downstream of the human ERα F promoter (Saos F-Luc). Cell over-confluence, which induced differentiation, caused a time-dependent increase of F promoter activity and inactivation of Protein Kinase C alpha (PKCα). Consistently, PKC down-regulation obtained by phorbol 12-myristate 13-acetate (PMA) treatment resulted in pro-

moter stimulation in sub-confluent, proliferating cells. The F promoter contains putative PMA-responsive AP-1 sites, but AP-1 activation was not higher at over-confluence. Beside these, the promoter harbours multiple Runx2 sites. The non-receptor tyrosine-kinase c-Src is a well-known negative regulator of Runx2-dependent transcription, so we tested promoter responses to c-Src inhibition. Treatment with the specific c-Src inhibitor, PP1, resulted in marked promoter stimulation. Interestingly, also PMA treatment was found to blunt c-Src activity, suggesting convergence of the two signals. The tyrosine kinase inhibitor 4-dimethylaminopyridine (DMAP) caused a decrease of active c-Src and stimulated the promoter, but caused no further promoter induction in PP1-treated cells. Exogenous, over-expressed hERα stimulated the promoter only slightly and hormone-independently, through its ligand-independent Activator Function 1. Conclusions: We showed a robust PKC/c-Src-dependent and estrogen-independent mechanism modulating transcription of Estrogen Receptor alpha in osteoblasts, probably affecting estrogen responsiveness during cell differentiation.

Conflict of Interest: None declared

P169-S

ROLE OF PARATHYROID HORMONE-RELATED PROTEIN AND VASCULAR ENDOTELIAL GROWTH FACTOR ON THE DECREASED OSTEOBLAST FUNCTION ASSOCIATED WITH DIABETIC OSTEOPENIA

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Type 1 diabetes mellitus (DM) is associated with bone loss, but the mechanisms of DM-related osteoporosis are poorly defined. Vascular endothelial growth factor (VEGF) has a role in diabetic nephropathy and retinopathy. Both VEGF and parathyroid hormone-related protein (PTHrP) are expressed in osteoblasts, and are coordinately decreased in age-related osteopenia. We examined here the roles of PTHrP and VEGF on the putative alterations of osteoblast function in DM. In vivo, we used a marrow ablation model in mice made diabetic by multiple streptozotocin injections. Mouse osteoblastic cells MC3T3-E1 were grown in differentiation medium (alpha-MEM with 10% fetal bovine serum, 50 mug/ml ascorbic acid and 100 muM beta-glycerophosphate) up to near confluence, with or without high glucose (HG) (25 mM) (or mannitol, as osmotic control). Growing cells in HG medium were supplemented (or not) with either PTHrP (1–36) (100 nM) or VEGF164 (0.5 nM). The gene expression of various osteoblastic products were analyzed by real-time PCR after total RNA isolation from mouse tibia and MC3T3-E1 cells. Diabetic mice showed a significant decrease (20%; $p < 0.05$ or less) in the gene expression of osteocalcin, PTHrP, the PTH/PTHrP type 1 receptor (PTH1R), VEGF, and its receptors 1 and 2 (VEGFR1 and VEGFR2, respectively); and a 4-fold increase in receptor activator of NF-kappaB ligand (RANKL)/osteoprotegerin (OPG) ratio, and 2-fold increase in peroxisome proliferator-activated receptor (PPAR-gamma). In vitro, HG medium did not affect significantly OC gene expression, but decreased (20–40%) mRNA expression levels of PTHrP and the PTH1R, as well as VEGF and its receptors; and increased (40%) the RANKL/OPG ratio. Moreover, RANKL secretion (by ELISA) was dramatically increased (5-fold over control) in the cell-conditioned HG medium. Continuous presence of PTHrP (1–36) or VEGF164 during cell growth reversed the changes in PTH1R, and RANKL/OPG ratio induced by HG medium. The former peptide also increased significantly the diminished VEGFR1 mRNA levels in this medium.

Conclusions: These findings strongly suggest that interaction between PTHrP and VEGF has an important role in the alterations of osteoblastic function associated with DM-related bone loss.

Conflict of Interest: None declared

P170-M

IN VIVO BONE FORMATION OF HUMAN EMBRYONIC STEM CELLS

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Human embryonic stem cells (hESC), with their versatile growth and differentiation potential, are ideal candidates for use in regenerative medicine protocols. The ability to form bone tissue based on differentiation of hESC into osteoblasts will be a useful tool for clinical applications such as repairing non-healed bone fractures or bone defects. However, there is no standard protocol for differentiation of hESC into osteoblastic cells. In addition, when implanted in vivo as undifferentiated cells, hESC forms teratomas with a mixture of various mesodermal, endodermal and ectodermal tissues and thus limiting their clinical use. The aim of this study was to design xeno-free protocol for directing the differentiation of hESC into osteoblastic cells and test the ability of these hESC-derived osteoblastic cells to form bone in vivo. We cultured hESC in absence of serum and mouse feeder layer and in suspension as 3D-aggregates known as embryoid bodies (EBs). We found that hESC when cultured as EBs, became enriched for cells expressing a range of mesenchymal stem cell (MSC) markers revealed by immunohistochemistry and RT-PCR. Time course quantitative analysis using flow cytometry revealed that hESC-derived MSC were enriched already at day 10 and increased significantly in number up to day 20. In order to test the functional ability of these hESC-derived MSC, we implanted day 20 EBs mixed with hydroxyapatite/tricalcium phosphate (HA/TCP) as an osteoconductive scaffold, subcutaneously in SCID mice and found that the cells formed bone and cartilage as well as fibrous tissue after 8 weeks. There was no evidence for differentiation to other non-mesodermal tissues. This is in contrast to non-differentiated hESC that formed a mixture of endodermal, mesodermal and ectodermal tissues or EBs implanted in absence of HA/TCP that formed fibrous tissues and muscle-like tissues. Our study demonstrates the feasibility of establishing a fast, reproducible and xeno-free system for differentiation of hES cells into osteogenic tissues. Future studies will aim at optimizing culture conditions and will test the functional ability of the cells in animal disease models.

Table: Characterization of in vivo implants of hESC

	Tissue	Staining	hEB - HA	hEB + HA
	Mesenchyme	H&E, Vimentin	++++	+++
Mesoderm	Chondrocyte	Alcian blue	–	++
	Bone	H&E	–	++
Endoderm	Colon Epithel	PAS, HNF4a	++	(+)
Ectoderm	Neuro-tissue	Tubulin-III-b	(+)	–

+ scored as 1/5 of section of implant that is positive

Conflict of Interest: None declared

P171-T

WHOLE GENOME EXPRESSION ANALYSIS OF MG-63 OSTEOBLAST-LIKE CELL LINE UNDER DIFFERENT PHISIOLOGICAL STIMULI

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We performed a comprehensive functional genomics analysis, using high-density oligonucleotide microarrays, in MG-63 osteoblast-like cell line upon exposure to different stimuli, in order to inquire the molecular basis of early events involved in osteoblasts response to specific physiological activators. MG-63 is a human osteosarcoma cell line widely used as *in vitro* model system for the study of osteoblasts functions. Here we report a systematic transcriptomes study in MG-63 cells stimulated for 1, 6 and 24 hours with either bone morphogenic protein 2 (BMP-2) or 1,25-dihydroxyvitamin D3 [1,25(OH)2D3] or IL1-alpha (IL1-alpha). Before proceeding with microarray experiments we verified the induction of known molecular markers of osteoblasts differentiation using real time qPCR; transcriptome analysis was performed using whole genome HG-U133 Plus 2.0 GeneChip® (Affymetrix®). Gene expression data were analyzed applying high level statistical analyses available in Bioconductor and Significance Analysis of Microarrays (SAM) software; pathways analysis was performed using Ingenuity®. Genes differentially expressed throughout the different treatment conditions and time points were used to perform hierarchical clustering. This analysis allowed to uncover the specific signatures generated by different stimuli and to evaluate their specific kinetics of action and biological effects. Only a restricted number of genes resulted to be modulated by either of the two known anabolic stimuli BMP-2 and 1,25(OH)2D3, while a higher number of genes was identified as specifically modulated by IL1-alpha. Interestingly, functional classification and pathway analysis revealed that many of these genes exhibited functions related to osteoclasts activation. The osteoblasts and marrow stromal cells have been already identified as the target cells for osteotropic hormones as well as cytokines (other than M-CSF) to induce osteoclast development. Our findings supports a possible role of the IL1-alpha pro-inflammatory cytokine in the stimulation of the cross-talk between osteoblasts and osteoclasts and the dataset generated allows both the global and detailed molecular inspection of this biological event. This experimentally controlled large-scale gene expression analysis provides a global view of genes and networks regulated by different physiological stimuli in MG-63 and may represent a powerful tool for dissecting the osteoblast activation components.

Conflict of Interest: None declared

P172-S

OSTEOPROTEGERIN PRODUCTION BY MG63 BUT NOT BY SAOS-2 OSTEOSARCOMA CELLS IS STIMULATED BY A FACTOR PRESENT IN SERUM

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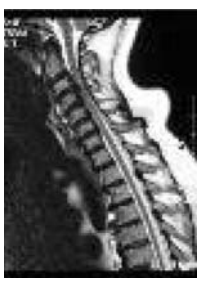
Osteoprotegerin (OPG) is produced by a variety of cell types including osteoblastic cells, fibroblasts, endothelial cells and B lymphocytes. OPG appears to have several important functions including inhibition of bone resorption, inhibition of vascular calcification and inhibition of apoptosis. While studying the regulation of OPG production in osteoblastic cells we observed an unusual dependency of OPG production by MG63 cells on the amount of foetal calf serum (FCS) present in the culture medium as distinct from the concentration of FCS. Thus, there was an increase in OPG production with volume of medium as well as with FCS concentration. This was not the case with Saos-2 cells. After a period of incubation (24 hours with 10% FCS) OPG production in MG63 cells came to a halt but could be restarted with the addition of FCS. Messenger RNA for OPG, measured using qRT-PCR, was increased following the addition of FCS and peaked at 6 hours then declined to starting levels by 24 hours. The factor apparently being consumed by MG63 cells had a molecular weight of over 30kD by ultrafiltration and was excluded from DEAE trisacrylamide ion exchanger but strongly adsorbed to carboxymethyl sepharose and was displaced by 1.5M sodium chloride at pH 7.2. The factor was labile at temperatures above 70°C. Human serum also contains a similar factor that stimulates OPG production in MG63 cells but sera from different individuals appear to have different concentrations. These findings suggest that FCS and human serum contain a large, heat labile protein with a net positive charge capable of inducing OPG production in MG63 cells. Further evidence of the nature of this protein and its possible significance for the survival of cancer cells will be presented.



Conflict of Interest: None declared

P173-M

FRACTURE OF OSSIFIED POSTERIOR LONGITUDINAL LIGAMENT CAUSING CENTRAL CORD SYNDROME – A CASE REPORT

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Introduction:

Ossification of the posterior longitudinal ligament (OPLL) is a disease found predominantly in the oriental population and is very rarely seen in non oriental ethnic groups. Radiologically, OPLL has been classified as continuous, segmental, mixed and localised types. Here we report the case of an ossified posterior longitudinal ligament presenting with acute central cord syndrome associated with fracture of the ossification.

Case: A 64 year old Caucasian farmer was transferred to our spinal unit with weakness and hyperaesthesia in the upper limbs following a road traffic accident where his car met with a head on collision. On examination he had hyperaesthesia in both upper limbs and motor power of grade 4 in the right upper limb with a distal motor power of grade 3 in the hand. There was no motor deficit in the left upper limb. All reflexes were normal and neurological examination of lower limbs revealed no deficits.

Investigations: Radiographs revealed an ossification of the posterior longitudinal ligament from C2 to C6 and CT and MRI scans confirmed the ossification at the same level causing significant canal stenosis. He also had exuberant bridging anterior osteophytes and soft tissue ossification in the cervical and thoracic spines, suggestive of diffuse idiopathic skeletal hyperostosis (DISH). There was also a break in the ossified ligament at C2 and C3 levels. He recovered completely in 6 weeks after being treated in a cervical collar and was discharged.

Discussion: Co-existence of DISH and OPLL is very rare and only four cases have been reported in literature so far. Fracture of the posterior longitudinal ligament has not been widely reported, although it is possibly more prevalent than is recognised. We report this case in order to highlight the importance of recognising this condition in non oriental populations and to demonstrate that non operative treatment has a good prognosis.

Conflict of Interest: None declared

P174-T

EFFECTS OF GLUCOCORTICOIDS ON OSTEOCYTIC GENE EXPRESSION IN VIVO AND IN VITRO

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The action of native or pharmacological glucocorticoid hormones, such as dexamethasone (Dex), is mediated via glucocorticoid receptors. Dex is recognized by multiple effects on a wide range of tissues and physiological conditions in the body, including bone metabolism. A low dose of Dex promotes osteogenesis *in vitro*, and induces the expression of osteogenic markers in mesenchymal stem cells. However, excess glucocorticoids can lead to osteoblastic dysfunction, enhanced bone resorption by osteoclasts, and induce osteocyte apoptosis. In this study we wanted to investigate how glucocorticoids directly affect osteocytes. We used both the MLO-Y4 cell-line and isolated primary rat osteocytes to study the effect of Dex treatment on osteocyte-specific gene expression. Another approach was to study the effects of a short intermittent dose of glucocorticoids *in vivo* on the expression of gene expression in bone. In both types of experiments we focused on measuring expression of those genes that are known being preferentially expressed in osteocytes, using mRNA-specific primers and real-time semi-quantitative RT-PCR. In our experiment using MLO-Y4 cells we observed a pattern indicating up-regulation of mRNAs important for bone formation, including osteocalcin (OC), pleiotrophin (PTN), dentin matrix protein-1 (DMP1) and phosphate-regulating endopeptidase homolog, x-linked (PHEX), 48 hours after exposing the cells to dexamethasone at a concentration of 10^{-8}M. We could also see a down-regulation of the estrogen receptor-α (ER-α) and ER-β mRNAs. In our in-vivo experiment BALB/C-mice were given an i.p. dose of methyl-prednisolone corresponding to 25 mg/kg body weight. Our expression data showed a transient increase in expression level of DMP1, receptor activator of NF-κ-B-ligand (RANKL), matrix extra-cellular phosphoglycoprotein (MEPE) and PHEX. This peak at 3 hours post injection was followed by down-regulation of sclerostin (SOST), fibroblast growth factor 23 (FGF23), ER-β and OC mRNAs at 6 hours post injection. In conclusion, our present results give quantitative data concerning the effect of glucocorticoids on osteocytic gene expression under different *in vitro* and *in vivo* conditions.

Conflict of Interest: None declared

P175-S

GLUCOCORTICOIDS REDUCE THE SECRETION OF BONE ANABOLIC SIGNALS FROM A SUBSET OF MATURE OSTEOCLASTS

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Glucocorticoids are known to exhibit deleterious effects on bone formation, and recent data demonstrated that this effect was mediated through the osteoclasts in vivo. In addition, recent data showed that osteoclasts secrete factors, which induce bone formation, i.e. possible coupling factors. We examined whether glucocorticoid treatment influences the secretion of bone anabolic signals from osteoclasts in vitro. This was evaluated by treatment of pre-osteoblasts with conditioned medium (CM) collected from mature osteoclasts treated with or without dexamethasone (DEX).

Mature human osteoclasts were seeded on plastic and cultured in the presence or absence of DEX (10, 30 and 90nM). CM was collected every 2nd–3rd day for a 20-day period, and divided into early (first 10 days) and late (last 10 days). MC3T3-E1 cells pre-osteoblastic cells were subsequently cultured with the different types of CM (60%), in the presence of 50 μg/ml ascorbic acid and 10mM β-glycerol-phosphate to allow osteoblast differentiation. BMP-2 (30ng/ml) was used as a positive control for bone formation. Bone nodule formation was evaluated by Alizarin Red staining and dye extraction after 2 weeks of culture.

CM (without DEX) from both early and late periods induced bone formation, to the same extent as BMP-2. DEX treatment of the osteoclasts in the early period did not change the ability of the CM to induce nodule formation. Interestingly, DEX treatment of the osteoclasts at the late stage significantly reduced the ability of the CM to induce nodule formation.

In conclusion, the secretion of anabolic factors from osteoclasts appears to be diminished by DEX treatment, but only in a subset of osteoclasts, whereas other osteoclasts are non-responsive to DEX with respect to secretion of anabolic factors. These data potentially explain the in vivo findings that the detri-

mental effects of glucocorticoids on osteoblasts are mediated through the osteoclasts.
Conflict of Interest: None declared

P176-M

REDUCTION IN MESENCHYMAL STEM CELL NUMBERS IN PREMATURE AGING DNA REPAIR DEFICIENT TTD MICE

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Background: Mice carrying mutations in DNA repair genes often show signs of accelerated ageing and therefore can be used as a model system to study age related diseases like osteoporosis. It has been shown that TTD mice, carrying a mutation in the nucleotide excision repair gene XPD (xeroderma pigmentosa group D), display features of ageing related osteoporosis as well as adipose tissue hypoplasia (de Boer J. et al, Science 2002). Since both cell types involved, osteoblasts as well as adipocytes, arise from the same mesenchymal stem cell population, the aim of the current project was to study the number, proliferation and differentiation potential of these cells in TTD compared to wild type (WT) mice. This might provide us with useful information concerning the mechanism behind age-related osteoporosis and the loss of adipose tissue.

Methods: Bone marrow from old (83w–105w) TTD and WT mice was cultured under osteogenic (TTD 10, WT 7) or adipogenic (TTD 5, WT 4) conditions and analysed for alkaline phosphatase activity (ALP), mineralisation (osteoblast) and lipid deposition (adipocyte).

Results: Under osteogenic conditions the number of ALP-positive colonies after 9 and 14 days of culture was significantly decreased ($p = 0.02$) in TTD compared to WT mice. The rate at which new ALP-positive colonies are formed between day 9 and day 14 of culture has not changed between TTD and WT mice, indicating that the decrease in colony number is not due to a delay in differentiation. Mineralisation of ALP-positive colonies did not seem to be affected, with a borderline significant decrease on day 14 at the onset of mineralisation but no significant changes on day 21 of culture. Lipid deposition was strongly reduced in TTD compared to WT mice ($p = 0.01$) after 35 days of culture.

Conclusions: The observed reduction in osteoblast and adipocyte differentiation indicates a reduction of mesenchymal stem cell numbers in TTD mice. This reduction in mesenchymal stem cell numbers and the corresponding decline in osteoblast differentiation could explain the premature osteoporotic features observed in TTD mice. In line with this, the reduction of mesenchymal stem cells and adipocyte differentiation may underlie the adipose tissue hypoplasia observed in TTD mice.
Conflict of Interest: None declared

P177-T

ZOLEDRONATE POTENTLY INHIBITS THE GROWTH, FUNCTION AND SURVIVAL OF NORMAL RAT OSTEOBLASTS

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Bisphosphonates are recognised not only as potent inhibitors of osteoclastic resorption but also for their toxicity to tumour cells. However, their actions on osteoblast function and survival are less well documented. We studied the effects of zoledronate, the most potent bisphosphonate, on primary osteoblast cultures derived from neonatal rat calvariae by trypsin/collagenase digestion. Osteoblast were cultured up to 14 days in DMEM (pH 7.35–7.40) supplemented with 2mM beta-glycerophosphate, 50mug/ml ascorbate and 10nM dexamethasone. Bone nodule formation was measured by image analysis of alizarin red-stained cell layers; cell number and viability were assessed colorimetrically. In long-term osteoblast cultures, zoledronate exerted dose-related cytotoxic effects. Treatment with 10muM zoledronate resulted in 40% and 99% reductions in osteoblast number, compared to controls over the first 4 and 7 days of culture, respectively; after 14 days of zoledronate treatment, no viable cells remained. Treatment with 1muM zoledronate did not affect osteoblast number during the first 7 days, but decreased cell number by 40% by day 14. Treatment with 100nM zoledronate did not affect osteoblast numbers up to 14 days. In control cultures, formation of "trabecular" bone nodules begins from day 10. Zoledronate potently blocked mineralised nodule formation: 65%, 95% and 100% inhibition of mineralised nodule formation was observed in cultures treated continuously with 10nM, 100nM and 1 μM zoledronate, respectively. Light microscopy revealed that in osteoblast cultures treated with 10nM and 100nM (but not 1muM) zoledronate, abundant, collagenous matrix was deposited in characteristic "trabecular" patterns but that mineralisation was blocked. For comparison, in this system the IC50 of the mineralisation inhibitor pyrophosphate, is ~2muM; thus zoledronate is about 200 times more potent. In conclusion, our results show that chronic zoledronate treatment blocks bone formation in vitro via inhibition of mineralisation in the low nanomolar range and a via general cytotoxic action on osteoblasts in the low micromolar range. The osteoblast cytotoxicity data are in line with previously reported IC50 of 10muM for the inhibition of breast cancer cell viability by zoledronate in 4 day cultures (Br J Cancer 82: 1459–1468; 2000). These findings raise the possibility that long-term exposure of osteoblasts (and possibly osteocytes) to zoledronate in vivo could have adverse consequences for bone health.
Conflict of Interest: T. R. Arnett, Novartis, Grant Research support A Brandao-Burch, Novertis, Grant Research support

P178-S

DIFFERING STRAIN REGIONS AND SHEAR FORCES UNDER CYCLIC BIAXIAL LOADING IN THE FLEXERCELL-STRAIN-UNIT

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Background: Mechanical stimulation of cells using the Flexercell device has become a well-established method. A number of culture plates and protocols have been tested with the goal of achieving highly reproducible and specific loading conditions. However, under cyclic biaxial loading both different micro strain regions and a homogeneous strain field upon the loading posts have been reported.

In our previous study we confirmed the homogeneously distributed effects of biaxial stretching over the stimulated area upon the loading posts for amplitudes ranging from 1–10% by Digital Image Correlation (DIC) using BioFlex 6-well culture plates coated with collagen type I (Ott CE et al., Calcif Tissue Int 78: suppl 1, 2006). As estimated by finite element analyses, radial and circumferential strains differ between on-post and off-post areas (Vande Geest JP et al., J Biomechanics 37, 2004).

To check whether unspecific off-post strains influence the Fos and Ptgs2 mRNA-expression pattern of mechanically stimulated MC3T3-E1 cells, we applied ring-shaped inlays to limit the cell culture to areas of the membrane that remained on-post with the straining protocols used. In addition, to assess the influence of shear forces under diverse cyclic biaxial loading regimens, we varied the viscosity of the culture media by adding different amounts of dextran. Cell culture conditions corresponded to those described previously.

Results: The application of the ring-shaped inlays as well as different viscosities of the culture media representing different shear forces in diverse stimulation protocols with variation in frequency and strain level had no significant influence on the Fos and Ptgs2 mRNA-expression analysed by quantitative PCR relative to Gapdh and Actb as endogenous controls. Similar results were obtained stimulating murine primary calvarial osteoblasts under the same conditions. In addition, there were no significant changes in Akt concentration and phosphorylation in the above experiments as measured by Western blot analyses.

Conclusion: Using BioFlex 6-well culture plates for biaxial loading the Flexercell-Strain-Unit is an appropriate tool for reproducible application of mechanical strains in vitro. At least in early responses on mRNA and protein level there was no significant impact of shear forces or less specific strained cells growing in the off-post area.
Conflict of Interest: None declared

P179-M

EXPRESSION OF METALLOPROTEINASE-14 IS PLEIOTROPIC ON ALKALINE PHOSPHATASE EXPRESSION AND AFFECTS OSTEOGENIC DIFFERENTIATION

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We show that modulation of the expression of the transmembrane enzyme MMP-14 affects the function of the membrane enzyme Alkaline phosphatase (AP) in early and pre-mineralization stages of osteogenesis. Both enzymes are developmentally regulated at the transcriptional level during osteogenesis, with a similar pattern of early up-modulation in differentiating osteoblast and down regulation concomitant to mineralization. There are no data on the requirement for MMP-14 expression for mineral deposition, and K.O. mice for MMP-14 have mild bone phenotype. The expression of AP is a known prerequisite for mineralization.

We report that in rat osteoblast differentiating in vitro the constitutive expression of MMP-14, obtained with an inducing antibody, is associated to constitutive expression of AP, to lack of nodules formation and of mineralization. Moreover, in clones of osteoblasts constitutive for MMP-14 also AP is expressed constitutively, no nodules form, no mineralization occurs. Chemical

inhibitors of MMPs inhibit also AP function, no nodules form, no mineralization occurs.

Thus radical changes in the timely expression of MMP-14, regulated either at the transcriptional or post-transcriptional level, affect the functionality of AP. Vice versa, modulation of the expression of AP by different modalities, with or without changes in the timely pattern of expression, weather at transcriptional or post-transcriptional level, do not affect the modulation of the expression and enzymatic function of MMP-14, but only the mineral deposition in nodules. Increased levels of expression of AP induced by F, by IGF II or by Endothelin (ET) does not affect MMP-14 level or timing while enhance nodule formation and mineralization.

Inhibition of AP by Levamisole or secondary to inhibition of ET, does not affect MMP-14 expression and nodules formation, while mineralization decreases. Inhibition of AP function in clones constitutive for MMP-14 and AP, has no effect on MMP-14 expression, while no nodules form and no mineralization occurs.

In conclusion, MMP-14 expression is pleiotropic on that of AP, but not vice versa. Transient upmodulation of both is required for the formation of nodules and mineral deposition. Down-modulation of MMP-14 is required for nodule formation. Down modulation of both enzymes, after upregulated expression is associated with mineralization. Upmodulation of AP increases the mineral deposition in nodules, when MMP-14 is down modulated at nodules formation.
Conflict of Interest: None declared

P180-T

HETEROTOPIC OSSIFICATION IN A RADIAL FOREARM FLAP: CASE STUDY

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Heterotopic ossification (HO) is a well documented phenomenon with many pathogeneses and sites of appearance. Here we report a patient who presented with HO to his radial forearm fascial flap.

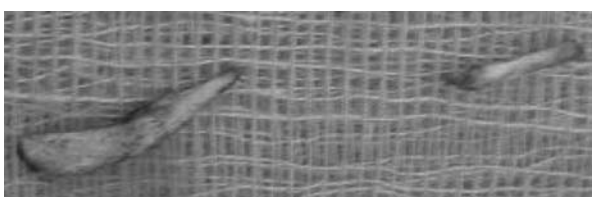
Pretreatment: A 22 year old right hand dominant male soldier was involved in an RTA in Kuwait. He suffered an intraventricular haematoma and crush injury to his left hand. His above injury and following surgery left a 15 × 6cm soft tissue loss from base of 3rd MCP obliquely to base of thumb which was treated with a distally based radial forearm fascial flap with skin graft. The wound was debrided and the flap was raised at the fascial level with some subdermal fat. Split skin graft from the thigh was used to cover the flap. The flap had an uneventful post operative course.

Diagnostic Tests: x-ray for two asymptomatic subcutaneous masses in fascial flap

Diagnosis and Treatment Plan: Heterotopic ossification. Booked for debulking of the RFF with excision of subcutaneous masses.

Treatment: The operation was performed ten months after the initial surgery and the masses appeared to be slivers of cortical bone (picture 1) measuring 3 × 0.5cm and 2 × .05cm. The histological report was that of 'devitalized cortical bone'. There was no confirmation of vascular origin.

Follow Up: There has been no recurrence of the masses ten months post debulking and excision.



Conflict of Interest: None declared

P181-S

AMELOBLASTIN (AMBN), A KEY FACTOR IN ENAMEL DEVELOPMENT AND MINERALIZATION, IS EXPRESSED AND REGULATED IN HUMAN MESENCHYMAL AND HEMATOPOETIC STEM CELLS, BONE CELLS AND IN ADIPOSE TISSUE

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The aim of the study was to examine the role of ameloblastin (AMBN) in cell recruitment and bone formation.

Human CD34+ cells isolated from bone marrow, mesenchymal stem cells (MSC) isolated from iliac crest, normal osteoblasts from femur and tibia (NHO, Cambrex), chondrocytes from the knee (NHAC, Cambex), subcutaneous adipose tissue from healthy undergoing mammoplastic surgery and osteoclasts differentiated from peripheral monocytes of various donors were examined. A murine ameloblast-like cell line (LS8) and human pulp cells (Dominon Pharmakine) were used as positive controls for AMBN expression. Expression of genes was measured by Real-time PCR and Affymetrix. Secretion of proteins to the culture medium was measured by Western blotting, ELISA and Luminex (kits from BioSource and Linco).

AMBN was found to be present in CD34+ cells, MSC, mature osteoblasts, osteoclasts, chondrocytes and in adipocytes. The expression of AMBN in bone forming cells was regulated by parathyroid hormone (PTH) and Emdogain (EMD) in a time- and dose-dependent manner. AMBN expression was reduced during differentiation of osteoclasts, however administration of rAMBN enhanced the number of differentiated osteoclasts. We also observed a positive effect on osteoblast differentiation, where AMBN increased the level of alkaline phosphatase (ALP), osteocalcin and CD44. AMBN had a profound effect on the expression of genes involved in immune responses in osteoblasts. To our knowledge no receptor has been identified yet, however, we found AMBN to induce the expression of genes involved in STAT signaling.

The present findings of AMBN expression and secretion from bone cells of both mesenchymal and haematopoetic origin demonstrate that AMBN may have important functions in bone homeostasis. AMBN expression in adipocytes may indicate a role in the delicate balance between resorption and formation.
Conflict of Interest: None declared

P182-M

THE COGNITION ENHANCER ANIRACETAM INCREASES THE NUMBER OF OSTEOBLASTIC COLONIES IN MSC CULTURES WHEN COMBINED WITH FLUID SHEAR STRESS

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Aniracetam is a cognitive enhancer drug whose actions in the CNS include increases in extracellular dopamine and serotonin and the facilitation of AMPA-type glutamate receptors by a reduction in the desensitisation rate. As receptors for dopamine, serotonin and glutamate are known to be expressed and have functions in osteoblasts, we investigated the effect of aniracetam on MSC cultures treated with osteogenic agents.

Rat marrow cells were extracted and cultured using the CFUF assay, and treated with aniracetam (50 and 100 μM) in the presence or absence of fluid shear stress applied for 5 minutes each day over the 14 day culture period. In control static cultures, aniracetam had no significant effects on colony formation. However, daily fluid shear and aniracetam treatment (100 μM) were associated with significant increases in the area of alkaline phosphatase positive colonies at 10 days (+30% $p < 0.001$). At 14 days, this difference in alkaline phosphatase was no longer significant, but later the markers of osteoblastic differentiation, type I collagen and mineral areas were increased by 40% and 50% respectively (both $p < 0.001$). At 14 days, the mean size of all colonies (identified by methylene blue staining) was calculated and found to be not different. In contrast, the number of colonies at 14 days was increased by 21% ($p < 0.05$) as a result of aniracetam and fluid shear.

These results show that aniracetam has the ability to enhance the number of precursor cells that undergo lineage commitment towards the osteoblastic lineage, but only in the presence of a mechanical stimulus. Whether these effects are due to the drug's actions on dopamine, serotonin or glutamate mediated signalling, or combined effects is not yet completely clear. Previous work showing that the response of osteoblasts to mechanical loading involves glutamate receptors and processes that are similar to long-term potentiation, suggests that at least some of these effects are due to enhanced glutamate signalling in loaded cultures. Aniracetam has been shown to improve cognitive function in elderly patients with Alzheimer's disease. Studies of their bone mass may be valuable to determine potential benefits of treatment for the human skeleton.
Conflict of Interest: None declared

P183-T

IMMUNOHISTOCHEMICAL STUDIES DEMONSTRATE THAT GAS6 AND AXL ARE EXPRESSED BY OSTEOBLASTS AND GROWTH PLATE CHONDROCYTES

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Endochondral bone formation occurs at epiphyseal growth plates situated at the end of long bones; in which chondrocytes are spatially and temporally dis-

tributed into precise areas of proliferation and differentiation. The differentiated, hypertrophic chondrocyte secretes a collagen-rich matrix which mineralises over time. The mineralisation process is essential for normal bone growth and is a prerequisite for vascular invasion of the growth plate. However, the precise signals regulating chondrocyte differentiation and growth plate cartilage mineralisation are not yet fully understood. Axl receptor tyrosine kinase (Axl RTK) and its ligand, growth arrest specific gene 6 (Gas6) has been identified as a modulator of osteogenic differentiation and mineralisation of pericytes (Collett et al, Circ. Res 92: 1123–9, 2003). Therefore, this present study aimed to determine if Gas6 and Axl RTK were expressed in the growth plate and if their expression was altered with the differentiation state of chondrocytes. Initial in-vitro studies using the ATDC5 murine chondrogenic cell line demonstrated the expression of both Gas6 and Axl RTK mRNA. This result strongly suggests that both Axl RTK and Gas6 are expressed by chondrocytes and this was investigated further using immunohistochemistry. Tibiae were dissected from one week old mice, fixed, embedded in paraffin and immunostained with antibodies specific to Gas6 and Axl RTK. Both Gas6 and Axl RTK were found to be expressed by proliferating and hypertrophic chondrocytes of the growth plate, and osteoblasts at the chondro-osseous junction. These results are consistent with the results from the cell culture studies. The distribution of staining suggested that there was stronger expression of both Gas6 and Axl RTK in the proliferating chondrocytes than in those of the hypertrophic zone. Laser capture microscopy will be utilised to isolate the RNA of the proliferating and hypertrophic cells, from which Gas6 and Axl RTK RNA expression will be quantified using RT-qPCR. These studies are the first to report the expression of both Gas6 and Axl RTK by growth plate chondrocytes and further studies will clarify the importance of this signalling pathway in chondrocyte differentiation and mineralisation.

Conflict of Interest: None declared

P184-S

BONE CELL GROWTH IS ENHANCED IN HISTOCARE™ GEL

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We have evaluated HISTOCARE™, a water-soluble cellulose gel (hydroxyl propel methyl cellulose (HPMC)) and its ability for the 3D culture of bone cells. HISTOCARE™ has previously been demonstrated to stimulate pancreatic beta-cells in culture. We have studied the effect of the gel on cytotoxicity, proliferation and differentiation of osteoblasts.

Murine preosteoblasts (MC3T3-E1, ATCC) were maintained in MEM-alpha (Gibson BRL, Life Technologies Ltd, Scotland) supplemented with 10 % fetal calf serum (FCS), and cultured with or without HISTOCARE™ gel (Amidic AB, Sollentuna, Sweden) in cell culture inserts. Morphology of cultured cells was examined by inverted light microscope. Effect on proliferation was evaluated by pulsing cells with 3H-Thymidine, and harvest after 1, 2 and 3 days. The effect on bone markers were evaluated after 1, 2, 4, 7 and 14 days and quantified by real-time PCR.

Cells cultured in gel formed evenly distributed cell clusters within 24 hours, whereas cells cultured without gel grew in monolayer on the insert membrane. Cell growth was more than 3-fold enhanced with gel ($p < 0.001$) compared to cells cultured without gel at all time points. We observed no cytotoxic effect of the gel on the osteoblasts, analyzed by lactate dehydrogenase (LDH) activity in the culture medium. The expression of the bone markers, collagen type 1 and osteocalcin, were significantly lower in cells culture in gel compared to without gel. However, the expression followed the same profile with gel as without gel, increasing 3 fold for collagen type 1 and 7–8 folds for osteocalcin during 14 days of cell culturing.

Cell culturing in HISTOCARE enhanced the number of osteoblasts, and reduced the expression of bone markers without affecting the expression profile during time.

Conflict of Interest: None declared

P185-M

ASSESSMENT OF PLASTICITY BETWEEN HUMAN MESENCHYMAL STEM CELL-DERIVED OSTEOBLASTS AND ADIPOCYTES BY MICROARRAY ANALYSES AND BIOINFORMATIC EVALUATION

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The age-related gain of fatty tissue in human bone marrow and the simultaneous loss of bone mass could contribute to diseases like osteopenia. Besides acceleration of adipogenic differentiation of mesenchymal stem cells (MSCs), transdifferentiation of pre-osteoblasts may contribute to this adipogenic degeneration. The detection of the hitherto largely unknown molecular basics is a prerequisite to uncover novel targets for therapeutic interventions that inhibit adipogenesis and enhance osteogenesis. Knowledge of the underlying molecular processes could also facilitate tissue engineering of bone in vitro. In our cell culture system of human MSCs, transdifferentiation from osteoblasts into adipocytes and vice versa has already been proven feasible. Here, we aim to identify gene products differentially regulated during transdifferentiation that could be involved in the initiation of this process. To examine adipogenic transdifferentiation of committed osteoblasts as well as osteogenic transdifferentiation of adipocytes, RNA specimens were isolated 3 h and 24 h after initiation of transdifferentiation and subjected to Affymetrix microarray analyses in duplicates. Signal log ratios and change p-values allowed for evaluation of reproducibility and distinct regulation as well as reciprocity of gene regulation between both transdifferentiation directions. 415 and 920 gene products showed reproducible regulation patterns in adipogenic and osteogenic transdifferentiation, respectively. Many of these transcripts displayed reciprocal regulation. In further bioinformatic analyses, reproducibility, high degree of regulation and distinct reciprocity served as input to develop a scoring scheme that provided the ranking of gene products due to their potential relevance. Some of the highly ranked gene products are components of signaling pathways like Wnt/β-catenin, IGF or FGF signaling. Functional examination of the influence of FGF1 as one of the highly ranked potential key factors confirmed its inhibitory effect on adipogenic development as expected by microarray results. Our findings serve as prerequisite for further functional examinations of promising candidates that could provoke transdifferentiation or even act as switches between the adipogenic and osteogenic lineage. Therapeutic interventions that affect the action of such factors could counteract age-related bone loss by specific enhancement of osteogenesis and inhibition of adipogenesis.

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Conflict of Interest: None declared

P186-T

EFFECTS OF RALOXIFENE AND ITS INTERACTION WITH HUMAN PTH ON HUMAN PRIMARY OSTEOBLASTIC CELLS

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In the last years, the development of combined therapies for osteoporosis based on the association of drugs that concurrently favour bone formation and inhibit bone resorption has aroused a great interest in clinical practice. In this direction, a number of studies have been conducted to investigate the potential synergistic effects of the biosynthetic active fragment 1–34 of PTH (teriparatide) and the selective estrogen receptor modulator raloxifene on osteoblasts using in vitro osteoblast culture systems. Although each single compound has revealed to induce anabolic effects on osteoblasts, the experimental conclusions about the combined use of these two drugs are far to be exhaustive. The variable results reported in literature are mainly referred to studies performed in different experimental conditions on heterogeneous cellular models of animal or human origins (murine cells, human transformed or immortalised cell lines, etc.). To avoid this, we performed a series of experiments on primary human osteoblastic (hOB) cells, testing a wide range of different raloxifene concentrations alone or in combination with different amounts of hPTH (1–34). Moreover, we tested different time-dependent experimental protocols for drugs administration to the cell cultures, in order to assess the interactive effects amongst the two drugs in the regulation of bone formation in vitro. Our results show that: 1) In comparison with control cultures, raloxifene and teriparatide alone did not change significantly hOB proliferation, while they can enhance osteocalcin production of about 4 folds and 5 folds respectively; 2) A combined treatment can led to an increase of hOB proliferation up to 30% and a further increase in osteocalcin production; 3) raloxifene led to a significant reduction in IL-6 production (up to -65%) in stimulated hOB cells, although this effect was more marked (-85%) if raloxifene is added in combination with hPTH (1–34). Further experiments are in course to demonstrate the effects of the associated treatment on mineralized bone nodule formation. However, our preliminary results support the use of the combined treatment for an optimal therapy of osteoporosis.

Conflict of Interest: None declared

P187-S

GENE EXPRESSION IN HUMAN OSTEOBLASTS AND OSTEOCLASTS IN A THREE DIMENSIONAL TISSUE CULTURE MODEL

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Bone is a mechanically sensitive tissue which is adversely affected by removal of loading forces. This atrophy emulates that of the type in a model of disuse. In order to elucidate bone loss manifested by different environments, we studied the effects of modeled microgravity (NASA bioreactor) on gene expression and proliferation in human osteoblasts and osteoclasts. Differentiation and signal transduction markers were evaluated by real-time PCR and immunohistochemistry. No apparent changes were observed in osteocalcin expression in osteoblasts in modeled microgravity. No apparent changes were observed in osteoclast differentiation. However the rate of proliferation in osteoblasts was markedly decreased (53%, $p < .05$)in modeled microgravity. This study indicates that modeled microgravity inhibits the growth of human osteoblasts and this could be one mechanism leading to bone loss in microgravity. Studies are ongoing to elucidate this mechanism. This study will also present work on the expression the effect of some natural compounds to enhance proliferation of human osteoblasts in modeled microgravity.

Conflict of Interest: None declared

P188-M

BMP-7, BISPHOSPHONATES AND PTH IN A RAT OPEN FRACTURE MODEL. FUNDAMENTAL DIFFERENCIES IN THEIR L EFFECTS ON UNION AND STRENGTH

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Introduction Open fractures show reduced rates of healing. Infection aside, this is likely to be due an impaired anabolic response caused by local damage to tissues and their blood supply. Delay in the anabolic response may result in a relative increase in catabolism, leading to a atrophic non-union. We performed a study using a rat open fracture model to examine the effects of anabolic and anti-catabolic treatments, and their combinations.

Method An open fracture model was used in 9 week old rats. A lateral approach was made to the right femur under anesthesia. The femur was stripped of periosteum and osteotomized with an oscillating saw without cooling an fixed with a 1.1mm K wire. The animals were followed for 6 weeks to assess union

Group N Treatment
Saline 29 Saline at 2W
PTH low 2W 29 Daily PTH g/kg$\mu$for 2W 10
g/kgμPTH low 6W 31 Daily PTH for 6W 10
PTH high 6W 30 Daily g/kgμPTH for 6W 50
Zometa 31 ZA 0.1mg/kg at 2W
PTH 2W Zometa 32 Daily PTH g/kg$\mu$for 2W 10
Zometa 29 0.1mg/kg at 2W
gμOP-1 29 OP-1 50
OP-1 + Zometa g + ZA 0.1mg/kg 2Wμ29 OP-1 50

All harvested femora were radiographed (Faxitron) and underwent QCT. Two thirds of the rats were allocated to mechanical testing and one third to histological analysis.

Results Radiology: no increase in union rates with any PTH regimen. ZA alone failed to produce any increase in the number of fracture unions in these open fractures. OP-1 administration increased union rate to 95% alone and 100% when combined with ZA

Mechanical union was defined as the ability of the femur to take load. Only those fractures that had united were mechanically tested. Energy absorption to failure was increased by 51% in OP-1 treated groups and by 85% in OP-1 ZA combined treatment ($p < 0.01$). No improvements in these parameters were seen with PTH or ZA treatment alone.

Conclusion Bone morphogenetic proteins are suitable anabolic agents in this model of open fractures. PTH failed to have any impact on fracture repair in this model, despite its previously noted positive effects in closed fractures. In open fractures, bisphosphonates may increase callus size and strength when combined sequentially with a suitable anabolic agent.

Conflict of Interest: D Little Consultant Novartis

P189-T

CELLULAR SCREENING OF BONE-RELATED DISEASES IN MICE

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Due to the aging populace and alterations in living standards, the incidence of skeletal diseases will rapidly increase. But despite vast efforts in bone research to date, most molecular mechanisms involved in skeletal diseases remain unclear. The mouse is one of the best model systems to study bone biology, and the German Mouse Clinic (GMC) is a mouse phenotyping center for large scale and comprehensive investigation of mouse mutants in different biological and medical fields, including bone and cartilage within the Dysmorphology Screen. To broaden the bone-related phenotyping options to the cellular level, we have established a cell culture system for osteoblasts and osteoclasts to analyze their function within mice that have shown striking differences from the Dysmorphology Screen.

Osteoblasts are isolated from neonate calvaria of mutants and their wildtype littermates, and kept in culture for 21 days after stimulation on day 5. During this culture period the cellular characterization is performed in a standardized manner on the RNA, protein and functional levels. We determine proliferation, apoptosis and metabolic activities of the cells as well as the activity of bone-relevant ALP. We measure expression levels of 15 bone-related genes by Real-Time PCR and investigate matrix mineralization and nodule formation in the culture. Additionally, we apply immunofluorescence and ELISA for bone-specific markers. All assays are performed at several defined time points to receive kinetic results for each parameter.

Our cell culture system has been validated by analyzing the AGA2 mouse, which is a model for osteogenesis imperfecta. We found increases in the cellular protein content, the metabolic and ALP activities, and concomitant decreases in matrix mineralization, nodule formation and collagen secretion. Consequentially this leads to a delay in the osteoblast differentiation process in AGA2, as also confirmed by Real-Time PCR. Additionally, results from other bone-related mutant lines will be discussed.

We have established a standardized cellular screening system for osteoblasts and osteoclasts. By determining and quantifying several cellular and molecular parameters, the comparison between diseased mice and their wild type littermates can provide a deeper insight into the cellular and molecular mechanisms / genesis of bone diseases. We have validated our system using a known bone mutant line, and plan to incorporate it in the GMC to analyze many other lines.

Conflict of Interest: None declared

P190-S

STEM CELL TRANSPLANTATION FOR TREATMENT OF CONGENITAL PSEUDOARTHROSIS OF THE TIBIA –PRELIMINARY RESULTS FROM 2 CELL TRANSPLANTATIONS

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Congenital pseudoarthrosis of the tibia (CPT) is a rare disorder with occurrence rate of 1 in 140,000 to 250,000 births in the general population. Approximately 50% of cases of CPT occur in children with neurofibromatosis. Orthopaedic treatment of congenital pseudoarthrosis of the tibia is difficult and currently, three surgical techniques are being used: free vascularized fibular grafting, intramedullary rodding and external fixation.

We represent two cases with mesenchymal stem cell (MSCs) transplantation to 6- and 12-year-old boys with CPT and neurofibromatosis type 1, who had gone trough various treatments and operations since birth. After several failed operations it was decided to transfer MSCs into the recurrent pseudoarthrosis sites after ethical committee's approval. After resection of the pseudoarthrosis, drilling of both bone marrow canals (proximal and distal ends of the tibia) was performed. Cultured MSCs were injected in solution into these canals and around the resection line or bone defect in collagen sponge. The transferred cells were partly undifferentiated MSCs and for 2 weeks differentiated osteoblast. After MSCs transplantation the patients were followed for ten months to see progression, totally 3 sites (2 + 1) were treated (pseudoarthrosis + bone defect, pseudoarthrosis).

In both cases, progressive angulation of the osteotomy and bone resorption at pseudoarthrosis site was observed despite external fixation. In patient 1, bone defect between vascularised fibula –distal tibia was healed, but the pseudoarthrosis re-evolved around fixation pin. In patient 2 the pseudoarthrosis showed some stability, which was later confirmed by ct, that showed comminuent bridging across pseudoarthrosis. For clinical reasons, both extremities were amputated and bone samples were prepared and analyzed by different histological methods. Preliminary results show that MSCs promote both bone formation and resorption in the re-created medullary cavity and improve the mineral content and lamellar structure of the bone. The number of osteoclasts in the cortical bone was six-fold higher compared to initial situation before MSCs transfer.

Our data suggests, that MSC transplantation is one of the potential possibilities in the treatment of CPT caused by neurofibromatosis type 1. One possible reason for not finding a desired clinical effect in the presented cases was that disease was advanced and MSC transfer was too late.

Conflict of Interest: None declared

P191-M

THE MECHANOSENSITIVE PART OF A SINGLE OSTEOCYTE, CELL BODY OR CELL PROCESS?

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Background: Osteocytes are the mechanosensors of bone. Their excitation mechanism might be due to a unique strain amplification resulting from the interaction of pericellular matrix and cell process cytoskeleton. Hence, it becomes important to delineate the role of different parts of the osteocyte, i.e. cell body and cell processes, in mechanosensing, and thus bone remodelling. Nitric oxide (NO) is essential for load-induced bone formation in vivo, and it is rapidly increased in response to mechanical stress in bone cells, including isolated osteocytes. Here, we demonstrate upregulation of intracellular NO production in single osteocytes after localised mechanical stimulation of cell body and a cell process.

Methods: A single DAR-4M AM-loaded MLO-Y4 osteocyte-like cell was subjected to a localized oscillatory mechanical stimulus of 10–20 nN using an Eppendorf micromanipulator. DAR-4M AM chromophore is an intracellular fluorescence NO indicator. Fluorescence images were recorded by using rhodamine filter (excitation $\lambda = 554$ nM; emission $\lambda = 572$ nM).

Results: Mechanical stimulation of the cell body of single osteocytes ($n = 2$) resulted in 15% fluorescence increase over the non-stimulated state, whereas the reference cell showed 1% fluorescence increase during the same time period. Mechanical stimulation of a single cell process resulted in a slow but prolonged 2–10% increase in fluorescence intensity in stimulated cells ($n = 2$) as compared to the respective fluorescence intensity just before stimulation. There was $< 1\%$ increase in fluorescence intensity in the non-stimulated reference cell.

Conclusion: NO is essential for mechanically induced bone remodelling, and is a meaningful parameter for measuring bone cell activation after mechanical loading. Here we demonstrate NO upregulation in individual bone cells after a localised mechanical stimulation by using DAR 4M AM chromophore. We also show that both cell body and cell processes of a single osteocyte might be involved in mechanosensing. This opens up the possibility to uncover the complexities and function of single osteocytes in the dynamic process of bone remodelling.

Conflict of Interest: None declared

P192-T

BMP-6 REGULATES GLUCOSE AND BONE METABOLISM VIA FATTY ACID AND INSULIN GROWTH FACTOR I SYNTHESIS

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We have recently shown that human recombinant BMP-6, given systematically, reduces glycemia in rodent models of diabetes and restores bone in animal models of osteoporosis (Simic et al, J Biol Chem 2006). To elucidate the underlying mechanisms of reduction of blood glucose levels following systemic application of BMP-6 we conducted gene expression experiments using liver samples of insulin free non obese diabetic (NOD) mice, a well established model of type I diabetes. Quantitative PCR (q-PCR) showed that one dose of BMP-6 (60 μg/kg i.v.) reduced gene expression of fatty acid synthase (FAS) by 2.5 fold. FAS is one of the key enzymes involved in de novo fatty acid synthesis.

Since it is well documented that fatty acids play an important role in bone metabolism, we investigated the influence of endogenous and exogenously applied BMP-6 on FAS gene expression in the bone tissue of wild type (WT) and BMP-6 Knock-out (BMP-6 KO) mice that have reduced number of Langerhans islands, increased levels of the blood glucose and reduced trabecular bone volume of the peripheral skeleton. Animals were OVX at 3 months of age and 3 weeks later therapy started and continued for the next 4 weeks as follows: (1) sham, (2) OVX + vehicle and (3) OVX + BMP-6 (10 μg/kg i.v. 3 $\times$ weeks) ($n = 15$). Total RNA was isolated from whole femurs and tibia and q-PCR analysis was performed. Sham BMP-6 KO mice had by 2.3 fold reduced expression of FAS as compared to wild type sham mice. Systemically applied BMP-6 did not alter FAS expression in bone tissue of either WT or BMP-6-KO mice.

Since dietary lipids alter the concentration of IGF-I in bone tissues, we analyzed gene expression of IGF-I in the bone by q-PCR and serum levels of IGF-I by ELISA. BMP-6 KO mice have reduced gene expression of IGF-1 by 4.65 fold. Systemic application of BMP-6 increased IGF-I gene expression in both WT and KO mice so that the difference in IGF-I level between WT and BMP-6 KO mice was reduced to 1.7 fold change. Serum concentration of IGF-I was lower in BMP-6 KO mice as well, and BMP-6 application reduced the difference between WT and BMP-6 mice.

In conclusion, systemic administration of BMP-6 decreases the expression of FAS in the liver of NOD mice and endogenous BMP-6 influences FAS expression in bone tissues. We suggest that reduced bone trabecular volume of BMP-6 KO mice could in part be a consequence of reduced levels of FAS that subsequently influence the IGF-I gene expression and serum levels.

Conflict of Interest: None declared

P193-S

OPTIMIZATION OF EXPRESSION AND PURIFICATION OF RECOMBINANT BONE MORPHOGENETIC PROTEIN-6 (RBMP-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). However, mice with BMP-6 gene knock-out do not show obvious anatomical deficiencies in contrast to pathological phenotypes of mice with BMP-2, BMP-5 and BMP-7 knock-out, respectively. In order to further investigate biological activities of BMP-6 in vitro and in animal models, we developed a practical method of BMP-6 production and purification. A BMP-6 expression vector was transfected via Lipofectamine into 293 cells for transient protein production. In addition, such a construct was transfected into dhfr(-) Chinese hamster ovary cells (CHO), for establishing stable producer lines, with subsequent growth in selective media and gene amplification using appropriate markers. Different culture media and growth temperatures and harvest times were explored to optimize the production levels. Harvest media were processed over Cobalt-IMAC resin resulting in highly enriched BMP-6 material with biological activity in the alkaline phosphatase assay using C2C12 cells. In the rat implant bioassay the protein successfully induced new bone formation. Final purification steps will be discussed.

Conflict of Interest: None declared

P194-S

ICAM-1 EXPRESSION IN A CO-CULTURE OF HUMAN BONE LINING CELLS AND OSTEOCLAST PRECURSORS INCREASES DURING OSTEOCLASTOGENESIS

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Adhesion between bone lining cells and osteoclast precursors is necessary for osteoclast formation. Some data suggest that this might be accomplished by Intercellular Adhesion Molecule-1 (ICAM-1) that is expressed by bone lining cells and binds to Leukocyte Function-associated Antigen-1 (LFA-1) expressed by osteoclast precursors. ICAM-1 is also expressed by osteoclast precursors, but whether it plays a role in the process of osteoclast formation is not known. In the present study we investigated the expression of ICAM-1 at different time points during osteoclastogenesis. We performed PCR-analysis and immunohistochemistry on (i) human bone lining cells, (ii) osteoclast precursors and (iii) osteoclast-like cells in a co-culture of bone lining cells and osteoclast precursors. During the first three days of co-culturing, no differences in ICAM-1 expression were observed. Most of the bone lining cells and osteoclast precursors were ICAM-1 positive. After one week, mRNA expression of ICAM-1 significantly increased in the co-culture. ICAM-1 immunostaining showed that this increase was due to an increase in ICAM-1 on osteoclast precursors which had migrated to the surface of the culture dish. A decreased expression was found by the bone lining cells surrounding the osteoclast precursors. When the co-culture was stained for ICAM-1 at the 3 week time point when osteoclast-like cells were formed, some of these cells were positive whereas others did not reveal ICAM-1 staining. Our data suggest that the role of ICAM-1 in a co-culture of bone lining cells and osteoclast precursors changes during osteoclast formation. The decreased expression of ICAM-1 over time by bone lining cells could indicate that ICAM-1 plays a role in the initial interaction of bone lining cells and osteoclast precursors and that at a later stage when the osteoclast precursors migrate to the surface such an ICAM-1-mediated interaction is no longer required.

Conflict of Interest: None declared

P195-M

THE UTILITY OF MEASURING TRACP5B TO MONITOR TREATMENT RESPONSE IN CLINICAL CONDITIONS AFFECTING BONE

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Bone turnover markers are used to monitor treatment of disorders affecting bone. N- and C-terminal telopeptides of type I collagen (NTX and CTX) and deoxypyridinoline crosslinks (DPD) are released following bone degradation. Tartrate resistant acid phosphatase isoform 5b (TRACP5b) is a marker of osteoclast number and activity. The aim of this study is to compare the treatment response of TRACP5b with established markers of bone resorption in patients with clinical conditions that affect bone.

The study groups were: primary hyperparathyroidism (PHPT) treated with parathyroidectomy and studied within 1 week or later after surgery ($n = 15$),

hyperthyroidism treated with carbimazole (n = 7), hypothyroidism treated with L-thyroxine (n = 4), Paget's disease treated with bisphosphonates (n = 4), and osteoporosis treated with teriparatide (n = 5). Serum resorption markers TRACP5b and βCTX and urine resorption markers NTX/Cr and fDPD/Cr were measured on fasting early morning samples pre- and post treatment.

In PHPT patients assessed within one week of surgery, the mean decrease from baseline in TRACP5b was 17% ($p < 0.005$), βCTX 51% ($p < 0.05$), fDPD/Cr 8% and NTX/Cr 60% ($p < 0.02$). Between one and ten months post surgery, TRACP5b decreased by 51% ($p < 0.002$), βCTX by 84% ($p < 0.05$), fDPD/Cr by 43% ($p < 0.01$) and NTX/Cr by 82% ($p < 0.02$). In successfully treated hyperthyroid patients, the change in TRACP5b was not significant, whereas βCTX, fDPD/Cr and NTX/Cr decreased by 44%, 63% and 48% respectively ($p < 0.05$). In successfully treated hypothyroid patients, TRACP5b increased from baseline by 28%, βCTx by 62%, fDPD/Cr by 72% and NTX/Cr by 25%. Paget's patients were treated for a mean period of 19 months. TRACP5b decreased by 16%, βCTX by 51%, fDPD/Cr by 51% and NTX/Cr by 77%. In osteoporosis patients treated with teriparatide for three months, TRACP5b increased by 47% ($p < 0.02$), βCTX by 108% ($p < 0.05$), fDPD/Cr by 13% and NTX/Cr by 122%.

This study demonstrates the importance of measuring a broad repertoire of bone resorption markers since different markers respond to a greater degree in different disease states. The percentage change in TRACP5b was less than for other markers, but did achieve statistical significance even though the numbers were small. TRACP5b can be measured reliably in serum, is not affected by fasting state and has low diurnal variability. Its utility in monitoring therapy of diseases affecting bone merits further study in larger patient groups.

Conflict of Interest: None declared

P196-T

THE REGULATION AND ENZYMATIC BASIS OF BONE RESORPTION BY HUMAN OSTEOCLASTS

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There have been considerable advances recently in our understanding of the mechanisms that regulate osteoclastic differentiation. However, much less is known of the means through which the resorptive activity of these cells is controlled. This is especially so for human osteoclasts. We have developed an assay that allows us to measure resorptive activity while minimising confounding effects on differentiation. We achieved this by optimising osteoclastogenesis, so that resorption could readily be detected over a very short incubation period (a few hours). Additionally, to minimise the variance of our results, we related the resorption that occurs in each culture during the test period to the resorption that had occurred in the same culture in a prior, control period. Using this approach, we found that Receptor-Activator of NF-kappa B Ligand (RANKL) strongly stimulated release of the collagen degradation fragment CTX-I by osteoclasts over a similar range to that over which it induced osteoclastic differentiation. This suggests that RANKL exerts an action on osteoclastic function distinct from its ability to induce differentiation. Calcitonin dose-dependently inhibited bone resorption, while PTH, IL-1, TNF-alpha, IL-6, IL-8, VEGF, MCP-1, MIP-1gamma, IFN–gamma and dibutyryl cyclic GMP showed no significant effect. Calcium ions, cyclosporin A, IFN-beta and dibutyryl cyclic AMP all strongly suppressed resorption. Bone resorption was also suppressed by the bisphosphonate alendronate. We next tested the relative role of Cathepsin K and MMPs in osteoclastic bone resorption. We found that the non-specific cysteine protease inhibitor E64, and the cathepsin K-inhibitor MV061194 strongly suppressed bone resorption. In contrast, inhibitors of matrix metalloproteases (MMPs) had no effect on CTX-I release. Although it is controversial whether or not CTX-I detects MMP products, MMPs have been shown to increase the amount of CTX-I released from collagen by Cathepsin K, so that this result does not support a role for MMPs in bone resorption by osteoclasts. Furthermore, release of the MMP-derived collagen fragment ICTP represented less that 0.01% of the quantity of CTX-I released in our cultures. This suggests that MMPs make at most a very small contribution to the bone-resorptive activity of osteoclasts. Thus, bone resorption by osteoclasts is overwhelmingly Cathepsin K-dependent.

Conflict of Interest: TJ Chambers, Medivir UK, Consultant

P197-S

C-SRC KINASE INHIBITOR AZD0530 INHIBITS THE FORMATION AND ACTIVITY OF HUMAN OSTEOCLASTS

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Targeted disruption of c-Src in gene knock out studies in mice primarily resulted in defective osteoclast function, with mice developing osteopetrosis. Therefore, drugs designed to selectively inhibit c-Src activity could interfere with osteoclast activity. We tested the effect of the dual Src/Abl kinase inhibitor AZD0530 on (i) osteoclast formation by peripheral blood mononuclear cells (PBMCs) co-cultured with osteoblasts, and (ii) osteoclastic bone resorption. Different concentrations (0.1–10 μM) of the inhibitor affected neither osteoblast morphology nor adhesion of PBMC to the osteoblasts. However, AZD0530 inhibited the formation of multinucleated osteoclast-like cells dose dependently. PBMC-osteoblast co-cultures were then exposed to different periods of time in the presence of 1μM AZD0530. AZD0530 was most effective in inhibiting the formation of osteoclast-like cells when added at the onset of osteoclastogenesis, suggesting that c-Src is important during the initial induction of osteoclast formation. Since formation of actin rings, to which c-Src co-localizes, is a prerequisite for osteoclastic bone resorption, the effect of AZD0530 on actin rings was analysed by employing the co-culture system on cortical bone slices, .AZD0530 did not only prevent migration of osteoclast precursors to the bone surface, but also the subsequent formation of actin rings. Following withdrawal of the drug, this process proved to be reversible. Culture conditions where actin rings were found (3 weeks without or 1 week with AZD0530 followed by 2 weeks without) coincided with cultures where bone resorption was observed. Our data suggest that c-Src activity is pivotal for the formation and activity of osteoclasts. The reversibility of AZD0530's effect on osteoclast formation and activity makes it a candidate drug to temper osteoclastic bone degradation in bone diseases with enhanced osteoclastic activity.

Conflict of Interest: Teun J. de Vries, Royal Netherlands Academy of Arts and Sciences Tim Green, AstraZeneca

P198-M

IL6 INHIBITS RANKL-INDUCED OSTEOCLASTOGENESIS BY DIVERTING CELLS INTO THE MACROPHAGE LINEAGE: IMPLICATION OF STAT3

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Osteoclasts are bone-resorptive multinucleated cells which differentiate from hematopoïetic precursors closely related to the monocyte/macrophage lineage in the presence of M-CSF and receptor activator of NF-kB ligand (RANKL). Previous studies demonstrated that IL6 indirectly up-modulates osteoclast differentiation through the production of RANKL by osteoblasts. However, only few data are available concerning the direct effect of IL6 on osteoclasts. To investigate the role of IL6 on osteoclast differentiation, we used the monocyte/macrophage cell line RAW264.7 which differentiates into osteoclast in the presence of RANKL. We showed that the addition of IL6 inhibited RANKL-induced osteoclastogenesis in a dose-dependent manner. Furthermore, this effect was irreversible as RAW264.7 were resistant to RANKL action after 3 days in culture with IL6. RT-PCR and Flow cytometry analysis showed that IL6 decreased the expression of osteoclasts markers such as TRAP, Calcitonin Receptor and CD44. Interestingly, RAW264.7 cultured in presence of RANKL and IL6 expressed macrophage markers such as CD11b and CD16/CD32. Thus, our study showed for the first time that IL6 inhibits RANKL-induced osteoclastogenesis by diverting cells into the macrophage lineage. We next investigated the mechanism involved in this inhibition. We focused on the transcription factor STAT3 which is the main signaling molecule activated after IL6 stimulation. We used 2 STAT3 inhibitors (AG490 and STAT3 inhibitor peptide) but any of them prevented the IL6 effect. However, these experiments revealed that STAT3 is mandatory for osteoclastogenesis. Indeed both inhibitors completely abolished RANKL-induced osteoclastogenesis of RAW264.7 cells. We demonstrated that a basal level of phosphorylated-STAT3 on serine727 associated to an absence of phosphorylated-STAT3 on tyrosine705 is correlated with osteoclastogenesis. Furthermore, stimulation with IL6 induced both serine and tyrosine phosphorylations, and consequently RAW264.7 generated macrophages instead of osteoclasts. With AG490, phosphorylated-STAT3 on serine727 considerably decreased, and no osteoclasts were formed. Finally, we confirmed the IL6 inhibitory effect on osteoclasts differentiation by using freshly isolated mouse bone marrow precursors and human PBMCs. In conclusion, we demonstrated the importance of the level of activated-STAT3 and its form of activation (tyrosine or serine phosphorylation) in the control of osteoclastogenesis.

Conflict of Interest: None declared

P199-T

THE LEVELS OF BONE TURNOVER MARKERS IN PERIPUBERTAL GIRLS

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Puberty is a time of large increase in bone mass and it is associated with high values of bone turnover markers. During puberty, bone turnover is affected by bone remodeling, bone modeling and growth. Little research has been conducted on serum levels of bone turnover markers of children at different ages. We studied the association of TRACP 5b with other biochemical markers of bone turnover and with bone mineral density (BMD). A total of 170 healthy peripubertal Caucasian girls aged 10–19 years were included in the study. The following serum markers of bone turnover were measured: tartrate-resistant acid phosphatase (TRACP) 5b, a marker of osteoclast number; C-terminal cross-linked telopeptides of type I collagen (CTX), a marker of osteoclast activity; bone-specific alkaline phosphatase (BAP), a marker of osteoblast number; and procollagen I N-terminal propeptide (PINP), a marker of osteoblast activity. The mean activity of a single osteoclast was determined at each age group using the resorption index CTX/TRACP 5b, and the mean activity of a single osteoblast using the formation index PINP/BAP. BMD was measured from lumbar spine (LBMD) and femoral neck (FBMD) at baseline and at 2 years. All bone turnover markers correlated significantly with each other and with LBMD and FBMD. Highest levels of all bone turnover markers were observed at the age of 10–12. The values decreased during aging until they reached the normal adult levels at the age of 17. BAP and TRACP 5b decreased at a similar rate, while PINP decreased at a higher rate and CTX at a lower rate. As a result, the resorption index increased and the formation index decreased up to the age of 17. These results demonstrate that bone turnover markers decrease and correlate significantly with BMD in growing children. The number of osteoclasts and the number of osteoblasts is decreased at a similar rate. However, osteoblast activity is decreased at a higher rate than osteoclast activity, and the mean activity of a single osteoblast is decreased, while the mean activity of a single osteoclast is increased up to the age of 17.

Conflict of Interest: None declared

P200-S

CHARACTERIZATION OF THE ROLE OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR (PPAR)-DELTA IN OSTEOCLAST BIOLOGY

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Peroxisome Proliferator-Activated Receptors (PPARs) belong to the nuclear steroid hormone superfamily and three isoforms have been isolated: PPARalpha, PPARgamma and PPARdelta, each presenting typical tissue distribution and specific and in some cases opposite roles. In this study we investigate the role of PPARdelta in osteoclast biology.

We propose that a conditional null of the nuclear hormone receptor PPARdelta in OCLs will dysregulate osteoclastogenesis and alter the skeletal structure of affected mice. Using this approach together with gene expression profiling we can identify novel targets of PPARdelta that are functional in bone resorption. We investigated PPARdelta function in osteoclast-like cells (OCLs) in vitro by using GW501516, a highly specific PPARdelta agonist. We use the RAW/C4 cell line, which is a sub-clone of the RAW264.7 macrophage cell line with enhanced potential to differentiate into OCLs. We found that PPARdelta is the prevailing isoform in these cells and that its expression is markedly upregulated during OCL differentiation whereas the other isoforms decrease in expression. GW501516 increases the expression of the typical OCL markers tartrate acid phosphatase (TRAP), cathepsin K and calcitonin receptor during OCL differentiation. Interestingly GW501516 decreases the levels of expression of all three PPAR isoforms during differentiation. The transcriptional regulation of target gene, peroxisome proliferator response elements (PPREs) by PPARdelta in RAW/C4 cells was found to require exogenous retinoid X receptor (RXR) and peroxisome-proliferator-activated receptor-gamma co-activator 1alpha (PGC-1alpha) for an optimal activation. Under these conditions the addition of GW501516 (1muM) maximises PPRE responsiveness to PPARdelta-mediated transcription. We also have generated transgenic mouse lines with the objective of conditionally knock-out PPARdelta specifically in OCL by using the Cre-LoxP system and use these to characterize PPARdelta-null phenotype in bone.

The characterization PPARdelta 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

P201-M

CHARACTERISATION OF A NOVEL ANTIGEN EXPRESSED IN OSTEOCLASTS AND OSTEOBLASTS

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Screening of a panel of monoclonal antibodies raised against human bone cells has identified a novel antigen expressed at a high level in osteoclasts with a lower abundance in osteoblasts, including osteoblast-like osteosarcoma cell lines. We are characterising this antigen using a combination of immunofluorescence, confocal microscopy, dot blotting and Western blotting.

Immunohistochemistry has revealed that this novel antigen can be detected in the human osteosarcoma cell lines, MG63, TE85, SaOS-2 but not rat UMR106 cells. Confocal microscopy has shown the antigen to be distributed throughout the cytoplasm in MG63, TE85 and SaOS-2 cell lines. Two patterns of staining have been seen, punctuate staining surrounding the nucleus and radiating throughout the cytoplasm and/or intracellular ribbon-like staining. Co-localisation studies have ruled out mitochondrial localisation of the antigen. Cells fixed at different stages of confluence do not explain the reason for variation in pattern of staining; however it is likely that the variation in staining pattern is dependent upon stage of differentiation of the cells.

We have utilised the rapid growth of osteosarcoma cells to produce sufficient antigen for protein characterisation. Dot blotting has shown that extracts of cellular protein from MG63, TE85 and SaOS-2express the antigen, with the greatest expression in TE85 cells. However, interestingly MG63 cell culture medium is the only one to contain the antigen. Western blotting has shown immunoreactive bands in MG63 and TE85 cell protein extract which vary between 70 and 250kDa possibly due to variation in cleavage of the antigen or antigen complex formation.

The variation in expression of antigen between cell protein extract and culture medium and in its intracellular distribution suggests it may be linked with membrane transport. The specific expression in osteoblasts and osteoclasts but not in non-skeletal cells suggests that the antigen may play important roles in bone formation and resorption.

Conflict of Interest: None declared

P202-T

RELEASE OF INFLAMMATORY CYTOKINES BY MONONUCLEAR CELLS FROM PERIPHERAL BLOOD UPON EXPOSURE TO METAL IONS

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Aseptic loosening of arthroplasties is a long-term complication in an otherwise most beneficial surgical procedure. The longevity of the implant can be affected by a number of factors, one being the sensitivity of the recipient to the alloy of the implant. In this study we continued our investigations on the release of inflammatory cytokines and the gene expression of peripheral blood mononuclear cells (PBMC) treated with Co^{2+} and Ni^{2+} salts. PBMC were isolated from buffy coats by Ficoll/Hypaque gradient centrifugation. The cells were seeded into culture dishes. Adherent (monocytes) and non-adherent (lymphocytes) cells were separated after 3h and cultured separately. Ni2+ and Co2+ were added at 1, 10, and 100 uM and a time course for the release of tumor necrosis factor-alpha (TNF), interleukin-1beta (IL1) and interleukin 6 (IL6) (ELISA) and the expression of the respective transcripts (RT-PCR) was established. Significant amounts of the cytokines were released only, when the cells were treated with Ni^{2+} and Co^{2+} at 100 uM. Levels of TNF in the culture media peaked after 7 – 10h, the concentration being higher in cultures of monocytes and lymphocytes alone as compared to cultures of total PBMC (450 pg/ml and 500 pg/ml vs. 60 pg/ml). Transcript levels mirrored the early release of TNF. The kinetics of the release of IL1 and IL6 were similar, highest levels were determined after 24h and 48h. The concentrations of IL1 and IL6 were similar in cultures of monocytes, lymphocytes and of total PBMC (IL1: 700 pg/ml, 600 pg/ml, and 600 pg/ml; IL6: 14,000 pg/ml, 14,000 pg/ml, and 14,000 pg/ml). In each of the 3 cell cultures, the release of TNF was highest with Co^{2+}, while the release of IL1 and IL6 after 24h and 48h was similar for Ni^{2+} and Co^{2+}. To assess, whether the release of IL1 and IL6 was caused by autocrine stimulation through TNF, the cells were treated with anti-TNF antibodies. The block of TNF, however, did not affect the release of IL1 and IL6. The data demonstrates that (i) the response of PBMC from healthy donors to an exposure to metal ions was divided into an early (TNF) and a late response (IL1 & IL6), and (ii) the release of TNF by monocytes and lymphocytes was higher than the response of total PBMC, suggesting an inhibitory interaction between the cells. Comparing the data from separate donors, large differences became evident, these differences in sensitivity possibly being responsible for the individual reaction of the recipient to an implant.

Conflict of Interest: None declared

P203-S

HYDROGEN PEROXIDE AS A REGULATOR OF RANKL-INDUCED CALCIUM OSCILLATIONS AND OSTEOCLAST DIFFERENTIATION

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Receptor activator of NF-kappaB ligand (RANKL) generates the intracellular concentration of Ca^{2+} ($[Ca^{2+}]i$) oscillations that activate calcineurin, a Ca^{2+}/calmoduline dependent phosphatase, and subsequently regulate the activities of NFATc1. However, the mechanism of RANKL-induced Ca^{2+} oscillations in osteoclast differentiation is unclear. Here, we investigated a role of reactive oxygen species (ROS) in the induction of Ca^{2+} oscillations and osteoclast differentiation in bone-marrow derived macrophage (BMM) and cell line. RANKL generated $[Ca^{2+}]i$ oscillations via the modulation of phosphorylation of phospholipase Cgamma1 (PLCgamma1), Ca^{2+} release from 1,4,5-trisinositolphosphate receptors of endoplasmic reticulum, and subsequent store-operated Ca^{2+} entry. The ROS was also generated from 24 h to 72 h after RANKL stimulation and affected the phosphorylation of PLCgamma1 and the generation of Ca^{2+} oscillations. Dominant negative form of Rac1 or peroxiredoxin II (PrxII) overexpression inhibited RANKL-induced Ca^{2+} oscillations and osteoclast activities. In contrast, constitutively active form of Rac1 or dominant negative form of PrxII overexpression generated $[Ca^{2+}]i$ oscillations, which mimicked RANKL-induced Ca^{2+} oscillations. Moreover, in PrxII knockout mice, Ca^{2+} oscillations were autonomously generated via the phosphorylation of PLCgamma1 and NFATc1, and great decrease in bone density was observed. These results suggest that RANKL induces hydrogen peroxide production through Rac1 activation and produced hydrogen peroxide regulates the generation of Ca^{2+} oscillations which finally trigger late-stage of osteoclast differentiation.

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Conflict of Interest: The Ministry of Health and Welfare, South Korea, Grants Research Support (A050002 and A060084)

P204-M

THE EFFECTS OF VARIOUS GROWTH FACTORS ON HUMAN MESENCHYMAL STEM CELL EXPOSED TO TITANIUM PARTICLES

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Back ground: Success of total hip arthroplasty is limited by periprosthetic osteolysis caused by particulate wear debris. Because mesenchymal stem cells (MSCs)have an osteoprogenitor function and are critical contributors to osseous tissue integrity, the disturbance in their functions can contribute to osteolysis. The purpose of this study was to confirm the hypothesis that various growth factors reduce the suppressive effect of titanium particles on MSCs Methods: Cultured human MSCs at passage 3 were challenged with the prepared cpTi particles at the concentration of ten million/ml along with one of growth factors: TGF-b, FGF-2, IGF-I and BMP-6. After various periods of time, their effects were measured. Cell proliferation and viability was assess with BrdU proliferation assay and MTT assay. Apoptosis was assessed using the TUNEL method. Osteoblastic differentiation was assessed with RT-PCR for type I collagen, alkaline phosphatase, osteocalcin and bone sialoprotein. Results: 1) Proliferation and viability of hMSCs: cpTi caused 10–15 % reduction on the BrdU uptake after 24h, 72h, 108h ($p < 0.05$). TGF-b (10ng/ml) gradually reversed this reduction, reaching 5% higher than the control at 108h. FGF (10ng/ml) greatly increased the uptake by 30–50% ($p < 0.01$). IGF (100ng/ml)and BMP-6 (50ng/ml reduced the suppressive effect of Ti from throughout the three time period ($p < 0.05$). 2) Viability of hMSCs. With Ti, 7% reduction in viability was observed compared with the control after 7 days. Growth factors reversed this reduction and enhanced the cell viability by 13% to 30%. 3) Apoptosis of hMSCs: 10% of cell showed signs of apoptosis in the control medium. With Ti particles, 40% of cells were apoptotic. With growth factors, the percentage of apoptotic cells were 8 to 17%. 4) Osteoblastic differentiation of hMSCs: The expression of type I collagen decreased with Ti challenge. TGF-b furthered this effect. However, IGF and BMP-6 reversed this negative effect of Ti particles. The expression of alkaline phosphatase and osteocalcin were not affected by Ti or growth factors. Interestingly, Ti particle increased the expressions of mRNA of BSP. TGF-b and FGF reversed this increase. With IGF and BMP-6, there were no notable change. Conclusion: This study showed that various growth factors mitigated the suppressive effect of Ti particles in cell proliferation and viability. IGF-I and BMP was effective in promoting the expression of osteogenic genes suppressed by cpTi particles.

Conflict of Interest: Grant/Research Support: Korean Science and Engineering Foundation (R01-2006-000-10432-0) .

P205-T

EFFECT OF THALIDOMIDE ON THE FORMATION OF MURINE OSTEOCLASTS IN VITRO

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Thalidomide is currently arousing much interest and hopes, despite its proven teratogenic action. In recent years, a number of cases of positive action of the drug have been reported in the treatment of multiple myeloma, chronic graft-versus-host disease, tumors of various organs, severe autoimmunological syndromes and many other diseases. It is administered, among others, in the treatment of erythema nodosum leprosum, aphthous stomatitis, sarcoidosis, atopic dermatitis. Because of a broad range of indications for thalidomide, it seemed purposeful to investigate effects of the drug on the bone tissue.

The aim of the present study was to examine the effect of thalidomide on the formation of osteoclasts from neonatal mouse bone marrow cells *in vitro*. Osteoclast formation was stimulated by 1,25-dihydroxyvitamin D_3 added to the culture media on the next day after plating, together with thalidomide or its solvent (DMSO). Thalidomide was used at final concentrations of 0.5 mug/ml, 2.5 mug/ml, 12.5 mug/ml and 25 mug/ml. The bone marrow cell culture lasted 9 days and then histochemical staining for tartrate-resistant acid phosphatase (TRAP) was performed. Multinucleated TRAP-positive cells were considered osteoclasts. Osteoclast number and size were determined.

Thalidomide caused concentration-dependent increases in the number and size of osteoclasts. Results of the present study indicate the possibility of increased bone resorption during thalidomide treatment.

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Conflict of Interest: None declared

P206-S

ROLE OF PLCGAMMA2 IN OSTEOCLASTS DIFFERENTATION AND FUNCTION

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Osteoclast function is important for normal homeostasis of the skeletal system. Previous studies suggested that PLCgamma2 may have a role in osteoclasts differentiation and function. This possibility was tested using mice genetically deficient of PLCgamma2. Furthermore, we attempted to test which of the known osteoclast signal transduction pathway requires PLCgamma2 for proper function.

To overcome the limited survival of PLCgamma2-deficient mice, PLCgamma2–/– bone marrow cells were transplanted into lethally irradiated wild type recipients. Non-adherent bone marrow cells obtained from femurs and tibias of such chimeras or of intact wild type or various other knockout mice were cultured in the presence of M-CSF and RANKL. Osteoclast differentiation was assessed by histochemical TRAP and DAPI staining and after 3 days of culture and microscopic observation of multinucleation. The bone-resorbing capacity of the in vitro differentiated osteoclasts was determined by parallel cultures on an artificial hydroxyapatite surface. For signaling experiments, bone marrow-derived macrophages were stimulated by M-CSF or RANKL in suspension or plated on tissue culture plastic surface. Phosphorylation of PLCgamma2 and other signaling molecules was tested by immunoprecipitation following Western Blotting.

In vitro culturing of wild type PLCgamma2–/– bone marrow cells revealed that PLCgamma2 was required for the development of multinucleated osteoclasts but not for the expression of TRAP, an early marker of osteoclast differentiation. PLCgamma2–/– bone marrow cells were also unable to resorb the artificial bone surface. PLCgamma2 was activated upon adhesion of the cells but not by stimulation with M-CSF or RANK ligand in suspension.

Taken together, these results indicate that PLCgamma2 plays a critical role of the development of multinucleated osteoclasts, likely because of a defect in adhesion-receptor (supposedly integrin) signaling.

Conflict of Interest: None declared

P207-M

A GAMMA-GLUTAMYL PEPTIDE FROM ONION INHIBITS THE DEVELOPMENT AND ACTIVITY OF OSTEOCLASTS IN VITRO

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Dietary consumption of vegetables and fruits was demonstrated to exert a positive effect on bone metabolism. It was shown that supplementing the diet

of rats with onion powder (Allium cepa L.) leads to a significant inhibition of bone resorption. The active compound was identified by bioassay-guided fractionation and was found to be the dipeptide gamma-glutamyl-propenyl-cysteine-sulphoxide (GPCS). In the present study we further characterized the effects of GPCS on the recruitment and activity of osteoclasts in vitro. Osteoclast development was examined by culturing bone marrow cells (BMC) supplemented with macrophage colony-stimulating factor (M-CSF; 30 ng/ml), receptor activator of NF-kB ligand (RANKL; 5 ng/ml) and varying doses of GPCS. Osteoclast activity in the presence of GPCS was assessed (I) by determining the number of actin rings in isolated rat osteoclasts attached to glass coverslips using histofluorometry and (II) by quantifying the number of pits formed by osteoclasts cultured on dentin slices. GPCS inhibited osteoclastogenesis from BMC at concentrations of 1 mM and 10 mM by 80–100 % ($n = 6$; $p < 0.05$). Calcitonin, which was used as a positive control, caused a reduction in the number of osteoclasts at concentrations higher than 10 pM (10 pM calcitonin: −50 %, 100 pM calcitonin: −70 %). The number of actin rings, which were formed after pre-incubation for 3 h, was not changed by treatment with 2 and 8 mM GPCS for 10 and 25 min. No actin rings were observed in cells exposed to 5 nM calcitonin for 10 and 25 min. When GPCS at concentrations of 2, 4 and 8 mM was added to osteoclasts cultured on dentin slices, 8 mM GPCS caused a significant reduction in bone resorption per osteoclast (number of pits/osteoclast; control: 100 % +/− 30,8 mM GPCS: 51 % +/− 27; mean +/− SD; $n = 16$; $p < 0.05$), while 10 pM calcitonin virtually abolished pit formation. In conclusion, the data demonstrate that the protective effects of onion on bone may be caused at least in part by a direct effect on the cells of the osteoclast lineage, causing an inhibition of osteoclastogenesis and of the activity of mature osteoclasts.

Conflict of Interest: None declared

P208-T

EFFECTS OF METAL IONS ON OSTEOCLASTOGENESIS

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Metal-on-metal bearing technology, made of cobalt chromium (Co-Cr) alloys, is being used in anticipation of extending the durability of hip replacements. Increasingly, concern has been expressed that long term exposure to Co^{2+} and Cr^{3+} ions could cause DNA damage and immune dysfunction; specifically a reduction in the circulating number of CD8+ cytotoxic cells. More recently, Co^{2+} and Cr^{3+} have been shown to down-regulate the specific markers of osteoblast-like cells. Despite these observations the effects of metal ions on osteoclastic formation and differentiation have not been investigated. The aim of the current study was to elucidate the effects of various metal ions on osteoclastogenesis *in vitro*.

Human peripheral blood mononuclear cells (PBMC) were cultured onto glass coverslips and dentine slices incubated in the presence of M-CSF (25ng/ml), sRANKL (90ng/ml) and 0, 1, 10 and 100 μM Co^{2+} and Cr^{3+}. The extent of osteoclast formation (expressed as the number of TRAcP+ multinucleated cells) and lacunar resorption was determined on coverslips and dentine slices, respectively. These data were correlated with the TNFalpha, IL-1alpha and LIGHT protein levels in 24h incubation of adherent and non-adherent PBMC in response to various metal ion concentrations. All experiments were repeated at least 3 times and the differences between each group were determined using Mann-Whitney test.

Compared to untreated PBMC cultures, in the presence of 100 μM Co^{2+}, the number of multinucleated TRAcP+ cells, number of nuclei and the size of osteoclasts formed were increased by 6-fold, 4-fold and 2-fold respectively ($7.78\% \pm 0.11$, 19 ± 4.83, $62.2\ \mu m \pm 3.52$). Extensive lacunar resorption was noted in PBMC cultures treated with concentrations higher than 10 μM Co^{2+}. A dose of 100 μM Co^{2+} significantly increased the release of TNFalpha whereas lower concentrations had no effect on the parameters studied. On the other hand, only in the presence of 100 μM Cr^{3+}, a 2-fold decrease ($0.57\% \pm 0.14$) in the number of osteoclasts was evident as compared to controls. Extensive and significant lacunar resorption was noted for all Cr^{3+} concentrations. TNFalpha and LIGHT concentrations were significantly increased only at 100 μM Cr^{3+}.

In conclusion, we have shown that Co and Cr ions can induce osteoclast differentiation and lacunar resorption and that Co ions are more potent in inducing the osteoclastogenic effects as compared to Cr.

Study was supported by Furlong Charitable Research Foundation.

Conflict of Interest: None declared

P209-S

N-ACETYLGALACTOSAMINE GLYCOCONJUGATES IN BONE RESORBING OSTEOCLASTS

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The expression of the aberrant N-acetylgalactosamine (GalNAc) glycoconjugates, detected by binding of the lectin from Helix pomatia (HPA), is associated with metastatic competence and poor prognosis in a range of human adenocarcinomas. However, whether they have a functional role in the metastatic cell function, remains unknown. We analyzed HPA binding site distribution in bone resorbing osteoclasts of human and rat origin cultured on bovine bone slices with confocal laser scanning microscope. Our results show that fluorescently conjugated HPA labels strongly transcytotic vesicles carrying degraded bone from the ruffled border to the functional secretory domain (FSD) in rat osteoclasts, and also the ruffled border and Golgi in human osteoclasts. It associates with tartrate resistant acid phosphatase 5b (TRACP 5b) and Cathepsin K –containing vesicles, but does not associate with endocytosed transferrin suggesting its association with late endosomal, lysosomal and post-lysosomal compartments, but not with the early endocytotic route in osteoclasts. Our results suggest a common vesicular glycoconjugate for penetrative cell types of both pathological and physiological background, whose function, however, in penetration to tissues remains unknown.

Conflict of Interest: None declared

P210-M

CALCITONIN PARTLY COUNTERACTS THE SUBCHONDRAL BONE REMODELLING UNDERLYING EXPERIMENTAL OSTEOARTHRITIS: MORPHOMETRIC DATA FROM PQCT SCANNING

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Aim: As subchondral bone is suspected of contributing to osteoarthritic cartilage breakdown, we assessed the influence of calcitonin (CT) on trabecular bone morphometric parameters of the tibial proximal epiphysis in the early stages of canine experimental osteoarthritis. Materials and methods: Twelve dogs underwent anterior cruciate ligament transection (ACLT) in the right knee. Thereafter each of them received a daily nasal spray delivering either 400 U of CT (CT group, $n = 6$) or a placebo (placebo group, $n = 6$). The animals were killed 84 days after surgery. Both proximal tibiae of each dog were embedded in methylmethacrylate and scanned in the frontal plane by peripheral Quantitative Computed Tomography (pQCT). The slices were assessed by morphometry of subchondral trabecular bone in both medial and lateral plateaus of the right and left tibiae. Quantitative data were analyzed with two-tailed t tests. A p value < 0.05 was considered statistically significant. Results: Macroscopic signs of OA were visible in all operated knees, but were less extensive in the tibiae of the CT group than in the placebo one. In the placebo group, morphometric analysis of the pQCT scans showed a significant decrease in trabecular bone volume (BV/TV) and in trabecular thickness (Tb.Th) of the right, ACLT medial plateau, associated with a significant decrease in nodes and node-to-node struts and an increase in free ends and free-to-free struts. In CT group, BV/TV in the medial plateau did not significantly vary between the right and left tibiae, although Tb.Th was significantly lower in the ACLT medial plateau than in the control one. However, no difference was observed in the free ends, nodes, node-to-node struts and free-to-free struts between the right and left tibiae of this group. Conclusions: These data confirm the contribution of epiphyseal trabecular bone remodelling to osteoarthritis genesis in this experimental model. They also suggest that calcitonin can impede the subchondral bone loss mainly by preserving trabecular continuity, thereby preventing osteoarthritis development.

Conflict of Interest: None declared

P211-T

BONE MATRIX EXTRACT DIRECTLY AND INDIRECTLY STIMULATES BONE RESORPTION

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Bone remodelling is a complex process, which involves the coupling of bone formation to completed foci of bone resorption. The balance between these 2 processes determines if bone is lost or gained at a particular site. During bone

resorption osteoclasts release growth factors sequestered in bone matrix; their release may subsequently initiate new bone formation. Conversely, osteoblastic cells can regulate osteoclast activity through the expression of the counter-acting cytokines, RANKL and OPG. The aim of this work was to determine if factors released from bone impact on the RANKL/OPG system or on osteoclasts directly to regulate bone resorption.

An EDTA bone extract prepared from normal human cortical bone powder was partially purified using hydrophobic interaction chromatography (C18 SPE). Protein was eluted from the column using a step-wise gradient of acetonitrile in 20% increments and the fractions freeze-dried to remove the solvent. The 40% acetonitrile fraction, BE40%, contained the majority of the protein recovered and was used to treat the osteosarcoma cell line MG-63 and a murine monocyte cell line.

OPG production was inhibited after 48 hours of treatment ($p < 0.05$) in proliferating cultures of the osteosarcoma cell line MG-63 with inhibition reaching 32% after 72 hours ($p < 0.005$). In confluent cultures BE40% inhibited OPG secretion for the first 48 hours ($p < 0.05$) reaching 54% of the control values after 48 hours. However in the subsequent 24 hours OPG levels rose by 1.9-fold compared to the controls ($p < 0.05$). A model of osteoclast differentiation was developed using a clone of the murine monocyte cell line RAW 264.7 (RAW-D), which form large TRAP+ multinuclear cells (MNC) when treated with RANKL and TNFa for 3 days. When the bone extract was added to the RAW-D cells in the presence of RANKL and TNFa the formation of TRAP+ MNC was increased by 69% ($p < 0.0001$) and TRAP activity by 32% ($p < 0.005$).

Therefore bone matrix constituents were able to influence osteoclast differentiation directly and indirectly through the inhibition of OPG in osteoblastic cells. The simplest mechanism for this co-ordinated response would be the presence of one factor in the extract that is able to influence both osteoblasts and osteoclasts. More evidence on the identity of this factor will be presented.

Conflict of Interest: None declared

P212-S

RANKL-INDUCED OSTEOCLAST SURVIVAL IS ASSOCIATED WITH ENHANCED LEVELS OF MCL-1

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Mcl-1 is an anti-apoptotic member of the Bcl-2 family of proteins and is expressed in cells of the myeloid lineage such as macrophages and neutrophils. In some cell types, Mcl-1 expression is highly regulated by survival-promoting factors and prevents activation of caspases via the mitochondrial pathway. However, the role of Mcl-1 in osteoclasts is unknown. We have previously shown that RANKL protects rabbit osteoclasts from the anti-resorptive and pro-apoptotic effects in vitro of bisphosphonate drugs such as alendronate (ALN) and decreases the activation of caspase-9, although the exact mechanisms underlying the protective effect of RANKL are unclear. We therefore examined whether the pro-apoptotic effect of bisphosphonates, or the anti-apoptotic effect of RANKL, is related to changes in the level of Mcl-1 in osteoclasts.

Isolated and purified rabbit osteoclasts were treated for 48 hours with 100uM ALN and/or 100ng/ml RANKL. ALN treatment caused a reduction of ~50% in the number of adherent osteoclasts, of which ~17% were apoptotic after 48 hours. This was associated with a dramatic decrease in the level of Mcl-1 protein in the remaining osteoclasts (assessed by western blotting), although the level of Bcl-2 was not affected. Treatment of osteoclasts with RANKL alone caused a 3-fold increase in the level of Mcl-1 protein (but not Bcl-2). In the presence of ALN, RANKL restored the level of Mcl-1 to that in untreated osteoclasts and significantly decreased the proportion of apoptotic cells. In J774 macrophages, treatment with nitrogen-containing bisphosphonate also dramatically decreased the level of Mcl-1 prior to the activation of caspase-3, without any change in Bcl-2 or Bcl-xL.

These observations suggest that loss of Mcl-1 may be a major route by which nitrogen-containing bisphosphonates cause osteoclast apoptosis, probably as a result of inhibiting protein prenylation. In addition, the pro-survival effect of RANKL on osteoclasts appears to be mediated, at least in part, by enhancing the level of Mcl-1.

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Conflict of Interest: M Rogers, Procter & Gamble, Roche, Novartis, AstraZeneca, Grant Research Support, Speakers Bureau, Consultant

P213-M

A NOVEL CYTOKINE FAM3C INDUCES FORMATION OF OSTEOCLAST-LIKE CELLS IN VITRO

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To identify new genes that are regulators of differentiation and function of osteoclasts we performed a suppressive subtraction hybridization comparing cDNAs prepared from rat osteoclasts cultured either on bone or plastic. Cells were isolated from newborn rat bone marrow using magnetic beads covered with monoclonal anti-b3 integrin. mRNAs were isolated and reverse transcribed to cDNAs for amplification. One of the messages which was differentially expressed being elevated in cells cultured on bone was found to be a rat homologue of recently described human Fam3C gene (1). We cloned the full length cDNA and the rat sequence was deposited in GenBank (2).

In this study we prepared recombinant protein of rat Fam3C in Pichia pastoris with N-terminal 6-His tag. Polyclonal antibodies using expressed recombinant Fam3C were aroused in rabbits applying standard immunization protocol. Screening of different adult mouse tissues for the expression with immunohistochemical stainings or RT-PCR revealed very low tissue expression in studied major organs. In order to study the in vivo functions of this new cytokine-like protein we injected different doses of recombinant protein into mice tail veins. There was no any recorded acute phase response reactions neither any changes in body temperature, body weight or behavior of the animals. To study the role of this novel cytokine in the regulation of osteoclasts we prepared bone marrow cultures from mice and studied the effect of the recombinant protein on the differentiation of bone marrow derived osteoclasts. There was a clear dose dependent increase in the number of large tartrate resistant acid phosphatase positive multinucleated cells. We have also knocked out this gene in mice. Preliminary analysis of heterozygotes does not reveal any major anatomical abnormalities. It is of interest that Waerner et al (3) recently identified, using microarray strategy, this same gene to be a major regulator of epithelial–mesenchymal transition and named it accordingly as ILEI (*i*nterleukin *l*ike *E*MT *i*nducer).

1. Zhu, Y. et al.: Genomics 80 (2) 144 (2002)
2. Buki, K. G. and Vaananen, K: GeneBank accession number AY228475 (2003)
3. Waerner, T. et al.: Cancer Cell 10 227 (2006)

Conflict of Interest: None declared

P214-T

THE EFFECTS OF EXHAUSTIVE RUNNING EXERCISE ON BONE METABOLISM

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Elevated bone resorption is implicated in the development of stress fracture injury during repetitive loading. The response of bone turnover to exhaustive exercise is unclear. The study aim was to examine bone metabolism in response to, and during recovery from, exhaustive running. Seventeen physically-active males followed a controlled diet (8 g carbohydrate•kg−1 FFM•d−1) and refrained from exercise for 8 consecutive days. On day 4, eleven males (Ex) mean (+/-1SD) age 29(3) y completed an exhaustive exercise protocol (60 min at 65% VO2max followed by an intermittent run to exhaustion at 70% VO2max) on a treadmill. Fasting blood was obtained immediately before (BASE), during, and for 2 h after exercise, and on 4 consecutive recovery days (R1 to R4). Six males (CON) aged 26(3) y rested on day 4 and provided blood samples at BASE and on R1 to R4. Second void urine was obtained from all subjects at BASE and on R1 to R4. Plasma was analysed for a marker of bone resorption, C-telopeptide (CTX), and markers of bone formation, procollagen type I N propeptide (PINP) and bone alkaline phosphatase (bone ALP). Albumin-adjusted calcium (ACa), parathyroid hormone (PTH) and osteoprotegerin (OPG) were also measured. Urine was analysed for free pyridinoline (fPYD/Cr) and deoxypyridinoline (fDPD/Cr). All analytes were analysed using two-way repeated measures ANOVAs with the Newman-Kuels post hoc test. The area under the curve (AUC) was also calculated for markers of bone turnover. In Ex, CTX increased by 43 (19) % ($P < 0.001$) from BASE to R1, remaining elevated at R4 (30 (19) %). AUC (BASE–R4) for CTX was greater for Ex compared with CON ($P < 0.01$). fPYD/Cr was lower at R2 compared with BASE (13 (11) %, $P < 0.05$), whilst serum PINP, bone ALP and fDPD/Cr were unchanged. PTH was 2.3 (0.7) fold higher after 20 min of exercise ($P < 0.001$), but not statistically different from BASE post-exercise. ACa (7 (2) %, $P < 0.001$) and OPG (24 (19) %, $P < 0.001$) peaked at the end of exercise, and remained elevated for 2 h compared with BASE ($P < 0.05$). Exhaustive, submaximal exercise is associated with an increase in bone resorption, but not formation, with implications for the development of skeletal injury. The OPG increase may be a response to activation of bone resorption. Exercise was associated with an increase in PTH and mild hypercalcaemia.

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Conflict of Interest: None declared

P215-S

IN SILICO PROMOTER ANALYSIS OF VACUOLAR PROTON PUMP SUBUNITS

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Proton (H+) pumps serve a number of important functions in eukaryotic cells, for instance, the vacuolar ATPase (V-ATPase) in osteoclasts acidifies the resorption lacuna. We have analysed the promoter sequences of V-ATPase subunits. We were interested in comparing the transcriptional regulation of individual subunits in order to discover any common or distinct features in transcription factor (TF) distributions due to their stoichiometric amount and tissue specificity.

Promoter regions, 500bp upstream and 100bp downstream from the transcription start site, of 23 V-ATPase subunit genes were analysed in silico using tools from the Genomatix Software GmbH and Vista pipeline. Conservation rates and distribution of transcription factor binding sites (TFBSs) in each promoter were compared. Furthermore, various modules composed of two or more TFBSs were predicted and used to scan the human genome and promoters.

Preliminary studies showed several common and distinct features in V-ATPase subunit promoters. The majority of promoters contained TFBSs for regulators connected with hypoxic conditions or pH dependent activation. There were also over-represented binding sites for other TFs with low p-values, such as Ets family members including PU.1, Kruppel-like or Myc-associated zinc finger protein factors. TFBS distribution for the membrane-bound subunit a isoforms a1, a2, a3 and a4 indicated that only a3, which is highly expressed in osteoclasts, contained putative binding sites for NF-kappaB within the analysed region.

Predicted models for V-ATPase contained different sets of various TFBSs, mainly Sp-1, Egr-1 and zinc finger protein families. The models extracted from the unspecified subsets of V-ATPase subunits were common in the whole human genome and thus they can not be assumed to operate as specific regulatory complexes for V-ATPase subunits. More specific models can be built for certain subsets of V-ATPase subunits according to tissue specificity or stoichiometry.

All the results mentioned above are computer-based and thus putative, but when linked to the biological context, predicted findings may provide useful information of the transcriptional regulation of vacuolar proton pumps during bone remodelling.

Conflict of Interest: None declared

P216-M

ALTERATION OF Ca^{2+} SIGNALING AND OSTEOCLAST DIFFERENTIATION IN SERCA2+/− MICE

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In osteoclastogenesis, receptor activator of NF-kappaB ligand (RANKL) induces many signaling processes including Ca^{2+} signaling for differentiation of osteoclast. However, whether Ca^{2+} signaling is essential for in vivo bone homeostasis and osteoclast differentiation is not clear. In the present study, we investigated an effect of a partial loss of sarco/endoplasmic reticulum Ca^{2+} ATPase type 2 (SERCA2), one of the Ca^{2+} signaling component, on Ca^{2+} signaling, osteoclast differentiation and bone remodeling in SERCA2 heterozygote mice (SERCA2+/−). The expression level of SERCA2b decreased to ~40%, but the level of plasma membrane Ca^{2+} ATPase was not altered from 0h to 48h after RANKL treatment in SERCA2+/− bone marrow macrophages (BMMs). Amount of Ca^{2+} in ER, released Ca^{2+} after stimulation with ATP were decreased in SERCA2+/−. RANKL-induced Ca^{2+} oscillations were not generated at 48h after RANKL treatment in SERCA2+/−. The expression level of NFATc1 was decreased at 48h after RANKL treatment and translocation of NFATc1 into the nucleus by RANKL were not observed in SERCA2+/−. Actin ring formation by RANKL and multinucleated cells were not observed in SERCA2+/− and bone resorption activity of osteoclast in SERCA2+/− reduced to ~70%. Finally, the bone density in the femurs of SERCA2+/− increased to ~40% compared to normal mice. These results suggest that alteration of Ca^{2+} signaling by a partial loss of SERCA2 expression inhibits osteoclast differentiation and the process of in vivo bone remodeling in SERCA2+/− mice.

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P217-T

HYPEROSMOLALITY DOWN-REGULATES 1ALPHA, 25-DIHYDROXYVITAMIN D3-INDUCED OSTEOCLASTOGENESIS, SUPPRESSING THE RANKL EXPRESSION BY INHIBITING RUNX2 IN CO-CULTURE SYSTEM

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The bone integrity requires a delicate balance between the bone-forming osteoblast and bone-resorbing osteoclast. During bone resorption, bone is dissolved into high concentration of Ca^{2+}/PO4(2−)in the microenvironment where it can reach concentration as high as 40 mM. Moreover, the dissolution of bone matrix was included organic materials. In this study, we investigated how hypertonic stress down-regulate osteoclastogenesis at the molecular level in co-culture system. The role of hypertonic stress on the bone metabolism was measured the osteoclast formation and functional activity in presence of 1alpha, 25(OH)2D3 and sucrose. RT-PCR and ELISA were performed to measure the RANKL, OPG, and M-CSF expression levels by hypertonic challenge. To determine expression of RANKL regulating hypertonic stress, we was examined immunoblot for expression of Runx2 (osteoblast differentiation factor) and TonEBP (hypertonic stress resistant gene). Moreover, we determined the role of Runx2 and TonEBP in osteoclastogenesis using the siRNA tools. Hypertonic stress was significantly inhibited the number of TRAP-positive cells, bone-resorbing pit area, and RANKL expression in osteoblastic cells. Hypertonic stress was decreased Runx2, but was increased TonEBP in osteoblastic cells. RANKL expression was inhibited by knockdown Runx2 and overexpression of TonEBP. Furthermore, Knockdown TonEBP induced Runx2 expression respectively. These findings indicate that TonEBP has a role which regulates the RANKL expression by attenuating Runx2. Thus, TonEBP and Runx2 must be involved in the process of 1alpha, 25(OH)2D3-induced osteoclastogenesis in terms of signal transduction pathway. As a result, hyperosmolality can be a new candidate for modulating osteoclastogenesis and bone resorption.

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P218-S

CCAAT/ENHANCER BINDING PROTEIN (C/EBP) BETA REGULATES OSTEOBLAST AND OSTEOCLAST DIFFERENTIATION

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The leucine zipper transcription factors CCAAT/Enhancer Binding Proteins (C/EBPs) regulate proliferation and differentiation in many mammalian cell types. C/EBPbeta is expressed as 3 different protein isoforms (LAP*, LAP, LIP), of which LIP (short isoform) lacks the regulatory and transactivation domain. Data obtained from transgenic mice and cell culture experiments indicated that C/EBPbeta might play a role in bone cells, however, its precise functions remained still unclear. Here, we show that during osteoblast differentiation C/EBPbeta protein isoform levels increase, whereas they decrease during osteoclast differentiation, suggesting functions of C/EBPbeta in both cell types. We used a genetic approach to address the function of C/EBPbeta in bone. C/EBPbeta-deficient mice (1) displayed a reduction in tibia length. Histomorphometry revealed a 1.6-fold decrease in bone volume and calcein labeling showed a diminished osteoblast function. Replacement of the endogenous C/EBPbeta gene with the LIP isoform restored bone growth and even increased bone volume and osteoblast function over wild-type controls. However, osteoblast numbers were unaltered in both mouse mutants. Osteoclasts showed an increased cell size and activity in vivo in both C/EBPbeta-deficient and LIP knock-in mice. Ex vivo bone marrow cell cultures confirmed the formation of larger osteoclasts of both mutant strains. Whereas OPG (decoy receptor of RANK-L) completely abolished osteoclast formation in wild-type cultures, multinucleated osteoclasts still formed in both mouse mutants. High levels of TNFalpha were produced in cell cultures of both mutant strains and a TNFalpha antagonist completely blocked the RANK-L independent osteoclastogenesis. This suggests that C/EBPbeta regulates osteoclastogenesis by controlling TNFalpha expression.

Taken together, our data show that disturbance of C/EBPbeta affects bone homeostasis by affecting both osteoblasts and osteoclasts. Our data suggest C/EBPbeta as a novel candidate involved in bone diseases such as osteoporosis.

(1) Generously provided by E. Sterneck (E. Sterneck, L. Tessarollo and P. F. Johnson. 1997. An essential role for C/EBPbeta in female reproduction. Genes Dev 11: 2153–62)

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Conflict of Interest: None declared

P219-M

ROLE OF THE GEMINAL HYDROXYL GROUP IN TARGET ENZYME INHIBITION BY BISPHOSPHONATES AND PHOSPHONOCARBOXYLATES

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Nitrogen-containing bisphosphonates (NBPs) inhibit bone resorption by inhibiting farnesyl diphosphate synthase (FPPS), thereby preventing the post-translational prenylation of Ras, Rho and Rab family proteins in osteoclasts. By contrast, 3-PEHPC (NE10790), a weakly anti-resorptive phosphonocarboxylate (PC) analogue of risedronate (RIS), acts by inhibiting Rab geranylgeranyl transferase (Rab GGTase), exclusively preventing the prenylation of Rab proteins. The anti-resorptive potency of NBPs and PCs is determined by both their affinity for bone mineral and their ability to inhibit the target enzyme. In addition to the nitrogen-containing side chain, potent NBPs have an –OH group attached to the central carbon that, in concert with the phosphonate groups, contributes to the bone affinity of N-BPs. However, the role of the –OH group in inhibition of FPPS is less clear, and its role in PCs (in which a carboxylate group replaces one of the phosphonate groups of BPs) is even less well understood. We have therefore examined analogues of these compounds in which the –OH group has been substituted with the electronegative halogens F, Cl or Br (halo-), or simply with –H (desoxy-). We found that all the halo- and desoxy- analogues had reduced mineral affinity compared to the parent compounds. Desoxy-RIS was approximately 4-fold less potent than RIS at inhibiting FPPS in vitro, while the halo- analogues also exhibited reduced potency for inhibition of FPPS, with decreasing potency as atomic size increases (i.e. F > Cl > Br). These trends correlated with the potency of the desoxy- and halo- compounds for inhibiting Rap1A prenylation in J774 macrophages, and reducing viability of these cells. By contrast, desoxy-3-PEHPC and 3-PEHPC were equipotent at inhibiting Rab GGTase, inhibiting Rab prenylation and reducing cell viability. Interestingly, although the halo-3-PEHPC analogues and 3-PEHPC were equipotent for inhibition of Rab GGTase in vitro, these compounds showed a similar potency trend to the halo-RIS analogues in cell-based assays (F > Cl > Br), with the fluorinated analogue more potent than 3-PEHPC and the others less potent. This data indicates that the –OH group plays a role in the interaction of NBPs with FPPS, an effect that cannot be explained simply by electronegativity, since substitution with halogens of similar electronegativity reduces potency. By contrast, the –OH group is not important for the interaction of 3-PEHPC with Rab GGTase.

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P220-T

DECREASING THE PREMATURE CATABOLISM BY A SINGLE BISPHOSPHONATE INFUSION SHORTENS THE HEALING TIME IN HEMICALLOTASIS OPERATIONS. A PILOT STUDY IN 24 PATIENTS

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Introduction: The search for compounds that can enhance fracture healing has focused on compounds that speed up or increase the anabolic response like the BMPs, but rarely on the possibility to stall premature catabolism. In gonarthrosis, hemicallotasis is an alternative in the younger patients. The proximal tibia is cut and the angular deformity is gradually corrected in an external fixator. Like in any fracture situation, bone healing problems might occur and prolonged or even absent osseous healing might occur making secondary orthopedic inventions necessary.

In the present study we are evaluating if a decreased catabolism increasing the amount of callus. We evaluated the effect of one single injection of a bisphosphonate during hemicallotasis healing related to time of frame removal.

Material and Methods: In 24 consecutive gonarthrosis patients, a proximal tibial osteotomy was performed and an external frame was mounted. After reaching an ideal new position of the knee, the frame was locked and the tissue, in the fracture gap left to heal. The patients were offered one single infusion of 4 mg Zoledronic acid intravenously to strengthen the forming bony callus. 12 patients, 7 men and 5 women accepted (49.5 y) and 12 patients, 7 men and 5 women (49.6 y) decided not to have the infusion. The patients were followed to clinical healing on the basis of the radiographic healing, ultra sound examination and a subjective stability test (weight-bearing test) by a blinded observer.

Results: The frame was removed after 78.5 (SD 12.6) days in the treated group compared to 91 (SD 13.4) in the non-treated group ($p = 0.02$). One patient in the Zometa group had a delayed union (16 weeks + 5 days) compared to none in the control group. In the Zometa group 11/12 were healed before twelve weeks, at the first examination considering frame removal, compared to 5/12 (ns) in the controls. 11/12 Zometa-treated patients reported an episode of flu-like symptoms after the injections.

Conclusion: In this pilot study it appears that bisphosphonate treatment increases the strength of healing callus and allows a shorter fixation time during bone healing. A randomized study has been started.

Conflict of Interest: None declared

P221-S

MANIPULATING THE ANABOLIC AND CATABOLIC RESPONSE IN BONE GRAFT REMODELLING. SYNERGISM BY A COMBINATION OF LOCAL BMP-7 AND A SINGLE SYSTEMIC DOSIS OF ZOLEDRONATE

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Background: Remodelling of a bone graft or bone formation during the healing of a fracture can be enhanced by an anabolic substance such as a Bone Morphogenic Protein (BMP). On the other hand, BMPs also boost catabolism and might cause resorption, both of the graft but also of the new-formed callus. Bisphosphonates inactivate osteoclasts and can be used to control the resorption and in the present study the combination of both drugs was tested. Methods: Cancellous bone grafts were treated with either BMP-7 (Osigraft) or saline and placed in a bone conduction chamber implanted in the proximal tibia of rats. After two weeks an injection of zoledronate 0.1 mg/kg was given subcutaneously. The rats were killed after six weeks and graft resorption and bone ingrowth distance into the graft were measured by histomorphometric analysis.

Results BMP-7 increased new bone ingrowth distance into the graft from 2.04 mm (SD 0.98) in the controls to 3.14 mm (SD 0.93, $p = 0.007$) but also increased the resorption of new formed and old graft bone. This was counteracted by zoledronate increasing bone density to 40 (SD 9) % compared to 14 (SD 10) % in the controls ($p < 0.001$). In total, the net amount of retained bone increased by 500% when BMP-7 and zoledronate were combined. Interpretation: A bone graft can be treated with BMP-7 to enhance anabolism and increase new bone formation and at the same time be protected against premature catabolism and decrease resorption by a bisphosphonate. This combination might be useful in various conditions in orthopaedics.

Conflict of Interest: None declared

P222-M

HYPOXIA INDUCIBLE FACTORS 1 ALPHA AND 2 ALPHA ARE STABILISED IN HUMAN OSTEOCLASTS EXPOSED TO HYPOXIA

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Bone loss in humans frequently occurs in association with local or systemic hypoxia, e.g., in inflammation, fractures, infections, tumours, anaemias, airway and cardiovascular diseases or ageing. We previously showed that pO_2 as low as 0.2% strongly increased osteoclast (OC) formation (and thus resorption) in mouse marrow cultures and in human peripheral blood mononuclear cell (PBMC) cultures. We also found that the resorptive function of mature OC (unlike osteoblastic bone formation) is unimpaired even in extreme hypoxia. HIF proteins are thought to be the key transcription factors directing cell responses to reduced oxygen. HIF1α is constitutively expressed in most cells but is rapidly degraded in normoxia following (oxygen dependent) hydroxylation of a prolyl residue and subsequent ubiquitination. HIF2α is less widely expressed than HIF1α and is also rapidly degraded in normoxia. In this study we investigated the involvement of HIFs in the response of OC precursors and mature OCs to hypoxia. Human PBMCs were cultured on ivory discs for up to 14 days in MEM with 15% FCS, 7.5 ng/ml M-CSF and 1 ng/ml RANKL in 25 cm^2 flasks. RNA was extracted 6 and 24 hours after commencement of gassing on day 4 with 0.2%, 2% or 20% O_2 (plus 5% CO_2, balance N_2) and after a further 3 or 10 days of continuous gassing. Cultures were also treated for 6 or 24 hours with 100mM cobalt chloride, a hypoxia mimetic, at days 4 and 13. RT-PCR showed that HIF1α mRNA was readily detectable in 20% O_2 and in hypoxia in both pre-OC (day 4) and mature OC (day 14). HIF2α mRNA was expressed in pre-OC but was not evident in mature OC in 20% O_2. Immunofluorescence and Western blotting indicated that pre-OCs stabilise both HIF1α and HIF2α proteins rapidly when exposed to hypoxia (2% or 0.2% O_2) or cobalt chloride. HIF proteins were undetectable in hPBMC cultures maintained in 20% O_2, consistent with the notion that these proteins are degraded rapidly in normoxic cells. Expression of the key HIF target gene, VEGF was upregulated in cultures exposed to 2% O_2, but no change was observed in the expression of TNFα , IL-1, RANK, c-fms, or M-CSF, as determined by RT-PCR. Our results suggest that OCs and their precursors use the HIF machinery to respond to changes in local oxygen tension. The role of HIF prolyl hydroxylases as primary oxygen sensors

in pre/OCs is currently under investigation. This work provides an important new 'paradigm' for understanding the regulation of bone resorption.
Conflict of Interest: T. R. Arnett, Novartis, Grant Research Support. A. Brandao-Burch, Novartis, Grant Research Support.

P223-T

BONE-HEALTH RISK OF CHLOROBENZENES TREATMENT

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Background: The environmental exposure can originate from agricultural, industrial and social sectors of the society. The chemical load of the environment can modify the structure of the liver, immune-, central nervous and endocrine systems and the bone. Halogenated hydrocarbons (chlorobenzenes/ClB/ bromobenzenes) carry important environmental effects due to their accumulation and lipid solubility.

Aim: The aim of this study was to investigate the effects of hexa- and trichlorobenzene mix (1:1) ClB treatment in subtoxic doses on the femur structure. Wistar rats (male, 100–250g/bw.) were treated with chlorobenzene mix /1, 0 and 0, 1 ¼ g/bw. kg) for 30 and 60 days through gastric tubes. Various controls were included : 1./absolute control: untreated rats, 2./negative control: treated with tap water, 3./positive control: treated with ethanol (the solvent of ClB). At the end of the experimental periods, tissue-samples were taken from the femur of the rats . The bone mineral content of the samples were checked by DEXA. Liver function were estimated by determination of ^{3}GT, SGOT, SGPT. Bone structure were investigated by morphometry. The results were analyzed with SPSS statistical program

Results: As a result of the chronic chlorobenzene treatment the bone mineral density of the femur decreased in a dose- and time-dependent manner. The morphological structure of the femur correlated with the mineral content. The liver enzymes were not altered by subtoxic doses of ClB.

Conclusion: These data suggest that very low doses of chlorobenzenes, as elements of environmental pollution may cause structural alterations in the skeletal system.

This work was supported by: ETT 450/2006.
Conflict of Interest: None declared

P224-S

BONE TURNOVER MARKERS AND EARLY DETECTION OF BONE METASTASES IN PATIENTS WITH PROSTATE CANCER; A LONGITUDINAL APPROACH

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An increase in bone turnover markers in patients with prostate cancer (PCa) may predict the development of bone metastases, but can also be the effect of androgen deprivation treatment (ADT). To assess the diagnostic efficacy for the early detection of skeletal metastases we retrospectively performed serial measurements of a bone formation marker [amino-terminal procollagen propeptides of type 1 collagen (PINP)] and a bone resorption marker [pyridoline cross-linked carboxyterminal telopeptide of type 1 collagen (ICTP)] in serum of patients with PCa.

Residual serum samples of a total of 64 patients with histologically confirmed PCa, treated between September 1999 and November 2004, were selected from our PSA serum archive. They were divided into 3 separate groups: patients with no evidence of metastases (N0M0), with lymph node metastases only (N1M0) and patients who developed skeletal metastases (M1). In the M1 group the T1 sample was collected near the first positive bone scintigram.

Results The N1M0 and the M1 group show elevated PINP levels compared to the N0M0 group (ANOVA T0, $p = 0.0345$; T1, $p = 0.0001$). The PINP levels in the M1 group further increase (paired t-test, $p = 0.028$), while no increase is found in both other groups. There is no significant difference between the number of patients receiving ADT in the N1M0 and M1 group. Elevated PINP levels in the M1 group are already detectable 8 months prior to the first visible metastases on bone scintigraphy. The same trend is found in the ICTP analyses. The increase in ICTP found in the M1 group differs significantly from the ICTP change of patients without bone metastases (N0M0 and N1M0 group, $n = 45$, $p = 0.029$), although we find no significant difference in ICTP levels when comparing the 3 groups separately. ROC analyses reveal that both increases in PINP and ICTP are sensitive and specific for differentiation between patients with or without skeletal metastases of PCa (AUC = 0.71, $p = 0.002$, respectively AUC = 0.64, $p = 0.045$).

Conclusions: The follow-up measurement of serum PINP and ICTP levels is useful in the early assessment of skeletal metastases in patients with PCa, regardless the confounding role of androgen deprivation treatment or other factors that might interfere with bone turnover.
Conflict of Interest: None declared

P225-M

UNIFYING MICRODAMAGE- AND DISUSE-TARGETED RESORPTION: A LACK OF OSTEOCYTIC INHIBITION

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Apart from calcium homeostasis, the process of bone remodeling serves two purposes: mechanical optimization and microdamage repair. Hence, osteoclasts target both disused and microdamaged bone for resorption. But these targets appear contradictory, as microcracks occur in high strain conditions. Can one mechanism explain both?

We propose that strain-induced osteocyte signals [1] inhibit osteoclasts [2]. This theory explains disuse-targeted remodeling via the absence of osteocytic inhibition. Several studies indicate that microdamage induces osteocyte apoptosis [3]. Hence, microdamaged regions would have a similar absence of osteocytic inhibition.

Our theory was previously [4] implemented in computer simulations to explain why osteons are aligned to the main loading direction [5]. We use FE-analysis to calculate tissue strains and a cell-simulation method [6] to model osteoclasts. Osteonal remodeling is simulated in a small piece of compact bone (Fig. 1A), containing a 'damaged' region with inactive osteocytes, and a cavity from which osteoclasts start. The piece is loaded in the vertical direction, and strains around the cavity are low in this direction (Fig. 1B). The resulting osteocyte signals (Fig. 1C) guide the resorbing osteoclasts in loading direction. But as the cutting cone nears the damaged region, it diverts to resorb it (Fig. 1D).

The model explains disuse-targeted resorption and osteonal load-alignment. It also guides osteoclasts to nearby regions of osteocyte death, thus providing an explanation for microdamage-targeted remodeling.

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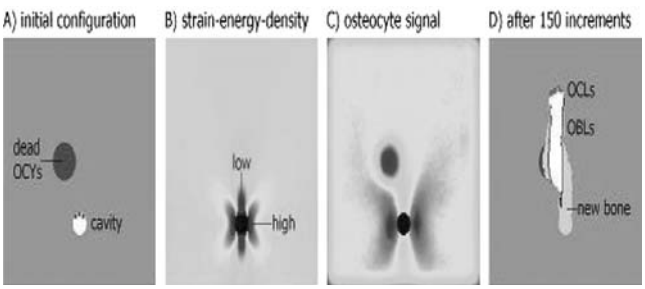

Conflict of Interest: None declared

P226-T

TRANSCRIPTIONAL REGULATION IN DEVELOPMENT OF HUMAN OSTEOCLAST

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The current belief in functional genomics is that genes expressed similarly are likely to be regulated via the same mechanisms. Also, the use of *in silico* methods for transcription factor binding studies has started to be more tempting because of the increasing amount of data and sophisticated programs available. These methods were used to study $CD14^{+}$ human peripheral blood mononuclear cells differentiating into osteoclasts, macrophages, endothelial and dendritic cells. Whole human genome microarrays were used to determine the genes highly expressed in osteoclasts compared to other monocyte–macrophage system cells studied. The experiment revealed a significant, osteoclast specific up-regulation of the genes that has not before shown to be specific for osteoclasts. The transcription factors, which were predicted to bind to most of these up-regulated genes, and whose corresponding genes are expressed in osteoclasts, were determined. The binding sites of these factors are good candidates for building putative regulatory modules. The *in silico* transcription factor binding site analysis revealed modules specific only for these up-regulated genes. The Ingenuity Pathway Analysis software was further used for interpreting the results. In

these analyses, the pathway networks were found to be centered around the known osteoclast related genes, like TP53, AKT1 and PTEN. In conclusion, systems biological approaches are powerful way to decipher the genomic events during the osteoclast differentiation. Although only putative, our target genes achieved by the use of bioinformatics strategies further reduce significantly the number of candidates for the functional evaluations of our findings, and finally, they serve as potential and promising new targets in the search of new drug target candidates.
Conflict of Interest: None declared

P227-S

EXPRESSION AND REGULATION OF TOLL-LIKE RECEPTOR 2 BY FIBRONECTIN FRAGMENTS IN HUMAN ARTICULAR CHONDROCYTES

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Background: Osteoarthritis is a complex disease involving both biomechanical and metabolic factors which alter the tissue homeostasis of articular cartilage and subchondral bone. Cartilage breakdown involves both extracellular matrix degradation and production of metabolically active breakdown products such as fibronectin fragments and activity of cytokines such as IL-1b and TNF-a produced by activated synoviocytes or by articular chondrocytes themselves.

We have previously reported that the expressions of TLR2 are regulated in human articular chondrocytes by IL-1b. In the present study, we investigated the mechanism of fibronectin proteolytic fragments induced TLR2 upregulation in primary cultured human articular chondrocytes.

Methods: Three fibronectin fragments were used in the present study including the 29 kD heparin binding fragment, the 45 kD gelatin binding fragment, and the 70 kD heparin and gelatin binding fragment. Following stimulation of chondrocytes in vitro by fibronectin proteolytic fragments, Immunohistochemistry, Western blotting, and RT-PCR were used to assess the expression of TLR2. MyD88 activation and nuclear factor-kB (NF-kB) translocation were evaluated by immunoprecipitation and electrophoretic mobility shift assay respectively

Results: All three fragments, in a similar fashion to IL-1b induced TLR2 upregulation although the 45 kD fragment appeared to have the greatest effect. Inhibition of this up-regulation of TLR2 gene expression by IL-1ra would be consistent with activation of an IL-1 autocrine/paracrine loop.

Conclusion: The results show for the first time that TLR2 expression in chondrocytes may be up-regulated by a non-infectious, inflammatory pathway and raise the possibility that this family of molecules, of recognized importance in a range of inflammatory conditions, may significantly contribute to the detrimental inflammatory/catabolic activities of chondrocytes in osteoarthritis.

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Conflict of Interest: None declared

P228-M

PROGRESSIVE NON-INFECTIOUS ANTERIOR VERTEBRAL FUSION, THE USEFULNESS OF THE CT SCANS TO FURTHER OUR UNDERSTANDINGS

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Background: Progressive non-infectious anterior vertebral fusion is a unique spinal disorder with distinctive radiological features. Early radiographic findings consists of narrowing of the anterior aspect of the intervertebral disk with adjacent end plate erosions associated with specific pattern of progression. The management needs a multi disciplinary approach with major input from the orthopaedic surgeon.

Context and Purpose: A single patient with progressive vicious kyphosis was undertaken and extensively investigated to evaluate a multiple malformation complex. Aetiological understanding was the main objective. Progressive non-infectious anterior vertebral fusion must be differentiated from other forms of vertebral fusions and particularly these resulted from failure of segmentation and from the unusual forms of congenital kyphosis.

Methods: Detailed clinical and radiological examination with emphasis on the remarkable role of the 3D-CT scans to further our understanding the course, and the different pathological stages of the development of progressive non-infectious anterior vertebral fusion. We compared our imaging outcome of our current patient with about 20 patients previously reported in Europe.

Results: Kyphosis in a 12-year-old-female secondary to progressive non-infectious anterior vertebral fusion was identified. This occurred at three vertebral levels. In the cervical spine there was progressive fusion of the lateral masses of the Axis with C3. Secondly, at the cervico-thoracic level, a severe, progressive, anterior thoracic vertebral fusion (C7–T5) and (T6–T7) resulted in the development of a thick anterior bony ridge and massive sclerosis and thirdly; progressive anterior fusion at L5–S1. Whereas at the level of the upper lumbar spines (L1) a split cord malformation was encountered. Situs inversus visceralis was an additional malformation. The role of the CT scan in detecting the details of the vertebral malformations was important. To our knowledge, neither this malformation complex and nor the role of the CT scan in evaluating these patients, have previously been described.
Conflict of Interest: None declared

P229-T

PROMOTER 2 -1025 T/C POLYMORPHISM AT RUNX2 IS ASSOCIATED WITH FEMORAL NECK BMD IN SPANISH POSTMENOPAUSAL WOMEN

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Runx2 is a runt domain transcription factor that is essential for osteoblast differentiation, bone remodelling and fracture healing. RUNX2-knockout mice show a complete lack of ossification, while overexpression of this gene in transgenic mice results in an osteoporotic phenotype. Thus, RUNX2 is a good candidate for the genetic determination of osteoporosis. In this association study, the effects of the −330 G/T polymorphism in promoter 1 and the −1025 T/C polymorphism (rs 7771980) in promoter 2 of RUNX2 were tested in relation to lumbar spine and femoral neck BMD in a cohort of 821 Spanish postmenopausal women. The minor allele frequencies of the two polymorphisms were 0.15 and 0.07, respectively. The two polymorphisms, located more than 90 kb apart, were not in linkage disequilibrium ($D' = 0.271$, $r^2 = 0.035$). In an ANCOVA test using age, weight, height and years since menopause, the −330 G/T polymorphism was not associated with any of the phenotypes analysed, while the −1025 T/C polymorphism was found to be associated with adjusted femoral neck BMD ($p = 0.002$). In particular, individuals carrying the TC genotype had higher mean adjusted femoral neck BMD values than those bearing the TT genotype. This result is in agreement with a recent study by Doecke et al (JBMR 21: 265–273, 2006) in which the C allele was overrepresented in a high FN BMD group. These authors also provided evidence for a functional role of this polymorphism. Our results constitute a replicate and highlight the importance of this RUNX2 promoter 2 polymorphism in FN BMD determination.
Conflict of Interest: None declared

P230-S

JUVENILE HYALINE FIBROMATOSIS. CASE REPORT

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Juvenile Hyaline Fibromatosis (JHF) is rare inherited recessive autosomal disorder characterised by multiple papilonodular lesions and gum hypertrophy, caused by a deposition of hyaline. Most common manifestation of diseases are in the children and newborn. Systematic diseases named Infantile Systemic Hyalynosis is characterised with head and body multicentric lesions , joint contractures, osteoporosis and mental retardation. Case report: A boy 5 years old with supraorbital tumefaction 2×2 cm size appeared four month ago, was seen on our clinic in August 2006. Patient was treated one year before with excision and pathohistological verification as Haemangioma in same place. After diagnostic and preparation we performed radical excision of tumor and PH diagnosis was Juvenile Hyaline Fybromatosis with clean surgically borders. Two months after tumor appeared again in the same place with more aggressive propagation to orbit and nose. Patient was sent to the irradiation therapy and Oncologic Institute. Discussion: Until 1985 30 cases of this disease had been published. We classified our patient as having a local type of disease. In this type we advice a large excision of tumors with precise pathohistological verification. If recidiv appeared, irradiation therapy is the method of choice.
Conflict of Interest: None declared

P231-M

CINACALCET AND HYPOCALCIURIC HYPERCALCEMIA WITH RECURRENT PANCREATITIS

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Introduction. Inactivating mutations in CaSR gene has been reported has the cause of hypocalciuric hypercalcemia (HH) with inappropriate PTH levels. Calcimimetic agents, allosterical activators of CaSR, have been successfully tested in the treatment of primary, as well of secondary, hyperparathyroidism. We report the effect of the calcimimetic Cinacalcet on mineral metabolism in a patient affected with HH and recurrent acute pancreatitis. Case report and

methods. A 16 year-old male presented with mild hypercalcemia and acute pancreatitis at Perugia Regional Hospital. The patient had a cholecystectomy and initiated a treatment with ursodesossicolic acid. After a second pancreatitis episode the patients was referred to our Centre. The patient's basal mineral metabolism parameters were assessed over 4 months using 4 different determinations. After basal investigations, cinacalcet 30 mg/day was started and administered for 15 days. On day 16, the dosage of cinacalcet was doubled to 30 mg twice daily. Results. The table shows parameters of mineral metabolism before the beginning of treatment with cinacalcet and during the 21 month follow-up period. Cinacalcet was well tolerated and no changes were observed in pancreatic enzymes and other laboratory parameters except for the indices of mineral metabolism. Patient has not had further episodes of pancreatitis and his serum amylase and lipase levels have remained within the normal range. Conclusion. The results of this case report show that the calcimimetic cinacalcet, 30 mg twice daily, normalizes serum calcium in a patient affected with HH. This encouraging case shows the need to evaluate cinacalcet as a potential therapy for HH.

Table: Biochemistry (mean +/−SE)

Parameter	Reference Range	Baseline	Cinacalcet 30 mg/day	Cinacalcet 60 mg/day
sCalcium mg/dL	8.5–10.2	11.42 +/− 0.1	10.1 +/−0.13	9.45 +/−0.11
sCa++ mmol/L	1.13–1.32	1.47 +/−0.01	1.35 +/−0.09	1.28 +/−0.02
sPO4 mg/dL	2.7–4.5	2.98 +/−0.19	4.02 +/−0.22	4.02 +/−0.12
IPTH pg/mL	10–78	79.1 +/−2.37	123.1 +/−14.1	72.6 +/−6.9
24-h uCa mg	50–400	245.0 +/−74.0	390	458 +/−36.8
uCa/Cr ratio mg	>0.01	0.0085 +/-0.00	0.0021	0.018 +/-0.001

Conflict of Interest: None declared

P232-T

POLYMORPHISMS ASSOCIATION WITH OSTEOPOROSIS STUDIES PRESENT A PROBLEM CAUSED BY POPULATION STRATIFICATION IN THE MESTIZO GROUPS

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Background/Aim: Population structure can lead to apparent associations with markers that are unlinked to disease loci. We analyze the influence of population stratification in the relationship between some polymorphisms and osteoporosis.

Methods: To analyze the allelic distribution and population structure, the Mexican population was divided in three different samples: the cases (70 osteoporotic women), the controls (70 non-osteoporotic women) and the general Mexican Mestizo population (frank population, 500 individuals). The screening was performed with 7 different polymorphic markers (4 STR's and 3 SNP's) associated with bone metabolism, using Capillary Electrophoresis and Real Time PCR. The population structure analysis was done by using the Structure software and the relationship between genotype and phenotype was performed by using the Strat software.

Results: We found an important stratification in the Mexican Mestizo population consisting of at least 3 different subpopulations, which increased the risk of spurious associations between OP and the genetic markers. In spite of this admixture, we found that the CT gene marker and the ER alpha gene marker have higher frequency in osteoporotic women than in non-osteoporotic women, which indicates a potential association between OP and such alleles. On contrary, we found any polymorphisms that the VDR gene marker and the IL6 gene marker may constitute protective factors against OP. These polymorphisms showed an allelic association (Strat) with the OP genotype (0.000000E+00, each one). Moreover, we analyzed several confounders for OP by a longitudinal multivariate model.

Conclusion: It is likely that false positive associations could be found in case–control studies when an admixture population is under analysis. It is known that stratification depends on Mestizo degree. Mexican population generally is composed by a differential mixture of European, Native American, and African ancestry, which complicates association studies of multifactorial diseases and could produce inconsistent results, and a frequent scapegoat for the lack of reapplication across studies. In our study, genes like a CT, ER-alpha, IL-6 and VDR displayed real difference between case and controls, which indicates a reliable association in spite of substructure; however, stratification provoked that genes like a STR: ER-alpha, ER-beta and IL-6 were found no related to OP in our population, contrary to that found in others population studies.

Conflict of Interest: None declared

P233-S

EFFECT OF RANK/RANKL/OPG HAPLOTYPES ON BONE PHENOTYPE AFTER TOTAL HIP ARTHROPLASTY

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The development of osteolysis or heterotopic ossification (HO) may have a major impact on implant survival and clinical function after total hip arthroplasty (THA). In both processes an imbalance between osteoblast and osteoclast activity contributes to the observed phenotype. Bone remodelling is regulated in part by RANK/RANKL/OPG signalling. Polymorphisms within the genes encoding these peptides are associated with diseases of bone remodelling. We studied whether single nucleotide polymorphisms (SNPs) in this signalling system were associated with osteolysis or HO after THA. Two SNPs within OPG (A163G and C1181G), 1 in RANK (T192C) and 3 in RANKL (T2230C, A9374G, and G36208T) were studied.

Genomic DNA was extracted from 609 white subjects 11 ± 4 years after cemented THA for idiopathic osteoarthritis. 268 subjects had failed implants due to osteolysis (osteolysis group) and 341 had radiologically intact implants (control group). The presence of HO after index THA surgery was assessed using Brooker's classification in 563 of the subjects in whom suitable radiographs were available for analysis. Haplotype analysis was made using Haploview 3.3 (Broad Institute, Cambridge, MA). The haplotype OPG 163G: 1181G, present in 7% of the population (osteolysis group 9.7%, control group 6.0%; $p = 0.03$), was associated with osteolysis (odds ratio 1.7; 95%CI 1.1 to 2.6). The haplotype RANKL 2230C: 9374A: 36208G, present in 6% of the population (osteolysis group 8.6%, control group 4.1%; $p < 0.01$), was also associated with osteolysis (odds ratio 2.1; 1.3 to 3.4). None of the haplotypes were associated with HO formation ($n = 299$ subjects with HO classified as Brooker grade 1 or greater). No differences in genotype or minor allele carriage rate for the individual RANK/RANKL/OPG SNPs were found between the osteolysis and control groups or between subjects developing HO versus those without HO after index THA surgery ($p > 0.05$ all comparisons)

We found that complex haplotypes within the genes encoding OPG and RANKL are associated with osteolysis, but not HO formation. However the frequency of these haplotypes in the population is <10%, and contribution to the overall population risk of osteolysis is likely to be low. Our data suggest that the individual common SNPs within the RANK/RANKL/OPG system do not have a measurable impact on the risk of osteolysis or HO formation.

Conflict of Interest: None declared

P234-M

FRIZZLED RELATED PROTEIN-3 VARIANTS AND PATTERNS OF HIP OSTEOARTHRITIS

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The wnts are regulatory factors for the differentiation and growth of osteoblasts. FRZB encodes for secreted-frizzled-related-protein 3 that antagonises wnt signalling. We examined whether 2 single nucleotide polymorphisms (SNPs), FRZB Arg200Trp and FRZB Arg324Gly, associate with the pattern of hip osteoarthritis (OA) in a retrospective case–control study.

The study sample comprised a subgroup of 301 subjects (152 male) taken from a study of 609 participants examining the role of these SNPs in the development of osteolysis after total hip arthroplasty (THA) for idiopathic OA. The subgroup was self-selected by the availability of suitable pre-operative hip radiographs for assessment using the American College of Rheumatology criteria. Genomic DNA was extracted from peripheral blood and genotyped using standard techniques.

281 subjects had femoral osteophytes, 267 had pelvic osteophytes and 252 subjects had both femoral and pelvic osteophytes. 267, 268 and 247 subjects had femoral, pelvic and both femoral and pelvic sclerosis, respectively. 277, 236 and 227 subjects had femoral, pelvic and both femoral and pelvic cysts, respectively. 278 subjects had superior joint space narrowing (JSN), 288 had axial JSN and 256 had medial JSN. 32 subjects had femoral head collapse.

The genotype distribution of the FRZB Arg200Trp SNP differed between subjects with pelvic osteophytes versus those without pelvic osteophytes (chi2 with test for trend $p = 0.03$). The carriage rate of the FRZB 200Trp allele was 21.3% in subjects with pelvic osteophytes versus 5.9% in those without pelvic osteophytes and was 21.8% in subjects with both femoral and pelvic osteophytes versus 8.1% in the remaining subjects (Fisher's Exact test $p = 0.037$ and 0.030, respectively). The odds ratio (OR) for pelvic osteophytes associated with carriage of FRZB 200Trp was 4.3 (95%CI 1.0 to 18.7, $p = 0.048$). The OR for combined femoral and pelvic osteophytes was 3.1 (1.1 to 9.1, $p = 0.035$) The

FRZB SNP variants were not associated with the other radiographic criteria of OA ($p > 0.05$).

In this study carriage of the FRZB 200Trp allele was associated with pelvic and combined femoral and pelvic osteophyte formation, suggesting that this locus may act as a marker for new bone formation in this setting.

Conflict of Interest: None declared

P235-T

THE GERMAN MOUSE CLINIC – DYSMORPHOLOGY, BONE AND CARTILAGE SCREEN

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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 osteoarthritis and osteoporosis. Mouse mutants are analyzed for medically relevant bone and cartilage parameters including morphological abnormalities. In the primary screen up to 26 mutant lines (i.e. 1500 animals) can be phenotyped. In addition, we have the capacity for another 1500 phenotypic analyses in secondary and tertiary screens for more detailed characterization of model systems of bone related human diseases.

For the primary screen, we cover a broad spectrum of parameters involving bone development, metabolism and homeostasis. We have implemented an experimental set-up utilizing DXA, (dual energy X-ray absorption) and X-ray imaging which enables us to perform high throughput non-invasive first-line phenotyping for bone and cartilage abnormalities. In addition, we developed a 54-parameter protocol for the rapid morphological observation of animals, which is able to detect and evaluate malformations and malfunction of the different organ systems. In secondary tests, we evaluate mutants with altered parameters from the primary screen in more detail using tests which include μCT and pQCT, markers of bone metabolism and hormonal regulation, fracture/stress parameters, and skeleton preparation.

Since the beginning of the GMC the Core Facility provided 60 mutant mouse lines and 12 inbred strains or hybrids for the primary screen. Most of the mutant lines provided by the Core facility for the primary screen have already finished the phenotypic analysis in the Bone and Cartilage module. In 15 lines we confirmed known bone phenotypes. 23 mutant lines showed interesting changes in a series of parameters and were further analyzed in secondary tests. We were able to characterize new mouse models for osteogenesis imperfecta, inflammatory arthritis, osteoarthritis and osteoporosis.

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

P236-S

HAPLOTYPES OF PROMOTER AND INTRON 1 POLYMORPHISMS IN THE COLIA1 GENE ARE ASSOCIATED WITH INCREASED RISK OF OSTEOPOROTIC FRACTURE

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Osteoporosis is a common age related disease. The pathogenesis is multifactorial but genetic factors are known to exert a significant influence. The COLIA1 is one of the most extensively studied candidate genes and has consistently been shown to be associated with bone mineral density (BMD) and fractures.

The aim of this study was to elucidate the effect of two promoter polymorphisms (-1997G/T and -1663IndelT), an intron 1 polymorphism (+1245G/T) and their haplotypes on vertebral fractures and BMD.

The study was a case–control study with 462 osteoporotic patients (men and post-menopausal women) and 336 controls. Analyses regarding T-score and fractures were conducted in age-matched sub-groups. Polymorphisms were examined using Taqman, sequencing and RFLP-assays and BMD was examined by DXA.

The −1663delT polymorphism was associated with decreased lumbar spine (ls) BMD $0.747 +/- 0.136$ g/cm^2 in individuals with the del/del genotype versus $0.829 +/- 0.179$ and $0.846 +/- 0.179$ in individuals with the del/T and TT genotypes, respectively ($p = 0.016$). The +1245G/T polymorphism was associated with decreased lsBMD $0.743 +/- 0.149$ g/cm^2 individuals with the TT genotype versus $0.853 +/- 0.180$ and $0.852 +/- 0.182$ in individuals with the GT and GG genotypes, respectively ($p = 0.008$), and increased risk of vertebral fractures OR = 2.9 ($p = 0.027$). The −1997G/T polymorphism was not associated with either BMD or fracture risk. The three most common haplotypes accounted for 91.7 % of the alleles. Individuals with one or two copies of haplotype 1 (−1997G/−1663insT/+1245G) had a significantly higher lsBMD $0.856 +/- 0.173$ g/cm^2 and $0.856 +/- 0.191$ versus $0.778 +/- 0.146$ in individuals with no copies of the haplotype respectively ($p = 0.003$), and a decreased risk of vertebral fractures OR = 0.6 ($p = 0.012$). Carriers of haplotype 2 (G/delT/T) had a reduced lsBMD ($p = 0.058$) although this did not reach significance. Previous functional studies suggest that the promoter allele combination in haplotype 1 is associated with increased transcription and therefore reduced BMD whereas the intron 1 allele in haplotype 1 is associated with the opposite. We therefore speculate that the intron 1 (+1245G/T) polymorphism is of greater significance than the promoter polymorphisms in an older population.

We have for the first time demonstrated that haplotypes of the COLIA1 are associated with fracture risk. Haplotype 1 protects against vertebral fractures in men and postmenopausal women.

Conflict of Interest: None declared

P237-M

THE ROLE OF VITAMIN D RECEPTOR GENE VARIATION AND ITS INTERACTION WITH IMMUNE REGULATORY MOLECULES IN BONE TURN OVER

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Aims: To investigate interactions between immune and endocrine systems in bone turn over and the role of Vitamin D receptor (VDR) gene variation on it.

Methods: The subjects for the study were 268 women randomly selected aged between 20–75 years form the participants of Iranian multicenter osteoporosis study. All the subjects had undergone bone mineral densitometry (BMD) measurements by dual energy X-ray absorptiometery at lumbar spine (vertebrae L2–L4) and hip (femur neck). Genomic DNA was isolated from peripheral blood leukocytes according to standard methods. RFLP-PCR was used and followed by sequencing several segment of ligand binding domain. After an overnight fast, blood was taken for measurement of serum parathyroid hormone, 25-hydroxyvitamin D, alkaline phosphatase, cytokines profile , leptin, adiponectin, osteocalcin and cross laps.

Results: Our results showed that a significant difference in frequency of the VDR allelic distribution in comparison with the Asian population. This finding is similar to Western populations in some of polymorphism. There were statistical differences in the allelic distribution of FokI and TaqI between osteoporotic patients and healthy population. After adjustment for age, body mass index, calcium and vitamin D intakes, statistical associations were found between TaqI VDR gene polymorphisms and BMD and were weakly correlated with serum concentrations of osteocalcin, alkaline phosphatase. Genotypes of the vitamin D receptor classified in seven major groups that only in three genotypic groups were associated with the serum concentrations of calcium or other biochemical values, calciotropic hormones, or markers of bone turnover. Serum concentrations of cytokines, adipokinase and bone turn over markers were different in these three groups.

Conclusion: Vitamin D receptor gene alleles accompany with cytokines predict the bone density and bone turnover. Based on these modulation effects of cytokines on bone turn over modified by genetic variations of VDR.

Conflict of Interest: None declared

P238-T

GAIN-OF-FUNCTION POLYMORPHISMS IN THE P2X7 PURINERGIC RECEPTOR ARE ASSOCIATED WITH INCREASED BONE MASS

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The $P2X_7$ purinergic receptor is an ATP-gated cation channel, which is expressed in many cell types including osteoclasts and osteoblasts. There is a wide variation in $P2X_7$ receptor function between humans. This may be explained at least in part by several loss-of-function and gain-of-function polymorphisms. Characterisation of $P2X_7$ knock-out mice has shown that they have reduced total bone mineral content and that they are less sensitive to mechanical loading compared with WT mice. In this study we investigated the effect of 8 loss-of-function or gain-of-function polymorphisms in the $P2X_7$ receptor gene on BMD and fracture risk in 462 osteoporotic patients and 336 normal controls. In addition to these SNPs we examined 2 non-conservative polymorphisms for which no functional data are available.

Seven of the polymorphisms, Val76Ala, Gly150Arg, His155Tyr, Arg270His, Arg307Gln, Thr357Ser and Ile568Asn were not associated with either BMD or fracture risk.

The Ala348Thr and Gln460Arg polymorphisms, which are in strong LD, were significantly associated with increased BMD. In individuals homozygous for the 348Thr allele BMD at the total hip was 0.820 ± 0.173 g/cm^2 compared

with 0.773 ± 0.158 g/cm^2 and 0.770 ± 0.156 g/cm^2 in individuals with Ala/Thr and Ala/Ala genotypes, respectively, p = 0.012. Similar results were found for the subregions of the hip and the lumbar spine. Furthermore, the odds ratio for vertebral fractures was 1.66, p = 0.07. In men the increased risk of vertebral fractures was significant, p = 0.002. The Gln460Arg polymorphism was not associated with vertebral fracture risk.

The Glu496Ala polymorphism was also associated with BMD. BMD adjusted for age at the total hip was 0.840 ± 0.147 g/cm^2, 0.803 ± 0.133 g/cm^2 and 0.889 ± 0.147 g/cm^2 in individuals with the Glu/Glu, Glu/Ala and Ala/Ala genotypes, respectively, p = 0,002. The findings in the heterozygotes are in agreement with Glu496Ala being a loss-of-function polymorphism, however, we have no explanation for the increased BMD in the homozygotes (n = 29). This may be a chance finding due to lack of statistical power, since it is not caused by LD with any of the other polymorphisms.

In conclusion, we found that two gain-of-function polymorphisms in the $P2X_7$ gene is associated with increased BMD. A loss-of-function polymorphism (Glu496Ala) may also affect BMD.

Conflict of Interest: None declared

P239-S

COMMON GENETIC VARIATION IN THE ESTROGEN RECEPTOR BETA GENE IS ASSOCIATED WITH INCREASED RISK OF OSTEOARTHRITIS

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Background: Estrogens are thought to be important in the pathogenesis of osteoarthritis (OA). Previous research found that Estrogen Receptors alpha and beta (ESR1 and 2) are present in chondrocytes, while several animal experiments showed a chondroprotective effect of estrogens. We and others reported an association between two polymorphisms (PvuII and XbaI) of the ESR1 gene and radiographic OA (ROA) of the knee.

In this study, we examined the relationship between common haplotypes of the ESR2 gene and radiographic hand-, knee- and hip osteoarthritis in a large population-based cohort study of elderly subjects aged 55 years and older.

Methods: In total, 4317 subjects (1806 males and 2511 females) had data available on genotype analysis and ROA outcomes. ROA was defined by the Kellgren/Lawrence score (K/L score), where a score >= 2 was defined as present OA. Genotypes for the variants were assessed using Taqman allelic discrimination assays. The program Phase was used to estimate haplotypes. For binary variables, logistic regression was used and for analysis of baseline characteristics and Urinary C-terminal cross-linked telopeptide of type II collagen (CTX-II), a measure of generalized cartilage degradation, ANCOVA analysis was used for statistical analysis.

Results: In our study we observed 3 common haplotypes: 1: CT (45.1%), 2: TC (37.1%) and 3: TT (17.1%). Female homozygote carriers of haplotype 1 had a 1.9 times increased risk for hip OA according to the K/L score (95% CI 1.2–3.0) and a 1.3 times increased risk for hand OA (95% CI 1.0–1.8), and female carriers of haplotype 1 had a 1.3 times increased risk for knee OA (95% CI 1.0–1.7). A similar trend was observed in females for total hip replacement and CTX-II levels, although not significant (p = 0.07 and 0.10 respectively). In males, no associations were observed. There was no interaction between genetic variation in the ESR1 and ESR2 genes in relation to OA-outcomes.

Conclusion: Female carriers of haplotype 1 of the ESR2 gene have an increased risk of OA, especially of the hand, knee and hip. This same ESR2 haplotype was previously found to be related to increased fracture risk in women, indicating a pleiotropic effect for ESR2 variants. The mechanism underlying this association is thought to involve lower expression of the ESR2 receptor in target cells, but this requires further study.

Conflict of Interest: None declared

P240-M

ASSOCIATION OF KIT GENE POLYMORPHISMS WITH BONE MINERAL DENSITY IN POSTMENOPAUSAL KOREAN WOMEN

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Bone mineral density (BMD) is a major factor for determining bone strength and osteoporotic fracture risk, and is determined by environmental and multiple genetic factors. KIT, which encodes a transmembrane receptor with tyrosine kinase activity, plays an important role in the differentiation of osteoclasts. We examined the associations between KIT gene polymorphisms and BMD in postmenopausal Korean women. All exons, their boundaries, and the promoter region (approximately 1.5 kb) from 24 individuals were directly sequenced. Eighteen polymorphisms were identified and three single-nucleotide polymorphisms (SNPs) were genotyped in all study participants (n = 946). BMD at the lumbar spine and femur neck was measured using dual-energy X-ray absorptiometry. The mean age of the study subjects was 58.9 +/− 7.5 years and the mean number of years since menopause was 9.6 +/− 7.9 years. None of the three SNPs (−694G > T, +41894A > G, and +49512G > A) was significantly associated with BMD value. However, multivariate analysis showed that the ht3 (−694T-+41894A-+49512G) was significantly associated with lower BMD at the femur neck (p = 0.007 in the recessive model). These findings indicate that KIT-ht3 may be a useful genetic marker for osteoporosis, and that KIT may have a role on bone metabolism in humans.

Conflict of Interest: None declared

P241-T

EFFECT OF PVUII AND XBAI POLYMORPHISMS OF ESR1 GENE ON BODY HEIGHT IN WOMEN DEPENDS ON MENARCHE – THE EPOLOS STUDY

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Aim: The aim of this study was to verify if menarche influence effect of PvuII and XbaI polymorphisms of ESR1 gene on body height in women.

Methods: The study group comprised 895 subjects, adult females, in age range 20–80 years, randomly selected in 7 centres from polish population. DNA was isolated from peripheral blood with using Gentra Isolation Kits. Polymorphisms were evaluated with RFLP technique, and determined by restrictive enzymes: PvuII and XbaI simultaneously. Body height was measured by stadiometer on standing without shoes, all data adjusted on age. Menarche data was captured from questionnaire filled by physician during the subject's visit. Statistical examination comprised ANOVA and T-Tukey tests.

Results: Haplotypes analysis has shown relationship between body height and haplotype 1 (px) which was influenced by menarche. Women with menarche under age of 13 years (n = 157), carriers of 2 copies of haplotype 1(px) have been 3,9 cm shorter comparing to women carrying none of copy of haplotype 1(px) (p < 0.01). In women with menarche in age between 13 and 15 years (n = 586), carriers of both copies of haplotype 1(px) have been 1,5 cm shorter comparing to carriers of any copy of haplotype 1(px), but result was on border of statistical significance (p = 0.055). In women with menarche above age of 15 years (n = 152) any haplotype-related body height differences have been observed. Analysis performed only in premenopausal population (n = 453) has shown that women with menarche under age of 13 years, carrying 2 copies of haplotype 1(px) have been 4,1 cm shorter comparing to haplotype 1(px) non-carriers (p < 0.05). In women with menarche in age between 13 and 15 years and in age above 15 years, any haplotype-related body height difference have been observed.

Conclusion: Polymorphisms PvuII and XbaI of ESR1 gene determine shorter body height in women, but the effect depends on menarche and is real in females with menarche under age of 13 years.

Conflict of Interest: None declared

P242-S

VITAMIN D AND ESTROGEN RECEPTOR-ALPHA POLYMORPHISMS ARE RELATED TO BONE DENSITY IN HEALTHY RUSSIAN ADOLESCENTS

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Osteoporosis represents a condition characterized by low bone mass, poor bone quality and an increased propensity to fracture. This disease develops more frequently in women after menopause and is considered to be related to hormonal changes at this time. However, predisposition for this disease is related to the peak bone mass, which develops during puberty. Peak bone mass value is a major risk factor of osteoporosis development later in life. It is under strong genetic control, however, little is known about the genes determining bone mass in humans. In the present study, we investigated the relationship between polymorphisms in the genes encoding the vitamin D receptor (VDR), namely BsmI, FokI, and estrogen receptor-alpha (ERalpha), namely XbaI, PvuII, as well as bone mineral density (BMD), and bone mineral content (BMC) in 80 Russian adolescent girls aged 14–19 years. Genotypes were determined by PCR-restriction fragment length polymorphism. BMD (g/cm^2) and BMC (cm^2) at lumbar spines (L1–L4) region and at the sites of proximal femur, including femoral neck (N), trochanter (Tr) and Ward's triangle (War) were measured by dual-energy X-ray absorptiometry. The significant association was found also for FokI polymorphism to BMCN and BMDN as well as BMCWar and BMDWar. Girls with Ff genotype had significantly higher mean value of BMCN, BMDN, BMCWar and BMDWar than girls with FF genotype, (4,38 vs. 3,94, P = 0,007 for BMCN; 0,894 vs. 0,819, P = 0,007 for BMDN; 1,059 vs. 0,946, P = 0,014 for BMCWar and 0,873 vs. 0,776, P = 0,002 for BMDWar). In addition significant association was observed between XbaI polymorphism to BMCN and BMDN. Girls with xx genotype had significantly higher mean value of BMCN and BMDN than individuals with XX genotype (4,3 vs. 3,86, P = 0,027 and 0,882 vs. 0,801, P = 0,019, correspondingly). However, no significant difference was found between above mentioned polymorphisms and BMC and BMD in L1–L4 and trochanter. In addition there was no association between Bsm I polymorphism of VDR gene as well as PvuII polymorphism in ERalpha gene with both BMC and BMD in every bone region we tested in this study. In conclusion, we show that in Russian adolescents VDR and ERalpha gene polymorphisms are related to bone density in the neck and Ward's triangle of the femur.

Conflict of Interest: None declared

P243-M

FRACTURE RISK IN MEN UNTIL ADULTHOOD VARIES TWOFOLD DEPENDING ON GC GENOTYPE – RESULTS FROM THE ODENSE ANDROGEN STUDY

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AIM: To examine the relationship between Gc genotype and bone fragility in men.

BACKGROUND: The multifunctional plasma protein Gc, also known as vitamin D-binding protein, DBP, or Gc-globulin, has two functions with relation to bone tissue: it is the major carrier protein of vitamin D in the circulation, and deglycosylation converts it into a very potent macrophage- and osteoclast-activating factor, Gc-MAF. In a previous study, female premenopausal fracture risk was associated with Gc phenotype.

METHODS: In 781 Caucasian 20 to 30 year-old men participating in the Odense Androgen Study, a population based observational study, we identified the Gc genotype by Real-time PCR melting curve analysis. Fracture history was obtained using a questionnaire. A physical examination was performed and various bone markers were measured. Bone mineral density (BMD) of the lumbar spine, hip, and whole body, was measured using a Hologic-4500 DXA-scanner.

RESULTS: The Gc genotype frequencies were in Hardy–Weinberg equilibrium, 53.4% of the men had the genotype Gc1-1, 39.2% Gc1-2, and 7.4% Gc2-2. A total of 359 (46%) of the participants had a history of one or more bone fractures. The risk of fracture varied significantly (p = 0,047, Chi-square test) according to Gc genotype, being highest in Gc1-1 (48.3%), intermediate in the heterozygous Gc1-2 (45.9%) and lowest in Gc2-2 (31.0%). We found no significant differences in BMD between Gc genotypes. By using logistic regression analysis and adjusting for several potential confounders such as BMD values and various biochemical and clinical variables, we found the relative risk of fracture to be 2.1 (1.2–3.8) for men with Gc1-1 compared with Gc2-2, and 1.9 (1.05–3.5) for Gc1-2 compared with Gc2-2.

CONCLUSION: The risk of fracture in men until the age of 20 to 30 years is associated with Gc genotype, as in premenopausal women being highest in Gc1-1, intermediate in Gc1-2, and lowest in Gc2-2.

Conflict of Interest: None declared

P244-T

A NOVEL MUTATION IN TYPE I PROCOLLAGEN C-PROTEINASE CLEAVAGE SITE CAUSING "DENSE" OSTEOGENESIS IMPERFECTA

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Background/aims: Osteogenesis imperfecta (OI) is a heterogeneous genetic disorder with a prevalence of approximately 1/15 000 individuals. OI leads to an increased susceptibility to fracture. Phenotypes range from mild to lethal. In more than 90% of cases the disorder is due to a dominant mutation in one of the two genes that encode collagen type I, Col1A1 and Col1A2. Mild OI is usually the result of a mutation that leads to a premature stop codon, which subsequently leads to too little, but structurally normal collagen. In contrast, the more severe OI phenotypes are often de-novo mutations leading to an exchange of a glycine residue for an amino acid with a bulkier side chain. The aim of this study was to sequence the collagen I genes of a 10 year old girl with an unusual clinical phenotype of suspected OI. She had suffered 15 fractures in response to mild trauma and been evaluated clinically without a definite diagnosis. Previous examinations had revealed the following: lumbar spine bone mass density (BMD) of +3, 4 SD in Z-score, and whole body BMD of +1, 9 SD in Z-score, no family history for OI and no other clinical signs of OI such as blue sclera, dentinogenesis imperfecta or hearing impairment. Repeated biochemical tests, including calcium, phosphate, parathyroid hormone, magnesium and vitamin D were all normal.

Methods: DNA was extracted from whole blood using a commercially available kit. Exons and flanking intron sequences of COL1A1 and COL1A2 were sequenced using standardised sequencing with ABI3130XL using 110 primer pairs.

Results: A novel heterogenous mutation, Asp1219Asn, was detected at the splice site of the carboxy-terminal propeptide of the COL1A1 gene. Aspartat is conserved at this site in collagen type I, II and III in several species indicating that the amino acid sequence in this region is important for normal collagen formation. A total of 72 controls were investigated at this site, but none differed from the control sequence ruling out the possibility of a naturally occurring polymorphism.

Conclusion: Sequencing of collagen type I is valuable in patients with suspected OI, especially when there is an unclear phenotype. Furthermore, BMD is not necessarily low or strictly correlated to bone strength in OI. This mutation may represent a new pathogenic mechanism leading to "dense" OI.

Conflict of Interest: None declared

P245-S

CHARACTERIZATION AND MAPPING OF ALI34: A NEW ENU-DERIVED MURINE MODEL FOR OSTEOARTHRITIS

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We have recently utilized the Munich N-ethyl-N-nitrosourea (ENU) mutagenesis program to identify and characterize new genes and alleles that regulate skeletal development and homeostasis in mouse. Subsequently, the ALI34 autosomal dominant mutant line was isolated depicting several features of osteoarthritis (OA) which is the most common type of arthritis affecting up to 30% of people over age 55. Here we provide preliminary characterization of the skeletal phenotype in ALI34 and report upon genetic mapping of the ALI34 locus.

Skeletal morphology was assessed via μCT and alizarin red/alcian blue staining. Dynamic histomorphometry was performed via tetracycline/calcin double-labeling. Proteoglycans were assessed using safranin O. Chondromodulin I (ChM-1) and type II collagen were detected using IHC. Bone and body composition parameters were measured via pDXA and pQCT. Linkage analysis was conducted using a high throughput SNP platform and an outcross-backcross (F1 X P) breeding strategy.

12-week-old ALI34/+ animals exhibit shortened tibiae and femora, and had ankylosis and osteophytes affecting the knee joint. Both periarticular and epiphysial bones displayed subchondral sclerosis, and ossification within the articular space. A decrease in the rate of new trabecular femoral bone formation was accompanied by a ~13% reduction in vBMD (295.4 ± 15.4 mg/cm^3 control n = 10 and 258.3 ± 30.5 mg/cm^3 ALI34/+ n = 8; p = 0.001) in the femur. Abnormalities of the growth plate were detected such as absence of the resting zone of chondrocytes, and disorganization of the avascular zone by ChM-1 and type II collagen reactivity. In ALI34 articular cartilage, the transitional zone was absent, and erosion of matrix was evident without fibrillation. The articular capsule contained increased numbers of mesenchyme cells, and tendon metaplasia was apparent. pDXA results showed that ALI34 males had increased lean mass (~21%, p = 0.001, n = 6) and lowered fat mass (~37.5%, p = 0.001, n = 6). ALI34 linkage was found on chr. 6 and the current critical region spans 5Mb.

Our observations demonstrate that the ALI34 locus plays a key role in regulating development of the growth plate, and knee joints in mice. The ALI34 mutant exhibits many features of OA and could represent a valuable animal model with which to investigate the pathogenesis of knee osteoarthritis. Further

studies are in progress to identify the causal mutation in ALI34 and to further characterize the skeletal and articular phenotype.
Conflict of Interest: None declared

P246-M

ASSOCIATION OF THREE POLYMORPHIC MARKER'S AT THE INTERLEUKIN 6 GENE WITH BONE MINERAL DENSITY IN MEXICAN POPULATION

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Background: Genetic factors may play an important role in the pathogenesis of reduced bone mineral density (BMD). Interleukin 6 (IL6) is a multifunctional cytokine that has been implicated in the pathogenesis of bone loss because of its important effects on osteoclasts differentiation, stimulation and function.

Methods: In this study, we evaluated the relationship between three DNA polymorphisms, two single nucleotide polymorphisms (SNPs) located in the IL6 promoter, and a CA repeat polymorphism at the IL6 gene locus, with osteoporosis disease, in 70 osteoporotic women, 70 non-osteoporotic women (control group) and 170 subjects from a Mexican general population. The SNPs were genotyped using the 5́exonuclease assay, while the dinucleotide polymorphism was genotyped using capillary electrophoresis. Lumbar BMD was measured using DXA only in osteoporotic and non-osteoporotic women.

Results: For the SNP located at −174 of IL6 promoter (GC), we found that the allele C frequency was significantly higher in non-osteoporotic women than in osteoporotic women (22% versus 10%; $p < 0.013$) and that the CC genotype was present only in non-osteoporotic women. The C allele was associated with higher BMD; therefore it may constitute a protective factor against osteoporosis (odds ratio 0.563; 95%CI 0.225–0.989). On the other hand, the Allele G seems to be associated with the presence of osteoporosis (odds ratio 2.14; 95% CI 0.86–5.33), however, it displayed the same frequency in general population. The importance of analyze the population genetics in a case–control study allowed us to associate adequately this polymorphism. For the SNP located at -572 of the IL6 promoter (GC) and CA dinucleotide polymorphism, we did not found any association with BMD when osteoporotic women, non-osteoporotic women and general population were analyzed. All of these results were adjusted with potential confounders for osteoporosis by a longitudinal multivariate model. We confirmed the association between the alleles of IL6 locus with the case–control phenotypes, using software STRAT for an admixture population.

Conclusion: The women with the C allele of IL6–174GC polymorphism had lower risk to present osteoporosis. These results suggest that genetic variation at the IL6 gene locus implies that this polymorphism may be useful marker for the genetic study of osteoporosis.
Conflict of Interest: None declared

P247-T

IL-1 GENE POLYMORPHISMS AND BONE TURNOVER AFTER THE MENOPAUSE

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Interleukin 1 (IL-1) gene variation has been implicated in the risk of osteoporotic fractures and of early postmenopausal bone loss. The aim of the study was to examine the relationship between IL-1 gene variations and bone turnover after the menopause. We recruited 136 postmenopausal Caucasian women (age 50 to 85 yrs) and measured bone turnover markers (bone ALP, OC, PICP, uPYD, uDPD, uNTX, sCTX). DNA was analyzed for common IL-1 polymorphisms IL-1A (+4845), B (+3954, −511, −1468, −3737) and RN (+2018). It has recently become apparent that haplotype analysis may be more informative than SNP analysis, so subjects were classified as IL-1 Os+ (homozygosity for the common allele at SNP A+4845 or B +3954) or IL-1 Os- (carriage of > 1 copy of the rare allele at +4845 and +3954).

Levels of biochemical markers, expressed as Z score (Z = result-mean/SD), did not differ between genotypes testing individual markers or for all markers combined. As there is accelerated bone loss in the early menopausal period, subjects were sorted by YPM quartile, and genotype groups compared within Q1 YPM (< 8.5 yrs) and within the pooled Q2–Q4 YPM (> 8.5 yrs). In the Q1 YPM group, the bone resorption Z score (4 markers) was significantly higher for IL-1 Os+ vs IL-1 Os- individuals ($p < 0.05$). Combined Z scores did not differ between IL-1 genotypes in the Q2–Q4 YPM cohort comparison, nor when the overall cohort was subdivided by age quartiles rather than by YPM. Within the Q1 YPM group, median value for each resorption marker was higher in IL-1 Os+ individuals, reaching statistical significance for serum CTX, the most dynamic resorption marker ($p = 0.002$), see table, median (SD) for bone markers.

Thus, IL-1 gene variation in the early postmenopausal period may be a determinant of bone resorption and this may explain previous findings of increased risk of osteoporosis. However, this conclusion must be interpreted with caution due to the small sample size and requires further investigation in a larger cohort.

Table:

	Q1 YPM IL-1 Os-	IL-1 Os+	P	Q2-4 YPM IL-1 Os-	IL-1 Os+	P
Bone ALP	32 (11)	35 (13)	0.7	41 (16)	36 (14)	0.6
OC	5.9 (3.0)	7.0 (2.1)	0.3	6.0 (2.5)	6.3 (2.8)	0.6
PICP	101 (38)	101 (25)	0.9	95 (24)	102 (34)	0.2
UPYD	38 (16)	48 (10)	0.3	46 (11)	43 (16)	0.5
UDPD	12 (5)	15 (4)	0.06	15 (5)	13 (6)	0.4
UNTX	44 (27)	67 (24)	0.2	43 (28)	49 (39)	0.6
SCTX	0.2 (0.1)	0.4 (0.2)	0.002	0.3 (0.2)	0.3 (0.2)	0.8

Conflict of Interest: K Naylor, Interleukin Genetics, Grant Research Support R Eastell, Interleukin Genetics, Grant Research Support, Consultant, Shareholder J Rogus, Interleukin Genetics, other (employee) K Huttner, Interleukin Genetics, other (employee) L Wilkins, Interleukin Genetics, other (employee)

P248-S

ANDROGEN RECEPTOR CAG REPEAT POLYMORPHISMS: ASSOCIATIONS WITH FEMORAL SHAFT BONE MASS AND BODY SIZE IN YOUNG MEN

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Introduction: Hypogonadism causes bone loss and increase fracture risk. Previously, analyses of the polymorphisms in the CAG-repeats in exon 1 of the androgen-receptor (AR) have revealed associations with reduced bone mass and increased fracture risk in women but, recently, no impact on BMD in 18–20 year old men. We examined the effects of the AR CAG polymorphism on bone mass in men participating in the Odense Androgen Study (OAS).

Methods: OAS is a population-based, prospective study on endocrine status, body composition, muscle function, and bone metabolism in young men. We invited a random sample of 3000 Danish men aged 20–30 years through the Central Office for Civil Registration to participate. Seventy-three per cent responded and 783 accepted further participation.

Results: Genetic analysis showed a median number of CAG repeats of 21. By DXA, no significant differences in BMD of the lumbar spine or hip were detected. By MRI, significant association existed between AR genotype and cross-sectional measures of femoral shaft cortical bone (CB) and total bone (TB) (table 1). In a subsequent regression analysis containing physical activity, smoking status, alcohol, age, BMI, free testosterone, the number of repeats remained significantly correlated to both CB and TB ($R2 = 20.5$; $p = 0.04$ and $R2 = 16.4$; $p = 0.04$). The association was no longer significant after correction for height, which could, at least in part, be explained by the inverse relationship between height and numbers of repeats ($\rho = -0.09$); $p = 0.019$). Accordingly, in partial correlation analyses, the effect of CAG repeats remained after correction for physical activity but not weight, height, or thigh muscle mass.

Conclusion: the number of CAG repeats in the AR was inversely related to CB and FS, however, the effect was small and can be accounted for by differences in height as men with many CAG repeats showed a trend towards being shorter and having lower lean body mass

Table:

Number of repeats n	Low < 21	High > 21	p-values
Weight	82.2 (12.3)	81.3 (11.9)	P = .32
Height	182.1(6.77)	181.2 (6.53)	P = .06
Free testosterone	0.47 (.12)	0.48 (.13)	P = .29
Femoral total bone	7.50 (.85)	7.31 (.76)	P = .017
Femoral, compacta g	6.22 (.73)	6.04 (.64)	P = .011

Conflict of Interest: Brixen K, Eli Lilly, Consultant Brixen K, Eli Lilly, Speakers bureau Andersen M, Ipsen, Grant/Research Support Andersen M, Ipsen, Speakers Bureau Brixen K, MSD, Grant Research Support Brixen K, Novartis, Consultant Brixen K, Novartis, Speakers Bureau Andersen M, Novo Nordisk, Speakers Bureau Andersen M, Pfizer, Speakers Bureau Brixen K, Servier, Consultant Brixen K, Servier, Speakers Bureau

P249-M

EFFECTS OF PRO12ALA AND C161T POLYMORPHISMS OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-GAMMA GENE ON THE BONE MINERAL METABOLISM IN KOREAN WOMEN

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Background Since osteoblasts share the same origin with adipocytes in bone marrow, it is assumed that PPAR, which is an important nuclear receptor for adipocyte differentiation, plays a role in the bone microenvironment. Recent evidences support the primitive roles of PPAR in osteoclasts differentiation as well as osteoblasts differentiation. Previously, we published that C161T polymorphism of PPAR-gamma gene was associated with serum levels of OPG, which plays an inhibitory role in osteoclastogenesis. Therefore, we investigated whether two common polymorphisms in the PPAR-gamma gene were related to the bone mineral metabolism in healthy middle-aged women. Methods In 239 healthy Korean women (mean age 51.4 ± 6.7 years), anthropometric measurements were done and lumbar spine and femoral neck BMD, bone turnover markers, such as serum ALP levels, urine deoxypyridinoline levels, and 24-hour urine calcium excretion were measured. Serum levels of OPG were measured with ELISA method. Genotyping of the polymorphisms in the PPAR-gamma gene was performed via an allelic discrimination assay with using a TaqMan probe. In addition, we examined the haplotype analysis between two polymorphisms of PPAR-gamma gene, Pro12Ala and C161T. Results In Pro12Ala polymorphism, allele frequencies were 0.950 for C allele and 0.050 for G allele, which was in Hardy–Weinberg equilibrium ($p = 0.716$). In Pro12Ala polymorphism, Mean levels of serum ALP ($p = 0.014$) was significantly higher and serum OPG ($p = 0.033$) was significantly lower in G allele carriers compared with non-carriers. After adjustment for age and BMI, serum ALP was persistent significant ($p < 0.01$), but serum OPG became marginally significant ($p = 0.063$). In haplotype analysis between two polymorphisms of PPAR-gamma gene, Pro12Ala and C161T, subjects with one minor allele showed significantly lower serum OPG levels than subjects with no minor alleles regardless of its allele types, moreover subjects with two minor alleles showed significantly lower serum OPG levels than subjects with one minor allele ($p = 0.005$). After adjustment for age and BMI, these trend was persistent significant ($p = 0.007$). Conclusion PPAR-gamma Pro12Ala polymorphism has influence on levels of serum ALP, and show a tendency to be associated with levels of serum OPG. Especially, the haplotype analysis with C161T variants reveals that subjects with more minor alleles tend to show lesser level of serum OPG.

Conflict of Interest: None declared

P250-T

EFFECTS OF THE ESTROGEN RECEPTOR, CALCITONIN RECEPTOR AND OSTEOPROTEGERIN GENES ON BONE MINERAL DENSITY, BONE-RELATED BIOCHEMICAL MARKERS AND FRACTURE INCIDENCE IN SLOVAK POSTMENOPAUSAL WOMEN

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Osteoporosis is a disease of low bone mineral density (BMD) and microarchitectural deterioration of bone with increased fracture risk. The estrogen receptor (ESR) and the calcitonin receptor (CALCR) are proteins which mediate hormonal action in target tissues. In bone the effect can result in bone resorption or formation. Osteoprotegerin (OPG) belongs to cytokines regulating osteoclastogenesis. Therefore, possible variability in the ESR, CALCR, and OPG genes could play a role in variability of BMD followed by variability in fracture risk.

In present study we analyzed effects of PvuII and XbaI polymorphisms in the ESR gene, AluI polymorphism in the CALCR gene, and AseI polymorphism in the OPG gene on variability in femoral and spinal BMD, as well as circulating alkaline phosphatase (ALP; formation marker), osteocalcin (OC; formation marker), beta-CrossLaps (CTX; resorption marker) and fracture incidence in 121 Slovak postmenopausal women (63.4 ± 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 analyzed by GLM procedure and covariance analysis after correction of the measurements for age and BMI. Frequencies of fractures were tested using the chi-square test.

We found a significant effect of ESR/pp genotype on ALP ($p = 0.034$), CTX ($p = 0.024$), and femoral ($p = 0.034$) and spine ($p = 0.041$) BMD. ESR/XbaI polymorphism did not affect any of the analyzed traits significantly. However, significant association of ESR/ppXx haplotype and ALP ($p = 0.020$), CTX ($p = 0.001$), femoral ($p = 0.046$) and spine ($p = 0.038$) BMD was observed. With the CALCR gene we did not find significant associations with the traits. The OPG gene influenced OC ($p = 0.046$) and CTX ($p = 0.010$), moreover, significant association between OPG-CALCR interaction and femoral BMD ($p = 0.015$) was found. Comparison of fracture incidence between the genotype groups showed significant differences ($p < 0.001$) for both ESR polymorphisms and ESR haplotypes.

The analysis of associations between candidate genes and BMD as well as bone-related biochemical markers can contribute to more detailed information 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

P251-S

EVIDENCE FOR ASSOCIATION BETWEEN THE ILE1062VAL POLYMORPHISM IN LRP6 AND BMD BUT NOT FOR INTERACTION WITH TWO LRP5 VARIANTS IN THE ODENSE ANDROGEN STUDY

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Osteoporosis is characterized by low bone mineral density (BMD) and microarchitectural deterioration of bone leading to an increased fracture risk. Heritability studies demonstrated that genetic factors may account for up to 80% of the variability in BMD. Multiple studies showed that the genes encoding low density lipoprotein receptor-related protein 5 and 6 (LRP5/6) are implicated in the regulation of bone homeostasis. Both LPR5 and LRP6 act as coreceptors for Wnt ligands in canonical Wnt signaling. It has already been demonstrated that natural variants in LRP5 and LRP6 are associated with BMD and susceptibility to osteoporotic fractures. We previously reported association between the Ala1330Val and Val667Met variants in LRP5 and peak bone mass in young, physically active men from the Odense Androgen Study (OAS).

The objective of our study is to evaluate whether LRP6 is a susceptibility gene for the regulation of bone density in the same cohort and whether there is a genetic interaction between LRP5 and LRP6 polymorphisms. The individuals form part of the OAS, a large prospective population-based observational study comprising 783 Caucasian men aged 20–30 years. BMD at several skeletal sites was carried out using DXA. Genotyping information of Val667Met (exon 9) and Ala1330Val (exon 18) in LRP5 and of Ile1062Val (exon 14) in LRP6 was obtained by fluorescence polarisation and Taqman assay. P-values less than 0.05 were considered significant.

We observed for the Ile1062Val polymorphism in LRP6 a significant association between genotypes and BMD at the FN in the whole population if we considered a recessive effect of the test allele (one-way ANOVA; $p = 0.03$). However, in contrast with the LRP5 polymorphisms no significant associations were found with Ile1062Val and BMD in the subgroup of non-sedentary men. We additionally studied possible genetic interactions between the LRP5 polymorphisms and the LRP6 variant using two-way ANOVA and multiple linear regression analyses for interaction. No evidence for interaction between LRP5 and LRP6 polymorphisms was found in the whole population neither in the subgroup of physically active men.

We conclude that natural variation in both LRP5 and LRP6 is involved in the regulation of BMD in subjects of the OAS but that in this population no genetic interaction between LRP5 and LRP6 polymorphisms could be detected.

Conflict of Interest: None declared

P252-M

SEQUENCING OF THE PROMOTER REGION AND EXONS 1A AND 1B IN THE HUMAN CALCIUM-SENSING RECEPTOR GENE

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Calcium homeostasis is a very exact homeostasis responding to very small fluctuations in the extracellular calcium ion concentration. One of the keys to the regulation of this homeostasis is the calcium sensing receptor (CASR). Mutations in the gene encoding the receptor could thus result in changes in the receptor function leading to changes in the calcium set-point.

Several studies have aimed at associating changes in the DNA sequence to a specific alteration in calcium homeostasis in vivo or the sensitivity of the receptor

in vitro, but none of these have focused on the promoter region and the untranslated regions . The aim of this project was therefore to sequence the promotor region and the untranslated exons 1A and 1B of the CASR gene, in order to determine whether DNA-variants or mutations in these regions are associated to a specific clinical diagnosis involving dysfunction of the calcium homeostasis.

DNA from 23 patients had previously been sequenced for mutations in exon 2 to 7 of the CASR without detecting an association of mutations to their calcium metabolic disease. In this study we sequenced the promoter regions and untranslated exons of the CASR gene using eight primers to amplify the DNA sequence of interest by polymerase chain reaction followed by direct sequence analyzing on an ABI 3100 Genetic Analyzer.

In 15 of the patients, we found a variation in the single nucleotide polymorphism (SNP) rs 9883981: 6 with |GGG|, 2 with |GAG| and 7 with |GG/AG| in the untranslated exon 1B, 111 nucleotides upstream of the splice site. Patients genotyped with the homozygote |GGG| sequence, had been given the clinical diagnosis of either familial hypocalciuric hypercalcemia (FHH), primary hyperparathyroidism (PHPT) or autosomal dominant hypoparathyroidism (ADH). Patients genotyped with the homozygote |GAG| sequence had been diagnosed as having either PHPT or ADH. The heterozygous genotype |GG/AG| leads to the phenotypes of FHH, PHPT and ADH. Thus the conclusion according to the results of this project is that the genotype of the CASR gene could not be directly associated to the diagnoses. A further study of the untranslated region of the CASR is required to find an association between mutations/polymorphism and the diagnosis. However, it is impossible to make any statements whether any one of these genotypes are just polymorphisms or a disease-causing mutation until the frequency of these DNA variants has been determined in the background population.

Conflict of Interest: None declared

P253-T

MUSCLE ASSOCIATED GENES ARE EXPRESSED IN HUMAN BONE BIOPSIES AND SHOW AGE AND DISEASE DEPENDENT CHANGES

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Background: Expression of muscle characteristic mRNAs have recently been described in human bone biopsies containing red marrow. Their expression were markedly altered already in patients with early primary hyperparathyroidism without clinical symptoms (J Bone Miner Res. 2006, 26 suppl1 SU430). One bone cell type expressing muscle characteristic mRNAs has been shown to be osteocytes. (J Bone Miner Res. 2006, 26 suppl1, 1006). Aims: To characterize the muscle associated mRNAs expressed in human pelvic bone biopsies in healthy and osteoporotic women in relation to possible age dependent variations. Methods: Healthy (40) and osteoporotic females (26) between 50 and 85 years of age had a transiliacal bone biopsy which were immediately frozen on liquid nitrogen for RNA isolation or fixed in paraformaldehyde for histological analyses. We use global gene expression profiling employing Affymetrix microchips technology and statistical analysis to identify categories of genes that were differentially expressed between younger and old women (group I: 50–65 and group II: 70–85 years of age). Cell morphology and specific biochemical markers were used to evaluate the population size of osteoclasts, osteoblasts, and osteocytes, and used to normalize the data. Results: 14 muscle related genes were statistically ($P < 0.05$, student t test) differentially expressed between younger and old healthy women with a mean correlation coefficient of 0.36. These mRNAs were: TTN, MEF2A, MYH10, TPM1, PPP1R12B, SSPN, MYO6, SORBS2, MBNL3, LOC129285, MYOD1, KCNJ12, MTPN, and PALLD; 11 were increased and 3 decreased in old healthy women. In osteoporotic women, the 3 muscle related genes ACTC, CXCL19 and MRAS were all increased at old age. Thus, the mRNAs which varied by age in healthy women, were distinct from those which were differentially expressed in patients with primary osteoporosis. The highest correlation (0.54) was found for the central myogenic differentiation factor MYOD1 which showed a 44% increase, and induces early myocyte differentiation from precursor cells (Annu. Rev. Cell Dev. Biol. 2002. 18: 747–83) Conclusion: the fact that muscle related mRNAs are expressed in human bone and are significantly altered as a function of age and disease may offer new paths for diagnosis and disease monitoring.

Conflict of Interest: None declared

P254-S

ALLELIC VARIANTS OF THE ENZYME MTHFR AND OSTEOPOROTIC FRACTURES

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Background: The C677T (rs1801133) polymorphism of MTHFR (methylenetetrahydrofolate reductase) results in an alanine to valine change that reduces the enzymatic activity and tends to increase homocysteine levels. Thus the TT genotype has been associated with an increased risk of cardiovascular events and also with bone mineral density in some studies, but the results are controversial. Our objective was to determine the relationship of the polymorphism with osteoporotic fractures.

Subjects and methods. C677T was analysed in 823 subjects, including 365 controls (age 76 ± 10 years), 136 with vertebral fractures (70 ± 8 yr) and 322 with hip fracture (81 ± 19 yr). DNA was isolated from the peripheral blood or buccal swabs and the alleles characterized by means of a Taqman assay. The association of genotypes with fractures was estimated by the odds ratio (OR).

Results: The distribution of MTHFR genotypes was similar in patients and controls, without statistically significant differences. In comparison with TC/CC genotypes, the age-adjusted OR for hip fractures of the TT genotype was 1.0 (95% confidence interval 0.6–1.7) in women and 0.7 (0.3–1.8) in men. The OR for vertebral fractures was 0.8 (0.4–1.7) in women and 1.7 (0.4–6.7) in men.

Conclusion: In this study we did not find an association of the C677T polymorphism of the MTHFR gene with the risk of osteoporotic fractures, either at the spine or the hip.

Conflict of Interest: None declared

P255-M

MULTI-LOCUS ANALYSIS OF ESTROGEN PATHWAY POLYMORPHISMS AND VERTEBRAL FRACTURE RISK IN ELDERLY WOMEN: AN EXERCISE

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Background: Common diseases like osteoporosis are thought to be caused by multiple genetic variants with each a small effect. Estrogen plays a role in preserving strength and integrity of trabecular bone in men and women. Estrogen receptors alpha (ESR1) and beta (ESR2) polymorphisms are associated with vertebral fracture risk in elderly women, with small yet consistent effects. Polymorphisms in other genes of the estrogen pathway (like RIZ1, COMT, CYP19) show inconsistent results. Applying multi-locus pathway analysis is expected to provide better insight in the complexity of common disease.

Objective: To study epistatic effects of 6 polymorphisms in 6 different genes involved in the estrogen pathway in relation to vertebral fracture risk in women.

Methods: 1327 elderly women (55 years and older) who had X-ray data on vertebral fractures (n = 149) were genotyped for ESR1 XbaI, ESR2 intron2, RIZ1 Pro704 Ins/Del, COMT Met158Val, CYP19 +1531, CYP3A4*1b polymorphisms. We applied 3 methods to identify epistatic effects: Multilocus FAMHAP analysis, Multifactor Dimensionality Reduction (MDR) and Focus Interaction Testing Framework (FITF).

Results: For the risk of vertebral fracture: FAMHAP identified as most significant interaction the genotype combination of the RIZ1 Pro704, CYP19 3'UTR +1531, and the ESR2 intron 2 polymorphisms (P-value: 0.001, adjusted for multiple testing); FITF identified CYP19 3'UTR +1531 and ESR2 intron 2 polymorphisms as the most significant 2-way interaction (P-value: 0.0006), and RIZ1 Pro704, CYP3A4*1b and the ESR2 intron 2 polymorphisms as the most significant 3-way interaction (P-value: 0.007); and MDR identified (though not significant) ESR1 XbaI, RIZ1 Pro704, CYP19 3'UTR +1531, and the ESR2 intron 2 polymorphism as the most predictive interaction.

Conclusion: We studied only few polymorphisms in few genes, but the major epistatic effects rose from the same 3 genes: RIZ1, CYP19, and ESR2. Only ESR2 was associated independently with vertebral fracture risk in women. These results illustrate that genetic association studies using pathway analysis can make hierarchies of genetic effects underlying complexity of osteoporosis.

Conflict of Interest: None declared

P256-T

TRAF6 GENE VARIANTS IN A FAMILY WITH A HIGHLY PENETRANT FORM OF OSTEOPOROSIS

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The search for genes responsible for most complex disorders such as osteoporosis has been of a major challenge to geneticists. A whole genome scan was performed in a Maltese family with a highly penetrant form of osteoporosis from

which a number of loci were indicated. In this study a gene present on one of the indicated loci on chromosome 11p12 was further investigated.

Based on prior knowledge of physiology and studies done on animal models, the TNF receptor-associated factor (TRAF)-6 gene located on chromosome 11p12 was chosen for sequencing. All exons together with intron–exon boundaries and 1.5kb upstream from the transcriptional start site were first amplified by PCR and sequencing using the BigDye® terminator technique.

Three sequence variants were identified in the TRAF6 gene, two of which had never been reported. An A to T transversion was identified at position -721 (5′ upstream) of the transcriptional start site, when compared to TRAF6 reference sequence (AY228337). Three affected members from this family were observed to be heterozygous for this variant that was not found in normal controls. After screening 398 chromosomes from the general population, only 4 alleles were observed (1.0%) with this variant. An already reported deletion of a T (rs3830511), was observed in a polyT region very close to the intron/exon boundary of exon 5. A transition G to A was found in intron 6, 110bp upstream of the intron/exon boundary. Only one heterozygote for this variant was identified within this family, while the rest were homozygous for the wild type allele G. This individual was also heterozygous for the deletion described above. When screening the population, the G/A variant was observed to be a common polymorphism.

Although these variants were not directly linked with the disease in this family, one cannot exclude the possible role of this gene in the pathogenesis of osteoporosis. Other variants might be present in other regions not sequenced within this gene. The rare variant found in the promoter region is being further investigated for its possible effect on gene expression.

Conflict of Interest: None declared

P257-S

RELATIONSHIP OF C420G POLYMORPHISM IN THE PROMOTER OF RESISTIN GENE WITH BONE MINERAL METABOLISM IN KOREAN WOMEN

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Background: Resistin is thought to be an important link between obesity and insulin resistance. Also, resistin gene is new candidate gene of insulin resistance and type 2 diabetes mellitus. The role of resistin in bone mineral metabolism has been recently reported on in vitro studies. Resistin is expressed in mature human osteoblasts. Recombinant resistin increased the number of differentiated osteoclasts and enhanced the proliferation of preosteoblasts. Previously, we published that serum levels of resistin was associated with lumbar spine bone mineral density (BMD) in middle-aged men. There has been no report on the association of resistin gene polymorphism and bone mineral metabolism in human being. Therefore, we investigated whether one common single nucleotide polymorphism (C420G) in the resistin gene were related to the bone mineral metabolism in healthy middle-aged women. Methods In 247 healthy Korean women (mean age 51.3 ± 6.8 years), anthropometric measurements were done and lumbar spine and femoral neck bone mineral density (BMD), bone turnover markers, such as serum alkaline phosphatase (ALP) levels, urine deoxypyridinoline levels, and 24-hour urine calcium excretion were measured. DNAs were extracted from the samples and the genotyping of the C420G polymorphism in the promoter of resistin gene was performed via an allelic discrimination assay with using a TaqMan probe. Results Allele frequencies were 0.672 for C allele and 0.328 for G allele, which was in Hardy–Weinberg equilibrium ($p = 0.420$). There were no differences in mean values for age and body mass index among different genotypes of C420G polymorphism in the resistin gene. In the bone turnover markers, there were no differences in mean levels for serum ALP and urine deoxypyridinoline among different genotypes of C420G polymorphism in the resistin gene. In addition, there were no differences in mean values for BMDs and urine calcium excretion among different genotypes of C420G polymorphism in the resistin gene. Conclusion: Thus, our results show that C420G polymorphisms in the promoter of resistin gene, which has previously been found to be associated with obesity and insulin resistance, are not associated with bone mineral metabolism in healthy middle-aged Korean women.

Conflict of Interest: None declared

P258-S

THE N-TERMINAL FRAGMENT OF PARATHYROID HORMONE-RELATED PROTEIN INDUCES EPITHELIAL–MESENCHYMAL TRANSITION BY TRANSACTIVATION OF THE EPIDERMAL GROWTH FACTOR RECEPTOR

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Recent data suggest that G protein-coupled receptors, including the parathyroid hormone (PTH)/PTH-related protein (PTHrP) type 1 receptor (PTH1R), might modulate osteoblastic proliferation and/or differentiation by transactivation of the epidermal growth factor (EGF) receptor (EGFR). The mechanisms of EGFR transactivation are complex, and might involve the release of EGF ligand by the action of metalloproteases (MMPs). Both PTHrP and the PTH1R are expressed in renal tubuloepithelial cells. In these cells, EGF and transforming growth factor-beta; induce epithelial–mesenchymal transition (EMT), a critical process in renal fibrosis. We hypothesized that activation of PTH1R can transactivate the EGFR and thus induces EMT in tubuloepithelial cells. Mouse renal tubuloepithelial cells MCT were cultured for 48 h in the presence or absence of PTHrP (1–36) (100 nM), pretreated or not with the MEK inhibitor U0126 (20 μM), the EGFR inhibitor tyrphostin AG1478 (5 μM), the protein kinase (PK) C inhibitor bisindolylmaleimide I (BIM) (25 nM) or the MMP-2/MMP-9 inhibitor I (Calbiochem) (10 μM) for 2 h. To determine phosphorylation of p42/p44 extracellular signal-regulated kinases (ERK 1/2), cells were serum-depleted 24 h prior to PTHrP stimulation. Protein expression of the EMT markers E-cadherin, alpha-smooth muscle actin (alpha-SMA), and integrin-linked kinase (ILK), as well as ERK 1/2 and phospho(p)ERK 1/2 protein levels were assessed by Western blot. We observed that PTHrP (1–36) significantly decreased E-cadherin protein levels, while it increased alpha-SMA and ILK protein expression (2-fold vs corresponding control value, $p < 0.05$) in MCT cells. Moreover, this PTHrP peptide induced a rapid stimulation of ERK 1/2 phosphorylation (5-fold over control, at 5 min; $p < 0.01$), decreasing to control values at 15 min in these cells. Preincubation with either BIM or AG1478 significantly ($p < 0.05$ or less) inhibited, but not abolished, both EMT alterations and ERK 1/2 phosphorylation induced by PTHrP (1–36) in MCT cells. In addition, pretreatment with MMP-2/9 inhibitor I significantly reduced the pERK 1/2 protein levels increased by PTHrP (1–36) in these cells. However, the latter effect was abrogated by U0126, which also significantly inhibited the PTHrP-induced changes in the EMT markers.

In summary, our findings suggest that the N-terminal fragment of PTHrP stimulates various EMT markers by ERK activation, in part through EGFR transactivation in tubuloepithelial cells.

Conflict of Interest: None declared

P259-M

THE ASSESSMENT OF BONE TURNOVER IN HYPOTHYROIDIAN MENOPAUSAL WOMEN TREATED WITH DIFFERENT LEVOTHYROXINE DOSES

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Hypothyroidism is a frequent pathology in women, prevalence increasing with age. Thyroxine's therapeutic criteria are primarily established according to the TSH (''thyroxine stimulating hormone'') seric levels, and the therapeutic benefit proves not to be the expected one. The aim of the study was to underscore the disturbances of bone metabolism in menopausal women receiving different doses of levothyroxine (L–T4).

The study was conducted on 64 patients with SCHT, mean age $52 +/- 1.8$ years, menopause being present. Based on TSH screening (N = 0.27–4.2 mU/L), the patients were divided in 2 groups: Group 1 (n = 34), with TSH > 10 mU/L (17, 4 +/– 2.1mU/L) and Group 2 (n = 30), with TSH < 10 mU/L (7.8 +/– 1.6mU/L). L–T4 was given based on TSH values: Group 1 = 98 +/– 4.5 μg/day and Group 2 = 56 +/– 11 μg/day.

The initial measurements were: BMD (DXA) appreciating T and Z score according to WHO criteria; osteocalcin—evidencing bone function; beta cross-lap marker of bone destruction.

Serum markers were repeated after 6 and 12 months and BMD after 12 months. In group 1 after 6 months there was an increase in the serum level of osteocalcin compared to the initial values ($p < 0.001$); serum levels of beta cross-lap were not significantly modified. In group 2 after 6 months serum levels of osteocalcin increased but had a poor statistical significance ($p = 0.05$); serum levels of beta cross-lap remained the same.

12 months after treatment: In group 1 were obtained significantly high levels of osteocalcin and beta cross-lap ($p < 0.001$), compared to initial serum levels. DXA results shown a decreased BMD in lombar region with 1.2% and 0.6% in the head of femur. In group 2 were obtained significantly high levels of osteocalcin ($p < 0.001$) with a poor statistical significance for beta cross-lap ($p = 0.05$). DXA results shown decreased BMD values: 0.8% in spinal vertebra and no change in femur.

Starting treatment with thyroxine in patients with hypothyroidism in menopause women requires a careful assessment of therapeutical guidelines. Increased bone turnover during this period can be accelerated by the administration of thyroxine in concordance to the dose and time of administration.

Conflict of Interest: None declared

P260-T

SEVERE VITAMIN D DEFICIENCY IN OSTEOARTHRITIC PATIENTS IS ASSOCIATED WITH LESS IMPROVEMENT IN DAILY FUNCTIONING AFTER TOTAL KNEE AND HIP ARTHROPLASTY

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Background: Vitamin D deficiency is common in the elderly and can result in muscle weakness and bone pain.

Objective: To determine the prevalence of vitamin D deficiency in severely osteoarthritic patients and the association between vitamin D status and determinants of the rehabilitation process after total hip or knee arthroplasty.

Methods: Between December 2005 and April 2006 patients aged > 50 years who underwent total hip or knee arthroplasty because of osteoarthritis were included. Serum 25-hydroxyvitamin D3 (25-OHD) levels, visual analogue scale (VAS) for pain and general health (GH) and health assessment questionnaire (HAQ) as determinants for daily functioning were recorded at baseline and four months post-surgery.

Results: 116 women and 45 men aged 69.7 ± 9.3 (mean ± SEM) years were included. 93% was Caucasian. Only 16 patients (10%) were vitamin D sufficient (VDS serum 25OHD > 50 nmol). 35 patients (22%) were severely vitamin D deficient (VLD: serum 25-OHD < 12.5 nmol/), 51 patients (32%) were vitamin D deficient (VD: serum 25-OHD 12.5–25 nmol/l) and 58 patients (36%) were vitamin D insufficient (serum 25-OHD 25–50 nmol/l).

After arthroplasty, lower serum vitamin D levels were significantly associated with less improvement in VASGH ($R2 = 0.09$, $P = 0.004$), independent of sex, age, arthroplasty type, ΔVASpain, ΔHAQ and comorbidity. VDS patients had highest improvement in VASGH (ΔVASGH -24.6 ± 10.8), but VLD patients had no clinical relevant improvement in VASGH (ΔVASGH -1.4 ± 5.8). After arthroplasty, all patients had improvement in VASpain (ΔVASpain – 28.3 ± 2.8), but VLD patients remained clinically relevant more painful than VDS patients (VASpain VLD 37.9 ± 5.6 and VASpain VDS 26.7± 7.3, NS). Vitamin D deficiency also tended to be associated with a lower level of daily functioning pre and post surgery. VLD patients had highest HAQ score at baseline (0.98 ± 0.12) and no clinical relevant improvement after surgery (ΔHAQ -0.14 ± 0.12). Higher serum 25-OHD levels were associated with lower HAQ scores at baseline (in VDS patients HAQ 0.66± 0.12) and clinical relevant improvement after surgery (in VDS patients ΔHAQ -0.35 ± 0.12).

Conclusion: There is a high prevalence of vitamin D deficiency in osteoarthritic patients. Low vitamin D levels seem to have a detrimental effect on daily functioning and improvement in general wellbeing after arthroplasty. Therefore, it is reasonable to measure serum vitamin D levels in osteoarthritic patients

Conflict of Interest: None declared

P261-S

SERUM ADIPONECTIN LEVELS ARE POSITIVLY ASSOCIATED WITH THE DIESEASE SEVERITY OF RHEUMATOID ARTHRITIS EVALUATED WITH THE EXTENT OF OVERALL JOINT DESTRUCTION

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[Objectives] Adiponectin is an adipose-derived hormone which exhibits various biological functions, such as increasing insulin sensitivity, protecting hypertension and suppression of atherosclerosis, liver fibrosis. It has been shown to possess anti-inflammatory activity by suppressing both TNF-α and IL-6 production in macrophage activated with lip polysaccharide. Furthermore, we have previously reported that adiponectin inhibit osteoclast differentiation and proliferation. From this point of view, we hypothesized that disease activity or severity of rheumatoid arthritis (RA) is affected by the serum levels of adiponectin. The objectives of this clinical study were to test this hypothesis. [Methods] Hundred and twenty seven female patients with RA were enrolled to the study. Mean age was 60.7 ± 11.9 (from 23 to 89), and the mean body mass index was 22.5 ± 10.6 (from 14.0 to 38.5). The severity of RA was evaluated according to the extent of overall joint destruction represented by number of joint with erosion evident on plain radiograms, i.e. least erosive subset (LES), more erosive subset (MES) and most erosive subset with mutilating disease (MUD) reported by T. Ochi et al. We classified LES for mild cases and MES/MUD for severe cases (53 mild cases and 74 severe cases). Serum adiponectin, biochemical markers of inflammation [CRP, MMP-3], rheumatoid factor (RF), hemoglobin (Hb), albumin, bone metabolic markers [bone specific alkaline phosphatase, intact osteocalcin (iOC), carboxyterminal crosslinked telopeptide of type1collagen (1CTP), urinary-deoxypyridinoline] were measured. We also assessed tender joint number, swollen joint number, Steinbrocker class, and the dose of prednisolone and methotrexate. [Results] There was no correlation between the serum levels of adiponectin and the biochemical markers of inflammation or clinical score of disease activity. On the other hand, there were significant difference between mild and severe subsets of RA in the serum levels of adiponectin, CRP, 1CTP, iOC, MMP3, RF, Hb, Alb, tender or swollen joint number, and dose of predonisolone. Multivariate logistic regression analysis revealed that only serum adiponectin levels significantly associate with the severity of RA. [Conclusion] These results suggest that adiponectin may have relation to increased number of joint destruction in RA.

Conflict of Interest: None declared

P262-M

GH TREATMENT INCREASES CORTICAL THICKNESS IN YOUNG ADULTS WITH CHILD-HOOD ONSET GH-DEFICIENCY

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Patients with childhood-onset growth hormone deficiency (CO GHD) are usually treated with GH until final height is reached, but the treatment is stopped long before peak bone mass is obtained. Since GH has well known effects on bone growth and bone mineral accretions, it might be useful to prolong GH treatment in CO GHD beyond final height. To evaluate the potential benefit of GH-treatment on cortical bone in this patient-group a randomized controlled, open-label study of young adults with CO GHD was performed. Material and methods: In a group of 160 patients with CO GHD, 109 were randomized to Norditropin® SimpleXx® and 51 patients were randomized to receive no treatment. Male/female ratio was 61/99, with a mean age of 21.2 (2.2) years. Mean (SD) height for males / females was 171.7 (7.5) / 157.5 (8.1) cm, mean weight was 69.0 (13.4) kg /56.2 (11.1) kg, corresponding to BMI 22.5 (3.4) / 23.3 (3.5). Patients were treated for 2 years, with a maintenance dose of 1.0 / 1.4 mg daily for males and females, respectively. For all individuals hand x-rays were obtained at 0, 6, 12, 18 and 24 months and send to a central reading facility where they were analyzed in a blinded manner, using digital x-ray radiogrammetry (DXR) of metacarpal 2–4. From this reading information on cortical thickness, bone width, endosteal diameter and metacarpal index was obtained. In the GH-treated group a significant increase in cortical thickness and metacarpal index was seen. The main cause of the increase in cortical thickness is a significant reduction of the endosteal diameter. In the control group the endosteal diameter did not change, leading to a much smaller increase in cortical thickness. Conclusion: I Treatment with Norditropin® SimpleXx® for 2 years in young adults with CO GHD led to a substantial endosteal bone growth with a reduction of the marrow space diameter and consequently an increase in the cortical thickness. Since cortical bone loss later in life is mainly caused by endosteal bone resorption, the effects of 2 years treatment with growth hormone (Norditropin®) before peak bone mass is reached may prove useful in preventing osteoporosis later in life.

Table: Results after 24 months

	Cortical thickness	Bone Width	Inner Diameter	Metacarpal Index
Norditropin®	1.10	1.02	0.96	1.08
Untreated	1.04	1.02	1.01	1.01
Effect (%)	6.43	0.68	-4.64	6.14
P-values	0.0001	0.4719	0.0006	< 0.0001

Conflict of Interest: L Hyldstrup, Eli-Lilly Denmark, MSD, Novartis, Nycomed, Consultant, Novo-Nordisk research grant

P263-T

EFFECT OF KOREAN RED GINSENG ON LIPID PROFILES AND AORTIC VASCULAR (ENDOTHELIAL) PATHOLOGIC CHANGES FOR 3 MONTHS COMPARED WITH CHINESE RED GINSENG AND CONTROL IN RATS

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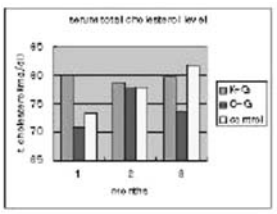
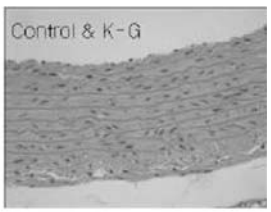
Background : Korean Red Ginseng has been shown to have an antioxidant effect on lipid peroxidation and endothelial cell calcification. However, there are few studies showing the effect of Red Ginseng on lipid profiles and vascular (endothelial) pathologic changes together.

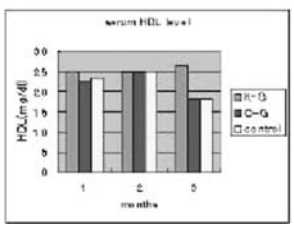
Methods : We studied the in vitro effect of Korean Red Ginseng compared with Chinese Red Ginseng and control by lipid profiles and aortic vascular (endothelial) pathologic changes during 3-month.

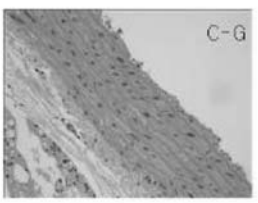
Results : HDL level was increased on 3rd month in Korean Ginseng group compared with other group by statistical significance ($p < 0.05$). In case of vascular (aortic endothelial) pathologic finding, endothelial wall thickening and elastic fiber tearing were noted in Chinese Red Ginseng group compare to Korean Red Ginseng group ($p < 0.05$).

Conclusion : These results suggested Korean Red Ginseng might have a beneficial effect on protection of lipid metabolism and vascular (endothelial pathologic) changes.









Conflict of Interest: None declared

P264-S

CARDIOVASCULAR DISTRIBUTION OF THE CALCIUM-SENSING RECEPTOR BEFORE AND AFTER BURNS

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Burned children have hypocalcemic hypoparathyroidism likely due to up-regulation of the parathyroid gland calcium-sensing receptor (CaR) (Crit Care Med 2000). CaR has also been identified in myocardial myocyte cultures (Biochem Biophys Res Comm 2006) and since burn injury decreases myocardial contractility, the aim of our study was to localize CaR within the heart and to determine if the receptor density or distribution is changed by burn injury. We obtained samples of heart and aorta from female Merino sheep sacrificed at 24h after being subjected to 40% burn injury under anesthesia or sham burn. Tissue was stained for CaR using the ADD antibody and fluorescently labelled secondary stain. Stain specificity was established using CaR peptide washout which resulted in 23% of fluorescence remaining. Tissue samples were examined using fluorescence deconvolution microscopy. In the heart tissue, CaR was localized to the endocardial endothelium, myocardial microvasculature and fibroblasts and vessels of the epicardium/adventitia. In the aorta, CaR was found in the endothelial intima, medial microvasculature, fibroblasts and vessels of the adventitia. No CaR was found in either cardiac myocytes or smooth muscle cells of the aorta. There were no differences in the density of either CaR or beta adrenergic receptors between burn injured and sham tissues, nor was there a difference in CaR distribution. This study demonstrates that in contrast to the parathyroid, where burn injury up-regulates CaR, cardiac and aortic CaR distribution and density are not different in burned animals when compared to sham-treated controls. We therefore suggest that CaR has a local, tissue-specific role and has a function in vascular Ca sensing, either for intravascular Ca deposition or for regulating other Ca channels to adjust ion concentrations and exchanges after trauma or burn injury.

Conflict of Interest: None declared

P265-M

EFFECT OF A SHORT-TERM VITAMIN D TREATMENT ON INFLAMMATORY BIOMARKERS AND OPG/RANKL SYSTEM IN POSTMENOPAUSAL WOMEN WITH OSTEOPENIA/OSTEOPOROSIS

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Introduction: Osteoporosis and cardiovascular diseases are linked by several common pathophysiological mechanisms and risk factors. Estrogens are one of important modulators in bone formation as well as in the development of atherosclerosis. They act through their effects on cytokines, such as IL-1, IL-6, TNFα and osteoprotegerin (OPG). The lack of estrogens induces an increase in these cytokines and decrease in OPG, both involved in the process of bone resorption and atherogenesis. Recently, the evidence of down-regulation of the expression of IL-6 and TNFα by vitamin 1, 25(OH)2D was shown. The aim of the study was to evaluate the possible impact of short term vitamin D treatment on some inflammatory biomarkers (IL-6, TNFalpha, CRP) and OPG/RANKL system.

Methods: 100 postmenopausal women with densitometrically confirmed osteopenia/osteoporosis were enrolled into the study. Patients were receiving calcium carbonate supplement (500 mg/d) and 20 000 IU vitamin D/week for six months. Circulating 25(OH)D, iPTH were measured by RIA methods; and hsTNFα, CRP, IL-6, OPG, and RANKL by ELISA at the beginning of the study and also after the treatment.

Results: Recommended 25(OH)D concentration above 30 ng/ml was detected in 45% of patients and 55% of patients suffered from vitamin D insufficiency. Vitamin D supplementation was reflected in significant increasing of 25(OH)D ($p < 0.001$) and decreasing of iPTH ($p < 0.05$). Estrogen and IL-6 levels did not change, but hsTNFα concentrations were markedly increased ($p < 0.002$) and CRP concentrations decreased ($p < 0.001$). OPG levels were slightly increased (NS) along with significant decrease in RANKL levels ($p < 0.05$). In a multivariate model both CRP and 25(OH)D were independently associated with IL-6.

Conclusion: Literary data indicate the potential role of vitamin D in regulation of inflammatory cytokines. Our data confirmed the possible association between 25(OH)D and RANKL, CRP and TNFα. Molecular mechanisms of the interaction between vitamin D and cytokines remain to be ascertained.

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Conflict of Interest: None declared

P266-T

THE LOWER DOSE OF OESTROGEN IS ABLE TO PREVENT POSTMENOPAUSAL BONE LOSS

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Although the minimal dose of 17β-estradiol in hormone replacement regimens was originally considered to be 2 mg/day, it is now increasingly accepted that a lower dose of 1 mg/day is effective in protecting women from the detrimental effects of the menopause and has a better safety profile. The aim of this study was to investigate effect of a lower dose of oestrogen (17β-estradiol 1 mg/day) on postmenopausal bone loss and turnover.

Methods: Study comprised 26 postmenopausal women aged 45–65 years with

T-score L2–L4 < 1.0 and > 2.5 SD. Treated group consisted of 16 women (average age 54.8 ± 5.59 years and postmenopausal age 6.81 ± 4.59 years) received oral 17β-estradiol 1 mg/daily continuously combined with dydrogesterone 5 mg/daily for 12 months. Control group included 10 subjects (average age 56.7 ± 4.11 years and postmenopausal age 11.5 ± 8.09 years). BMD and biochemical parameters of turnover and calcium homeostasis were measured at baseline and after 6 and 12 months.

Results: Increases in BMD were seen in lumbar spine $+2.7\%$ in 6 months and $+3.4\%$ in 12 months ($p < 0.01$), in total proximal femur $+1.4\%$ in 6 months ($p < 0.05$) and $+2.1\%$ in 12 months ($p < 0.01$), in Ward's triangle $+3\%$ in 12 months ($p < 0.05$) and in trochanter $+2.5\%$ in 6 months and $+3.1\%$ in 12 months ($p < 0.05$) in treated group. BMD significantly decreased in total proximal femur -1% in 6 months and -1.3% in 12 months ($p < 0.05$) and Ward's triangle -2% in 6 and 12 months ($p < 0.01$) in control group. There was a lowering in PTH from 72.7 ± 23.2 to 58.2 ± 12.8 in 6 months ($p < 0.05$) and to 52.9 ± 12.5 pg/ml in 12 months ($p < 0.01$), alkaline phosphatase from 78.2 ± 21.1 to 69.8 ± 19.1 U/l in 12 months ($p < 0.05$ vs baseline, $p < 0.01$ vs control) and osteocalcin from 33.0 ± 10.1 to 25.4 ± 9.28 ng/ml in 6 month ($p < 0.05$) and to 20.0 ± 7.03 ng/ml in 12 months ($p < 0.01$) in treated group. We also found an increase in PTH from 46.9 to 54.9 pg/ml in 6 months ($p < 0.05$) and to 54.9 ± 10.5pg/ml in 12 months ($p < 0.001$) and alkaline phosphatase from 86.0 ± 15.6 to 100.0 ± 15.0 U/l in 12 months ($p < 0.05$) in controls.

Conclusions: The lower dose of oestrogen effectively increases BMD, improves calcium homeostasis and lowering bone turnover in postmenopausal women with osteopenia.

Conflict of Interest: None declared

P267-S

EFFECTIVENESS OF HIGH AND MODERATE DOSES OF VITAMIN D3 IN PREVENTION OF OSTEOPOROSIS IN POSTMENOPAUSAL WOMEN

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Moscow region is highly deficient in vitamin D due to only 3% of postmenopausal women have 25(OH)D serum level more than 100 nmol/l (Toroptsova N. V., Benevolenskaya L. I. et al., 2005). But it is no a consensuses what daily dose of vitamin D is an adequate for prevention of postmenopausal osteoporosis in Moscow region.

The aim of study was to assess and compare effects of high dose (800 IU daily) and moderate dose (400 IU daily) of vitamin D3 with calcium supplementation for prevention of osteoporosis in postmenopausal women with spine osteopenia.

Methods: Study comprised 30 postmenopausal women aged 45–70 years with lumbar spine BMD (L2–L4) $< -1{,}0$ and $> -2{,}5$ SD, who were divided in 3 equal groups. Women in group 1 were administered vitamin D3 400 mg + calcium 1000 mg daily, women in group 2 received vitamin D3 800 mg + calcium 1000 mg daily and patient in control group did not take any medication influence bone remodeling. Duration of the study was 12 months. Effectiveness of therapies was evaluated by measurement of BMD and biochemical markers of bone turnover and calcium homeostasis.

Results: There was an increase vs. baseline in BMD in lumbar spine ($+1.9\%$, $p < 0.05$) and in trochanter ($+5.5\%$, $p < 0.01$) in group 1 and no any significant change in all skeletal sites in group 2. But BMD significantly decreased vs. baseline in lumbar spine (-11.9%, $p < 0.05$), Ward's triangle (-2.3%, $p < 0.05$), trochanter (-5.8%, $p < 0.05$) and total proximal femur (-2.4%, $p < 0.001$) in control group.

We found a significant lowering in serum PTH from 69.3 ± 13.0 to 42.3 ± 18.7 pg/ml ($p < 0.001$) in group 1 and from 78.9 ± 10.3 to 46.0 ± 28.8 pg/ml ($p < 0.05$) in group 2 and a decrease in serum alkaline phosphatase from 88.4 ± 32.9 to 70.3 ± 25.5 U/l ($p < 0.01$) in group 1 and from 88.4 ± 26.7 to 73.9 ± 12.3 U/l ($p < 0.01$) in group 2. There was an increase in serum PTH from 46.9 ± 14.8 to 55.1 ± 12.4 pg/ml ($p < 0.01$), in serum alkaline phosphatase from 81.0 ± 18.8 to 92.3 ± 24.1 U/l ($p < 0.01$) and in serum osteocalcin from 24.9 ± 12.5 to 29.4 ± 12.5 ng/ml ($p < 0.01$) and a decrease in serum calcium from 2.31 ± 0.09 to 2.25 ± 0.12 mmol/l ($p < 0.05$) and daily calcium excretion from 4.32 ± 2.46 to 2.96 ± 1.53 mmol/day ($p < 0.05$) in control group.

Conclusion: High and moderate doses of vitamin D3 are both improve calcium homeostasis and lowering bone turnover in postmenopausal women, but vitamin D3 in high doses better increases BMD in spine and femur.

Conflict of Interest: None declared

P268-M

CHANGES IN BMD AND CALCITROPIC HORMONS ACCORDING TO LENGHT OF BREASTFEEDING PERIOD

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Background: Prospective studies have demonstrated a loss of bone mineral density (BMD) during breastfeeding and a gain post weaning. The hormonal mechanisms responsible for these changes are less well described.

Aim: To determine whether changes in BMD postpartum are explained by changes in calcitropic hormones.

Material and methods: We followed 88 healthy women for 9 months postpartum (pp) with baseline at median 21.7 days pp and first and second follow up at 120.14 and 270.14 days pp, respectively. Subjects were categorised into three groups (gr.) according to the length of breastfeeding period (Gr. 1 ($N = 42$) continued breastfeeding; Gr. 2 ($N = 35$) stop breastfeeding at median 211 (IQR, 130; 279) days pp, and Gr. 3 ($N = 8$) stopped breastfeeding at 59 (0; 92) days pp. At each visit we performed DEXA scans of the whole body, the lumber spine, femoral neck and the hip. We also draw blood samples and collected 2h-urine for determination of plasma levels of prolactin, FSH, calciotropic hormones (vitamin D metabolites, PTH, PTHrP) and bone turnover markers (BTM).

Results: The length of breastfeeding (from birth to each visit respectably) was significantly associated with changes between 1st and 2nd visit in BMD at the whole body ($R = 0.24$; $p = 0.030$), and between 2nd and 3rd visit with changes in BMD at the total hip ($R = 0.27$, $p = 0.001$) and at the lumbar spine ($R = 0.34$, $p = 0.002$). Moreover, breastfeeding period correlated significantly with changes in biochemical markers of bone turnover as determined by levels of bone alkaline phosphatase ($R = -0.32$, $p = 0.003$), osteocalcin ($R = -0.25$, $p = 0.020$) and U-Ntx ($R = -0.42$, $p < 0.001$), and with changes in plasma levels of FSH ($R = -0.22$, $p = 0.042$) and prolactin ($R = -0.30$, $p = 0.005$). At 1st visit, a positive correlation was found between plasma levels of PTHrP and osteocalcin ($R = 0.29$, $p = 0.001$) and U-NTx ($R = 0.36$, $p < 0.001$). However, at subsequent measurements, PTHrP as well as levels of PTH, 1.25(OH)2D and prolactin were not associated with levels (cross-sectional) or longitudinal changes in BMD or BTM

Conclusion: Although a long breastfeeding period is associated with a decreased BMD and increased levels of BTM, these changes are not fully explained by changes in plasma levels of prolactin or calcitropic hormones. Further studies should focus on other mechanisms of action by which post-partum changes in bone may be explained.

Conflict of Interest: None declared

P269-T

VITAMIN D METABOLITES AND SKELETAL CONSEQUENCES IN PRIMARY HYPERPARATHYROIDISM

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Background: Plasma 25-hydroxyvitamin D (25OHD) levels are typically reduced and plasma 1.25-dihydroxyvitamin D (1.25(OH)2D) slightly increased in primary hyperparathyroidism (PHPT). PHPT is associated with reduced bone mineral density (BMD) mainly at sites rich in cortical bone, whereas successful parathyroidectomy causes an increase in BMD especially at sites rich in trabecular bone.

Aim: To investigate relations between preoperative vitamin D metabolites and skeletal consequences in patients with untreated PHPT and to appraise the influence of preoperative vitamin D metabolites on postoperative changes in BMD.

Design: Cross-sectional and cohort study.

Materials: 246 consecutive Caucasian PHPT patients aged 19–91 yrs. (median 63, 87% females).

Methods: Plasma intact PTH was measured by IMMULITE® automated analyzer (Diagnostic Products Corporation, Los Angeles, CA, USA), plasma 25-OHD by enzyme-immunoassay (IDS, Phoenix, Arizona, USA), and plasma 1,25(OH)2D radioimmunoassay (IDS, Phoenix, Arizona, USA).

Results: BMD was reduced at the femoral neck ($p < 0.001$) and forearm ($p < 0.001$) but normal at the lumbar spine ($p = 0.11$). Levels of biochemical bone markers were associated with high plasma PTH, high plasma 1,25(OH)2D and low plasma levels of 25OHD. Moreover, low plasma 25OHD was associated with low levels of BMD at the femoral neck ($rp = 0.23$), the forearm ($rp = 0.19$), and the whole body ($rp = 0.30$) whereas plasma 1,25(OH)2D was inversely associated with BMD at all regional sites and the whole body. Plasma PTH only showed an inverse association with BMD at the forearm ($rp = -0.21$). No association was observed between biochemical variables and prevalent spinal fractures. The annual increase in BMD after surgery at the spine was positively associated with preoperative plasma PTH ($rp = 0.40$) whereas the annual increase in whole body BMD was inversely associated with plasma 25OHD ($rp = -0.32$). No change in BMD at the femoral neck and forearm was observed one year after surgery.

Conclusion: Low vitamin D status and high plasma 1,25(OH)2D are associated with increased bone turnover and decreased bone mineral density in patients with primary hyperparathyroidism.

Conflict of Interest: None declared

P270-S

ESTROGEN STRONGLY ATTENUATES COLLAGEN-INDUCED ARTHRITIS IN RATS

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AIM: To investigate how estrogen therapy influences the progression of Collagen-Induced Arthritis (CIA) in rats in terms of disease incidence, inflammatory signs and joint erosion measured by enzymatic collagen type II degradation, compared to that of the conventionally used anti-inflammatory corticosteroid, prednisolone.

METHODS: Fifty female Lewis rats (150–175g) were randomized into 5 groups. Arthritis was induced in 4 groups. One group was treated with 17-beta-estradiol (0.18mg pellet/60 days release) and two groups with either a low or high dose of oral prednisolone (0.6mg/kg/day or 3mg/kg/day). Disease onset was registered at the first sign of swelling and redness of one hind paw, and the swelling was measured. Urine and serum for measuring the biomarker of collagen type II degradation (CTX-II) was collected at baseline and 7, 14, 21 and 28 days after immunization. At termination (day 28), one hind paw was snap-frozen in liquid nitrogen, homogenized and extracted for proteins, while the other hind paw was fixed in formaldehyde and decalcified in EDTA for histology and immunohistochemistry.

RESULTS: In non-treated animals, disease incidence occurred on day 11 and increased rapidly to 100% on day 15. Estrogen treatment both delayed the onset and reduced the overall incidence of disease by 33%. The respective reductions to high and low-dose prednisoloone were 100% and 66%, respectively. The anti-inflammatory effects of the different interventions were also confirmed by significant reductions ($p < 0.01$) in paw volume compared with untreated rats. Reductions of CTX-II in both serum ($p < 0.01$) and protein extracts from hind paws ($p < 0.01$) revealed effective prevention of the significant increases seen in untreated animals, regardless of the intervention used. Zymographic assessment of MMP

activity in protein extracts indicated that MMP-2 and MMP-9, two enzymes with direct implications for the generation of CTX-II fragments, were decreased in both estrogen and prednisolone-treated animals compared with untreated CIA rats.

CONCLUSIONS: These findings suggest that estrogen therapy could be a useful supplement to disease modification of different immune arthritis conditions, nurtured by the herein demonstrated anti-inflammatory effects. Secondly, cartilage degradation products, which can be measured both in serum and protein extract from affected joints, can provide important information when assessing disease activity and severity in different models of arthritis.

Conflict of Interest: Morten A Karsdal, Nordic bioscience, shareholder Per Qvist, Nordic bioscience, Shareholder Claus Christiansen, CCBR and Nordic Bioscience, Shareholder

P271-M

EVALUATION OF JOINT PATHOLOGY BY MEASURING CARTILAGE DEGRADATION PRODUCTS IN JOINT EXTRACTS

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PURPOSE: Severity of joint lesions is traditionally quantified by histological assessment of sections subjected to various staining techniques. Whether simple extraction of proteins/peptides from knee joints could be a useful approach for the quantification of joint pathology has not previously been investigated. In the present study, we investigated the utility of measuring the concentration of degradation peptides from collagen type I and II measured in protein extract from joints using specific ELISA techniques for quantifying joint pathology in the frequently used anterior cruciate ligament transection model of OA in rats.

METHODS: Twenty-four female Wistar rats (150–175g) were randomized into 3 groups and subjected to ACLT in one knee joint and sham operation in the contralateral knee. Groups of rats (n = 8) were terminated 7, 14 and 28 days after surgery. ACLT- and sham-operated knee joints were either snap-frozen in liquid nitrogen, homogenized and extracted for proteins in an extraction buffer, or fixated in formaldehyde and decalcified in EDTA. Levels of c-terminal telopeptides of type II collagen (CTX-II) and collagen type I (CTX-I), as well as hydroxyproline, were measured in protein extracts from knee joints using specific ELISAs. Activity of matrix metalloproteinases (MMPs) in extracts was visualized by zymography. Immunostaning of sections using specific anti-CTX-II antibodies was also performed.

RESULTS: The articular cartilage and bone in sham operated knees appeared histologically normal in the timeframe of the experiment. Whereas degradation products of collagen type I were similar in ACLT and sham-operated knees, we were able to detect significantly increased levels of CTX-II in ACLT-compared to sham-operated knees. Thus, the relative increases of CTX-II in joint extracts on day 7, 14, and 28 were +230% ($p = 0.07$), +300% ($p = 0.04$), and +270% ($p = 0.04$), respectively. MMP-2 and MMP-9 were slightly but notably increased in extracts from ACLT- compared with sham-operated knees. Immunostaining of paraffin-sections from ACLT-operated knees indicated that CTX-II positive staining could be linked to superficial erosions of articular cartilage, which was not visible in sham-operated animals.

CONCLUSIONS: These findings suggest that the measurement of collagen type II degradation products in protein extracts from joints is a useful methodological approach to obtain a objective quantitative measure of total joint damage.

Conflict of Interest: Morten Karsdal, Nordic bioscience, shareholder Per Qvis, Nordic Bioscience, shareholder Claus Christiansen, CCBR and Nordic Bioscience, shareholder

P272-T

DEVELOPMENT OF THE LIAISON® 25-OH VITAMIN D TOTAL ASSAY WITH IMPROVED SENSITIVITY AND PRECISION

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25-hydroxyvitamin D (25-OH Vitamin D) is the most abundant form of vitamin D in circulation, and the best available index of vitamin D nutrition. The importance of the vitamin D endocrine system in mineral homeostasis and bone metabolism, cardiovascular health in chronic kidney disease, neuromuscular function and the prevention of certain cancers has been demonstrated, while Vitamin D deficiency is prevalent world wide. Therefore, it is desirable to have a means of measuring 25-OH Vitamin D with excellent sensitivity and precision.

We report here the development of an improved assay for 25-OH Vitamin D on the LIAISON® analyzer. In the assay, a 25-OH Vitamin D-chemiluminescent tracer competes with 25-OH Vitamin D in the sample for binding to antiserum coated on paramagnetic beads. The polyclonal antiserum is the same as that used for the previous DiaSorin 25-OH Vitamin D assays. This antiserum has equal recognition of both the D3 and D2 forms of vitamin D, but does not react with 3-epi D3. The assay range is 3–150 ng/mL (7.5–375 nmol/L) and the method does not require any off-line extraction or pretreatment of samples. Time to first result is approximately 40 min and throughput is about 90 results per hour.

Analytical sensitivity, defined as the minimum detectable dose distinguishable from zero by 2 standard deviations, is < 2 ng/mL and functional sensitivity (LoQ) is < 4 ng/mL. Inter-assay precision, measured in quadruplicate over 40 runs, is 11% for values < 10 ng/mL, 8% for values 10–20 ng/mL, and 7% for values > 20 ng/mL. A comparison of results to the DiaSorin RIA gives linear regression of LIAISON® = 1.19*(RIA) + 0.31 ng/mL, $r = 0.95$. Linear regression analysis of expected vs. observed values from dilution linearity yields a slope of 1.03 and $r = 0.98$.

In a clinical study at Henry Ford Hospital, Detroit, MI, the improved assay, LIAISON® Total, was compared against the previous LIAISON® 25-OH Vitamin D assay. The resulting patient correlation with 164 patients gave a linear regression of LIAISON® Total = 0.89*(Previous LIAISON®) + 4.8 ng/mL, $r = 0.91$. A comparison of 5 day precision yielded: LIAISON® Total = 5–8%, previous LIAISON® = 8–14%. The functional sensitivity achieved was LIAISON® Total = 2.8 ng/mL and previous LIAISON® = 5.0 ng/mL.

In conclusion, these preliminary results demonstrate that the new LIAISON® 25-OH Vitamin D assay offers a method for routine assessment of 25-OH Vitamin D with improved sensitivity and precision.

Conflict of Interest: All authors except D. S. Rao work for DiaSorin.

P273-S

DEVELOPMENT OF BONE AND MINERAL ASSAYS FOR THE LIAISON® IMMUNOASSAY ANALYZER

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Reliable and convenient methods for measuring serum markers of bone turnover are a valuable aid in the management of osteoporosis and other diseases related to bone and mineral metabolism. DiaSorin offers sensitive and specific assays for 25-OH Vitamin D and N-Tact'' PTH on the LIAISON® automated immunoassay system. We report here the development of immunoassays for Osteocalcin, Calcitonin, and 1,25-dihydroxyvitamin D for the LIAISON analyzer.

The Osteocalcin and Calcitonin assays are two-site sandwich assays that use specific monoclonal antibodies for capture and detection. The assay for 1,25-diOH vitamin D is a competitive assay that uses the same polyclonal antiserum as the DiaSorin 1,25-diOH vitamin D RIA, with an improved offline sample extraction procedure similar to that used with the RIA. The Osteocalcin assay measures in the range of 0.3–300 ng/mL with analytical sensitivity < 0.3 ng/mL, functional sensitivity < 1.5 ng/mL, and inter-assay precision $\leq 6\%$. The Calcitonin assay measures in the range of 1–2000 pg/mL with analytical sensitivity < 1 pg/mL, functional sensitivity < 4 pg/mL, and inter-assay precision $\leq 10\%$. Including sample extraction, the 1,25-diOH vitamin D assay measures up to 200 pg/mL with analytical sensitivity of 3 pg/mL and inter-assay precision $\leq 20\%$. Each method demonstrates good correlation to a predicate device. For Osteocalcin, (LIAISON) = 0.92x + 0.46 ng/mL, $R = 0.993$; for Calcitonin, (LIAISON) = 1.06x + 8 pg/mL, $R = 0.965$; for 1,25-diOH vitamin D, (LIAISON) = 1.03x + 10.9, $R = 0.938$.

In conclusion, these results demonstrate that the LIAISON Osteocalcin, Calcitonin and 1,25-diOH vitamin D assays are rapid, precise and sensitive methods for the measurement of important serum markers of bone metabolism in an automated system.

Conflict of Interest: All authors work for DiaSorin.

P274-M

DOES TOTAL KNEE REPLACEMENT INFLUENCE THE LEVEL OF INTACT-PARATHYROID HORMONE IN POST-MENOPAUSAL WOMEN SUFFERING FROM END-STAGE KNEE OSTEOARTHRITIS? INTERMEDIATE RESULTS

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Background/aims. Aim of this prospective cohort study was the assessment of the impact of Total Knee Replacement (TKR) on the level of Intact-Parathyroid Hormone (I-PTH) in post-menopausal women suffering from primary end-stage

osteoarthritis of the knee (Grade IV according to the Outerbridge Classification). These are the intermediate results of this study, as the latter is expected to finish with the enrollment of another 11 patients. Methods. Between November 2004 and November 2006, one hundred and nineteen patients were enrolled in this study. The patients' mean age was 69.99 years (49–81). The years that had passed since menopause ranged from 7 to 30 (mean of 18.8 years). The serum values of I-PTH, Calcium (Ca) and Phosphorus (P) were evaluated pre-operatively and on the 4th postoperative day. A patient was excluded from the study if there was a history of an osteoporotic fracture, had received any anti-osteoporosis treatment in the past or suffered from any metabolic disease. Furthermore the I-PTH, Ca and P values had to be within normal range pre-operatively in order a patient to participate in the study. Results. The incidence of increased I-PTH values was 10.92% (13 out of 119 patients). Typical Secondary Hyperparathyroidism was found in 9 out of 13 patients. Three patients had Phosphorus level just below the normal range and one patient had Calcium level lower than normal. All these values were found to be normal the next day upon re-evaluation of the patient with new blood tests. Conclusion. TKR seems to play a potential pivotal role in the postoperative development of elevated I-PTH values (or secondary hyperparathyroidism) in patients suffering from end-stage knee osteoarthritis that had I-PTH, Ca and P values within normal range pre-operatively. As possible causes for this might be considered: the insufficient post-operative calcium or/and vitamin D intake, the temporary immobilization and diminished workload on both legs of the patient and the bone cuts (acting as a fracture?) that are required during this operation.
Conflict of Interest: None declared

P275-T

GLOBAL SERUM 25-HYDROXY-VITAMIN D LEVELS: A META-ANALYSIS

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Aim: To study the levels of serum 25-hydroxy-vitamin D (25OHD) in native subjects in all countries of the world to assess if the serum levels varied by latitude.

Subjects and methods: A search was performed of the PubMed, Embase, and Web of Science using the search term: serum vitamin D. The search spanned the time interval January 1, 1970 to November 1, 2004 and resulted in 5,855 papers. Inclusion criteria were papers reporting original cross-sectional data on serum 25OHD levels in subjects who were native inhabitants of the area where the measurements were performed, i.e. immigrants were excluded.

Results: A total of 433 studies including 110,528 subjects from all over the world were included in the study. The mean serum 25OHD level was 54 nmol/l (SEM 1.3 nmol/l, 95% confidence intervals for the mean 51–57 nmol/l). The 25OHD levels tended to be log-normally distributed. Unadjusted, there was a trend towards lower serum 25OHD with increasing latitude with large variations in mean values. However upon adjustment this trend disappeared. The meta-regression showed that women tended to have higher mean serum 25OHD (56 +/−1.6 nmol/l) than men (49 +/−2.4 nmol/l, $p < 0.01$). People with white skin had significantly higher serum 25OHD levels than people with black skin. However, there was a trend towards an interaction ($p = 0.07$) between skin colour and latitude because in most areas except USA, people with different skin colour lived in different areas. The change in serum 25OHD levels with latitude was similar in people with white and black skin. There was a significant decrease in 25-OHD levels with age ($p < 0.01$) Conclusion: Age and skin colour are major determinants for serum 25OHD levels. Upon adjustment for confounders no change in serum 25OHD was present with latitude. This may mean that the dressed human race has a "set point" for serum 25OHD at around 50 nmol/l, and that genetic and environmental factors, life-style and supplementation and fortification policy tend to maintain serum 25OHD levels at a certain level around the globe. This is an interesting feature as serum PTH levels tend to increase at serum 25OHD levels below this set point of 50 nmol/l, but the inter-individual variability is large. Our results suggest that geographic variations in various diseases with latitude more likely are explained by variations in age, sex and race than by variations in serum 25OHD.
Conflict of Interest: None declared

P276-S

DEXAMETHASONE INTERFERES WITH THE P2 RECEPTORS-INDUCED CELL SIGNALLING

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Glucocorticoid-induced osteoporosis may be due, at least in part, to inhibitory effects on osteoblasts proliferation, thus leading to a decreased bone formation.

P2 receptors signalling, induced by extracellular released ATP, plays a significant role in bone biology modulating osteoblasts functions. We and others have previously shown that P2 receptors signalling by osteoblasts is activated in response to mechanical stimulation. When exogenously applied to osteoblasts, ATP exerts a mitogenic action, stimulates activation of different transcription factors, and promotes active ERKs phosphorylation and Hsp90 expression, a molecular chaperone playing a central role in the control of cell proliferation.

Here, by using different osteoblast-like cell lines, we investigated the hypothesis that glucocorticoids may affect osteoblast proliferation through P2 signalling. We found that dexamethasone significantly reduced Erk-1/2 activation in human osteoblast-like cells. Moreover, at doses inhibiting osteoblastic cells proliferation, it significantly ($p < 0.05$) reduced (about 30%) the non-lytic ATP release induced by a mechanical stimulus, thereby interfering with nucleotide receptor signalling. This effect was associated to a reduction of the Erk-1/2 activation induced by P2 signalling, suggesting that the negative effect of glucocorticoids on osteoblast proliferation might result from reduced purinergic-induced cell signalling. This hypothesis was further confirmed by the observation that dexamethasone produced a significant reduction in the basal Hsp90 protein expression. In conclusion, these data suggest a new molecular mechanism explaining the negative effect of glucocorticoids on osteoblasts proliferation, through interfering with Hsp90-mediated MAPK activation and with the P2 receptors signalling.
Conflict of Interest: None declared

P277-M

VITAMIN D STATUS IN PATIENTS WITH CARDIAC INCOMPENSATION

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Introduction: Vitamin D deficiency is prevalent in the elderly population. Calcium absorption, bone mineralization and muscle function may be impaired. Vitamin D receptors have been demonstrated in skeletal as well as cardiac muscle and vitamin D deficiency has been related to degree of cardiac incompensation.

Aim: Investigate if patients with cardiac incompensation have vitamin D deficiency and investigate if degree of incompensation is related to the level of vitamin D.

Material and method: We included 149 men and women with varying degree of cardiac incompensation. Ejection fraction (EF) was evaluated by echocardiography and serum- parathyroidhormone (PTH), -25-OH vitamin D, -pro-Brain Natriuretic Peptide (-pro-BNP) as well as standard biochemistry was measured. Patients were classified according to New York Heart Association Classification (NYHA) and number of medicament taken for the heart disease was registered.

Results: The population had a mean age of 68 ± 12 years, serum-25-OH vitamin D was 57.0 ± 23.0 nmol/l, ejection fraction 35 ± 10%, pro-BNP 249.3 ± 435.9 pg/ml and 3 ± 1 medicaments were taken. No sex difference was demonstrated.

Patients with se-25-OHD < 50 nmol/l (41%) tended to have lower EF, higher se-proBNP and higher NYHA classification but the relation was not significant. Se-PTH was higher in patients with increasing NYHA classification ($p < 0.05$) and se-proBNP ($p < 0.01$).

Conclusion: In general patients were not vitamin D deficient. Vitamin 25-OH-D was not related to cardiac function in these patients whereas se-PTH significantly increased with cardiac incompensation evaluated by proBNP and NYHA class.
Conflict of Interest: None declared

P278-T

TYPE OF HIP FRACTURE AND VITAMIN D STATUS IN ELDERLY HIP FRACTURE PATIENTS

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Hip fracture rate increases yearly by 1–3% in the developed countries. Patients with pertrocanteric fracture (PF) have poorer prognosis and higher mortality rate during the first postfracture year.

The aim of this study was to assess vitamin D status of elderly hip fracture patients and evaluate whether it differed in patients with distinct fracture types.

Patients: 119 consecutive elderly hip fracture patients, aged 72.7 ± 9.46; 31 (26.1%) men, 88 (73.9%) women. Laboratory evaluation: 25(OH)D3 serum levels and routine biochemical tests.

Results: Serum 25(OH)D3 concentration was 2.4–20.7 ng/ml, mean 15.14 ± 18.58: in 40 patients (32.8%) < 10; in 31 (26.1%) between 10–15.9 ; in 45 (37.8%) between 16–29.9; in 3 (2.5%) > 30 ng/ml.

Sixty nine patients (58%) sustained PF and 50 (42%) subcapital fracture (SF). At admission 25(OH)D3 levels were 12.39 ± 6.32 in PF patients, 15.29 ± 6.16 in SF patients, $p = 0.016$.

Of 38 patients with initial 25(OH)D3 levels less than 10 ng/ml 29 pts (76.3%) sustained PF, and 9 (23.7%) sustained SF, p = 0.018. Among patients with higher 25(OH)D3 levels there was no significant difference between fracture types.

Of 23 patients younger than 65 years, 18 (78%) had PF, vs 5 (22%) that had SF, p = 0.035. No such difference was observed in the age group of >65. There was no significant difference in the mean age between patients with two fracture type: 71.97 ± 10.14 in PF, 74.04 ± 7.88 in SF.

Presence of concomitant diseases and number of them didn't correlate with hip fracture type.

Conclusion: Majority of elderly hip fracture patients had inadequate vitamin D status, while about third of them were vitamin D deficient. Patients with lower vitamin D levels were at higher risk for pertrochanteric hip fracture. Patients younger than 65 had relatively higher risk for pertrochanteric fracture than older patients.

Conflict of Interest: None declared

P279-S

EFFECTS OF INTERMITTENT PTH ADMINISTRATION ON OPG, RANKL AND SOST MRNA AND PROTEIN IN PRIMARY AND SECONDARY METAPHYSEAL TRABECULAR BONE OF THE RAT

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It is unknown why PTH chronic excess and intermittent administration are associated with bone catabolism and anabolism, respectively. Recently, the SOST gene and its protein product sclerostin were identified as potent negative regulators of bone formation (Winkler et al., 2003). SOST is strongly expressed in osteocytes; it is structurally related to the BMP antagonist DAN/Cerberus family and acts by binding to BMPs and/or Wnt signaling cascade, inhibiting bone formation. Moreover, Wnts seem to regulate osteoclast formation and bone resorption by a repression of RANKL transcription (Spencer et al., 2006), and positively regulate OPG gene in osteoblastic cells (Jackson, 2005).

It has been shown that chronic elevation of PTH reduces SOST expression by osteocytes, suggesting that SOST regulation may play a role in mediating PTH activity in bone. Aim of this study is to evaluate how intermittent PTH affects SOST, OPG and RANKL mRNA in the primary and secondary metaphyseal trabecular bone of PTH-treated rats in comparison with controls.

Eight male Wistar rats were s.c. injected with 80 mg/Kg PTH (1–34) for three weeks, three times a week; control rats were s.c. injected with PBS. The distal right femurs were embedded in glycolmethacrylate for bone histomorphometry; left femurs were split in two halves, one half was paraffin embedded for SOST, OPG and RANKL immunodetection and the other half was frozen for RNA isolation. Osteoclasts were identified by TRAP staining in paraffin sections.

Histomorphometric variables of both bone formation and resorption were increased after PTH intermittent administration. Sclerostin was mainly expressed by osteocytes and rarely by osteoblastic cells; osteoclasts were negative. OPG and RANKL proteins were co-expressed in osteoblasts, lining cells, some osteoclasts, young osteocytes and in some bone-marrow cells. In the PTH group, SOST mRNA fell in both primary and secondary trabeculae; RANKL mRNA values fell in primary bone and rose in secondary bone, while OPG mRNA trend was opposite to that of RANKL. In conclusion, the anabolic effect of PTH is in line with the fall of SOST mRNA. The PTH-mediated opposite trend of OPG and RANKL mRNA in the two bony areas might be related to their different behaviour: trabecular formation is the main activity in primary metaphyseal bone, while osteoclast resorbing of trabeculae is prevalent in secondary metaphysis to allow longitudinal bone growth.

Conflict of Interest: None declared

P280-M

BMP-6 PASSES THROUGH THE GASTROINTESTINAL SYSTEM IN NEWBORN RATS

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We demonstrated that systemically administered BMP-6 restores bone in the osteoporotic rat model and that endogenous BMP-6 is required for treatment of osteoporosis (Simic et al., J Biol Chem, 2006). Here we show that 35% of orally administered BMP-6 in the formulation with absorption enhancers at low pH passes through the duodenum into the blood stream of adult rats and increases bone mineral density in osteoporotic rats. Since the gastrointestinal system of newborn rats is adjusted to absorption of colostrum containing proteins, we tested whether they can absorb BMP-6, which we have previously detected in the colostrum. Next, we used chelated human recombinant BMP-6 to mercaptoacetyltriglycine (MAG3) labeled with 99mTc-pertechnetate (BMP-6-MAG3-99mTc) and showed that newborn rats absorb about 9% of unmodified orally applied BMP-6, suggesting an important role for BMP-6 in the mother's colostrum. On the contrary, adult rats absorbed only 0.04% of the applied BMP-6 dose. As a positive control we delivered insulin duodenally to both newborn and adult rats and showed its effect on blood glucose only in infants. The reasons for the possible physiologic absorption of proteins in newborn rats could reflect the lack of gastrointestinal (GI) enzymes or increased permeability of the gut wall. Using a everted gut sac method we show that newborn rats have less enzymes than adult rats and increased permeability of the gut wall. Forty percent of BMP-6-MAG3-99mTc passed from the mucosal to serosal side of the gut in the absence of enzymes, while 24% passed through the duodenal wall taken from the adult animals. These results justify the presence of BMP-6 and eventually other morphogens from the TGFβ superfamily in the colostrum during early postnatal life.

Conflict of Interest: None declared

P281-T

CALCIMIMETIC DRUG AMG 073 INDUCES RELAXATION OF THE ISOLATED RAT AORTA

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The calcium receptor (CaR) is a seven transmembrane receptor expressed in most tissues, including those that regulate extracellular calcium homeostasis. The most important physiological function of the CaR is its suppressive regulation of the parathyroid hormone. Recently, we and others have demonstrated the expression of a functional CaR on cardiomyocytes as well as on endothelial cells. Several compounds that modulate CaR function have been developed. Calcimimetics are class of compounds that positively modulate the CaR by allosterically increasing the affinity of the receptor for extracellular Ca^{2+}. The calcimimetics have been shown to be a promising approach in the treatment of uremic hyperparathyroidism. Furthermore, new data suggest that the use of calcimimetics is beneficial on cardiovascular events in patients with renal failure. In this study we have investigated the effects of the clinically used calcimimetic, AMG 073, on contractility of the rat aorta by wire myography. AMG 073 significantly attenuated the contractile responses to phenylephrine or 125 mM KCl. Moreover, AMG 073 elicited a concentration-dependent vasodilatation of the aorta precontracted with phenylephrine, KCl or the blocker of potassium channels, tetraethylammonium chloride (TEA). Intriguingly, CaR agonists, neomycin and gadolinium, did not have any effect on the contractility of the rat aorta. AMG 073 also induced relaxation of the aorta precontracted with L-type calcium channel activator BayK 8644, suggesting inhibition of L-type calcium channels by AMG 073. Inhibition of endothelium function by L-NAME and indomethacin impaired AMG 073-induced relaxation of the vessel precontracted with phenylephrine, but not with KCl, suggesting involvement of hyperpolarising factor (e.g. potassium channels) in endothelium-dependent relaxation. Our data demonstrate that AMG 073 causes both endothelium-dependent and -independent vasodilatation, which may contribute to understanding the cardiovascular protective effect of AMG 073. The effect could be due to a direct action on the ion channels or/and via the CaR.

Conflict of Interest: None declared

P282-S

CALCITONIN INCREASE PROTEOGLYCAN LEVELS IN HUMAN OSTEOARTHRITIC ARTICULAR CARTILAGE BY STIMULATION OF PROTEOGLYCAN SYNTHESIS AND INHIBITION OF AGGRECANASE ACTIVITY MEDIATED IN PART THROUGH INDUCTION OF INCREASED CAMP LEVELS

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Aim: Calcitonin has previously been speculated to have chondroprotective effects on articular cartilage both *in vivo* and *in vivo*. We wanted to investigate possible direct anabolic effects of calcitoin in human OA articular cartilage with focus on proteoglycan turnover.

Methods: Human OA cartilage was obtained from knee arthroplasty operations, which was dissected into 12–14 mg explants and cultured in four replicates with refreshment of medium every second or third day in the presence or absence of salmon calcitonin [$100E^{-18}$ M–$10E^{-9}$ M]. Effects of calcitonin was evaluated using 1) Proteoglycan synthesis radioactive labeled $^{35}SO_4$ [20 μCi] after 6 days of culture 2) measurement of sulphated glycosaminoglycans (sGAG) in N_2 pulverized explants extracts by the alcian blue binding assay after 21 days of culture, 3) investigations of metabolic activity by the viability assay AlamarBlue, 4) aggrecanase mediated proteoglycan degradation was quantified by the ^{374}ARGS-G2 ELISA. Direct effects of calcitonin on isolated human chondrocytes were inves-

tigated through measurement of intracellular and extracellular cAMP levels in concentrations from 1 pM–100 nM.

Results: Isolated human OA articular chondrocytes stimulated with salmon calcitonin responded concentration-dependently with a significant ($P < 0.001$) increase 150% in cAMP levels in doses from 1 pM–100 nM. In terms of functional activity, calcitonin significantly ($P < 0.001$) and concentration-dependently [$100E^{-18}$ M–$10E^{-9}$ M] induced proteoglycan synthesis measured by radioactive sulphate incorporation with a 180% maximal induction at $100E^{-15}$ M. In alignment, calcitonin treatment resulted in significant ($P < 0.01$) 80% increased levels of proteoglycan content in the articular explants. Calcitonin concentration-dependently inhibited aggrecanase mediated aggrecan degradation as measured by the ^{374}ARGS-G2 ELISA, ($P < 0.05$).

Conclusion: Calcitonin treatment increased proteoglycan content of human OA cartilage through a dual action of increased proteoglycan synthesis and in part through inhibition of the aggrecanase activity. This mode of action may be mediated through cAMP signalling, as non-specific stimulators of cAMP (Forskolin and IBMX) in others settings have been shown to stimulate proteoglycan synthesis. In conclusion, calcitonin may provide benefit to the management of joint diseases via the direct effects on chondrocytes in addition to the well-established osteoclast mediated effects on subchondral bone.

Conflict of Interest: None declared

P283-M

CONCENTRATIONS OF CALCIUM, PHOSPHORUS, CARBOXY-TERMINAL TELOPEPTIDES OF TYPE I COLLAGEN AND 1.25 DIHYDROXY VITAMIN D3 AND ACTIVITY OF BONE ALKALINE PHOSPHATASE IN BLOOD SERUM OF PERIPARTURIENT DAIRY COWS

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In the present study, influence of lactation on bone metabolism parameters in dairy cows was investigated. Concentrations of calcium (Ca), phosphorus (P), carboxy-terminal telopeptides of type I collagen (CTX) 1.25 dihydroxy vitamin D3 (1.25 (OH)2 D3) and activity of bone alkaline phosphatase (BALP) were measured in blood serum of periparturient dairy cows, to investigate of bone metabolism in the beginning of lactation. The research was carried out on dairy Holstein cows and heifers between 2 and 7 years of age. Samples were taken from heifers ($n = 6$) and cows in three experimental periods: 14 days before calving ($n = 24$), 10th day ($n = 24$) and 30th day ($n = 24$) after calving. For determination of 1.25 (OH)2 D3, n were 6 (heifers), 10 (14 days before), 10 (10th day after) and 8 (30th day after calving). The investigated biochemical parameters were measured by standard methods and commercial kits. Significance of differences was tested by paired T-test and for heifers by independent T-test. A significant increase of CTX ($p < 0.0001$) concentrations was found on the 10th and CTX and 1.25 (OH)2 D3 ($p < 0.0001$ and $p < 0.05$) on the 30th day after calving, as compared to the 14th day before calving. In heifers, significantly higher concentrations/ activities of P ($p < 0.01$ to $p < 0.0001$) and BALP ($p < 0.0001$) considering all three experimental periods in cows, were determined; concentration of CTX was significantly higher considering the cows 14 days before calving ($p < 0.0001$); concentration of 1.25 (OH)2 D3 was equivalent to the period around the 14th day before calving in cows and significantly lower considering the group of cows on the 30th day after calving ($p < 0.05$). The results indicate high rate of bone resorption in the beginning of lactation, as a source of calcium and phosphorus for compensation of these minerals lost by milk. Increase of 1.25 (OH)2 D3 to 30th day after calving could implicate to gradual adaptation of calcium absorption from the digestive tract. Higher concentrations/ activities of CTX, P and BALP in heifers confirm the high rate of bone remodelling during growth and formation of skeleton.

Conflict of Interest: None declared

P284-T

ABSTRACT WITHDRAWN

P285-S

24 MONTHS OF CINACALCET IN HEMODIALYSIS PATIENTS: IMPACT ON CALCIUM SALTS AND CALCIUM-FREE PHOSPHATE BINDERS

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Background: The long-term impact of Cinacalcet on dosage of calcium salts (CaCO3) and calcium-free phosphate-binding agents (Sevelamer HCl) has not been assessed precisely in the long term treatment of secondary renal hyperparathyroidism (SPTH).

Methods: Hemodialysis patients treated with Cinacalcet (baseline iPTH > 300 pg/ml and corrected calcemia > 2.37 mmol/l) were observed during 24 months. iPTH, corrected calcemia (Ca), phosphatemia (Pi) and Ca × P product, were performed every month initially, and every three months when iPTH achieved 150–300 pg/ml. Alfacalcidol (Vit D), CaCO3 and Sevelamer HCl were adapted to maintain Ca, Pi and Ca x P product in K/DOQI targets.

Results: 36 patients (17 F/19 M) were observed, 66.7 +− 12 y.o., diabetic in 11% of cases, on dialysis for 91 +− 16 months with 3 x 4 hr schedule using a dialysate calcium > 1.5 mmol/l.

The average daily dosage of Cinacalcet remained stable after 5th month (41 +− 19 mg/day), no hypocalcemia < 1.87 mmol/l was observed. After 24 months of treatment, Cinacalcet allowed for a significant decrease of iPTH, Ca x P product and an increase the number of patients to K/DOQI targets achieved. Sevelamer use and average daily dosage of Vit D could be reduced significantly in less number of patients. The average daily dosage of CaCO3 was increased significantly (Table 1)

Conclusion: Cinacalcet is very effective in long-term SPTH treatment, and may facilitate the achievement of K/DOQI targets. The efficiency of lower dose of Cinacalcet is associated with more daily dosage of CaCO3 and reduction of Vit D-treated and Sevelamer-treated patients

Table 1.

	T0	T12	T24	p*
iPTH pg/ml	920 +−251	436 +−126	227 +−104	0.0001
Ca mmol/l	2.57 +−0.1	2.24 +−0.1	2.31 +−0.1	0.0001
Pi mmol/l	2.21 +−0.4	1.21 +−0.2	1.32 +−0.2	0.0001
Ca x P prod	5.67 +−0.7	3.15 +−0.4	3.04 +−0.4	0.0001
Doqi targ %	–	48	61	0.0001
CaCo3 mg/d	1.15 +−0.44	2.56 +−0.72	2.62 +−0.65	0.0001
Sevelamer%	94.4	13.8	11.1	0.0001
Vt D %-μg/w	58.3–4.37	47.2–2.62	36.1–0.90	< 0.005

*T24 vs T0

Conflict of Interest: None declared

P286-M

FREE TESTOSTERONE AND PHYSICAL ACTIVITY ARE IMPORTANT FOR BONE IN WOMEN AND MEN

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Background: Anabolic hormones have been tried in order to treat osteoporosis in women and men and moderate, weight-bearing physical activity seems to have beneficial effects on the skeleton.

Aim: The aim was to study the endogenous, serum free testosterone and free estradiol in relation to body composition and bone as well as to smoking and physical activity in women and men.

Methods: A randomised population sample of 226 women and 184 men aged 25–64 years from the World Health Organisation, MONICA (MONItoring of trends and determinants for CArdiovascular disease) Project, Göteborg, Sweden, was studied. In menstruating women, blood samples were taken on cycle day 7–9. Among women 45–64 years, 36% had hormone replacement therapy (HRT). Calcaneal ultrasound was performed with LUNAR, Achilles. Smoking was estimated in the number of cigarettes or corresponding gram tobacco/day. Physical activity was graded 1 (sedentary) to 5 (heavy regular activity). Lean body mass (LBM) and body fat was measured with bioimpedance.

Results: Bone mass decreased with increasing age in all. After correction for age, free testosterone correlated positively with weight, body mass index, LBM and smoking but not to bone in women. Age-corrected free estradiol correlated negatively to physical activity in women. In women > 45 years without HRT both free testosterone and estradiol correlated to bone. In women with HRT free testosterone correlated positively to weight.

In men, age-corrected free testosterone correlated negatively to weight, body mass index and LBM. Bone was positively correlated to both free testosterone and estradiol in men.

In a multivariate analysis, bone was independently and positively correlated to free testosterone and physical activity in women without HRT and in men.

Conclusion: The endogenous sex hormone, serum free testosterone, and physical activity were of importance for bone in women without HRT and in men.

Conflict of Interest: None declared

P287-T

BONE LOSS IN PREMENOPAUSAL WOMEN WITH HYPERTHYREOSIS

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Introduction: Hyperthyreosis causes accelerated bone loss by increasing bone turnover. Elevated levels of the thyroid hormones T3 and T4, as well as decreased TSH levels have been connected with a high bone turnover rate, leading to reduced bone mineral density (BMD). Up to date, most studies have been conducted on postmenopausal women.

Aim: To examine the changes in bone metabolism in premenopausal women with hyperthyroidism.

Patients and methods: We enrolled 38 premenopausal women with hyperthyreosis (median age 40,5) in our study. Blood and urine samples were collected and analyzed for specific markers of bone turnover (B-ALP, bone alkaline phosphatase; U-DPD, urinary deoxypiridinoline) as well as biochemical markers of Ca metabolism using standard laboratory methods. BMD was measured by dual-energy X-ray absorptiometry (DEXA) using a Hologic QDR densitometer. Measurement sites were the lumbar spine (L1–L4), hip area and forearm. Statistical analysis was performed with MedCalc Version 9.2.0.0 software.

Results: The area that was most affected by osteoporosis was the lumbar spine (34%), followed by hip (29%) and forearm (18%). In the group of patients with decreased TSH levels (< 0,03 mIJ/l) B-ALP(M = 41.54U/l, SD 18.79) and U-DPD (M 11.49 nM/mM, SD 10.89) were higher compared to patients with TSH within the normal range (M = 29.09 U/l SD 20.51 for B-ALP, ; M = 6.38 nM/mM, SD = 4,20 for U-DPD) but not significantly (P = 0.081 for B-ALP; P = 0.076 for U-DPD).

We found no correlation between TSH levels and BMD. T4 exhibited a significant negative correlation with hip BMD (rs = −0.8, P = 0.044).

TSH was negatively correlated with B-ALP (rs = −0.45, P = 0.009) and U-DPD (rs = −0.34; P = 0.052). T3 showed a highly positive and significant correlation with U-DPD (rs = 0.596; P = 0.04).

Conclusion: Bone metabolic markers were highly negatively correlated with TSH, suggesting that low TSH level is a marker of increased bone turnover. Our results showed that these patients had a significant bone loss. This suggests a possible need for screening for osteopenia and osteoporosis in premenopausal women suffering from hyperthyreosis.

Conflict of Interest: None declared

P288-S

THE PREVALENCE OF OSTEOPOROSIS AND VITAMIN D DEFICIENCY/INSUFFICIENCY AMONG ELDERLY PEOPLE WITH FALLS IN DENMARK

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Background: In Denmark it has been shown that 40 % of postmenopausal women and 80% of elderly above the age of 65 suffer from Vitamin D insufficiency. Insufficiency of Vitamin D is a risk factor for falls. Among community-dwelling, generally healthy people above the age of 65, 1 in 3 will fall at least once a year. The presence of osteoporosis increases the risk of injury among patients with falls. We present data on the prevalence of osteoporosis and deficiency or insufficiency of Vitamin D among elderly patients with falls.

Material and Methods: In a randomized controlled intervention study on multifactorial fall prevention, including 400 patients above the age of 65 years, who have had an injurious fall, participants in the intervention group are offered a measurement of Vitamin D and a BMD-measurement.

Results: At this point 116 participants have gone through intervention, mean age 74,1 years, 70 % women. 35% and 34% of participating men and women respectively suffer from insufficiency of vitamin D (25OHD < 50 nmol/l), 4% suffer from deficiency (25OHD ≤ 25nmol/l). 35% of participating men (mean age 75) and 33 % of the women (mean age 74) have osteoporosis, defined as a T-score of less than or equal to −2,5 at the hip or lumbar spine. Of the 39 patients diagnosed with osteoporosis, 10 also suffer from Vitamin D insufficiency, and 15 had a fracture at the fall that lead to entering the study.

Conclusion: Among elderly Danes with falls we observe much less insufficiency or deficiency of vitamin D than expected from previous studies. This observation might be explained by oral calcium and vitamin D supplementation among the elderly in Denmark. It is important to focus on several risk factors of falls in the elderly. The presence of osteoporosis among elderly people with falls should be considered, and they should be examined and treated, also when fractures are not present.

Conflict of Interest: Peter Schwarz, Lilly Denmark, Consultant

P289-M

VITAMIN D3 8400 IU ONCE WEEKLY IN ELDERLY SUBJECTS WITH VITAMIN D INSUFFICIENCY: EFFECTS ON SERUM 25-(OH)D LEVEL

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Vitamin D deficiency is common in the elderly, and correction of very low levels is frequently done with large intermittent doses of vitamin D. This study examined if treatment with vitamin D3 8400 IU once weekly for 16 weeks would increase serum 25(OH)D to adequate levels in subjects 70 years or older who had vitamin D insufficiency: 25(OH)D ≥ 6 but ≤ 20 ng/mL (15 to 50 nmol/L).

Generally healthy subjects (n = 226) from five countries, mean age ± SD: 78.0 ± 6.4 years, were recruited between October 2005 and February 2006. Subjects were randomly allocated in a 1: 1 ratio to receive either vitamin D3 8400 IU once weekly or placebo; 25(OH)D serum levels were analyzed at a central laboratory by HPLC.

At baseline, mean (SD) serum 25(OH)D levels were similar in the 2 treatment groups: 14.3 (3.9) ng/mL in the vitamin D group and 14.4 (4.3) ng/mL in the placebo group. Following 16 weeks, 25(OH)D levels increased from baseline by 11.9 ± 0.6 ng/mL in the vitamin D group and decreased from baseline by 1.1 ± 0.4 ng/mL in the placebo group (p < 0.001). The between-group difference was 13.0 (95% CI, 11.6; 14.4). At 16 weeks 90% of treated subjects reached 25(OH)D levels ≥20 ng/mL vs. 8% of subjects on placebo; none of the treated subjects had 25(OH)D levels < 9 ng/mL vs. 14% of subjects on placebo. Treatment with vitamin D3 was safe and well tolerated. No subject reached 25(OH)D levels > 60 ng/mL through the study. No treatment differences were observed for serum calcium, phosphate, creatinine, 24-hour urine creatinine and creatinine clearance after 16 weeks of treatment relative to placebo. PTH levels decreased by 7.5 % in the vitamin D group and increased by 9.2% the placebo group (between-group difference −16.8%, p = 0.003). 24-hour urine calcium levels increased by 16.5% in the vitamin D group and decreased by 1.3% in the placebo group. A non-significant trend was observed between groups (p = 0.080). A similar percentage of subjects had abnormal laboratory at baseline and at week 16. Hypocalcaemia, hypercalciuria, and abnormal creatinine levels did not differ between groups.

In conclusion, treatment with vitamin D3 8400 IU effectively increased 25(OH)D levels and was safe in this study population.

Conflict of Interest: This study was sponsored by Merck & Co., Inc. and all authors were investigators and/or Merck employees.

P290-T

EFFECTS OF VITAMIN D3 8400 IU ONCE WEEKLY ON BODY SWAY OF ELDERLY SUBJECTS WITH VITAMIN D INSUFFICIENCY

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Vitamin D status has been shown to be related to body sway (a measure of neuromuscular stability) and the probability of falling in the elderly. A recent study showed that subjects with mediolateral body sway ≥0.46 cm have ~ 3-fold risk of recurrent falling over the next 12 months.

We examined the effects of treatment with vitamin D3 8400 IU once-weekly for 16 weeks on mediolateral body sway in subjects 70 years or older who had vitamin D insufficiency (25(OH)D ≥6 but ≤ 20 ng/mL). The study was conducted in 226 generally healthy subjects [mean age ± SD: 78.0 ± 6.4] recruited between the months of October 2005 and February 2006. Subjects were randomly allocated in a 1: 1 ratio to receive vitamin D3 8400 IU or placebo once-weekly and were stratified in a 2: 1 ratio based on baseline 25(OH)D levels (≤ 15 or > 15 ng/mL). Mediolateral sway was assessed using the AccuSway platform.

Baseline serum 25(OH)D levels were similar [vitamin D = 14.3 ± 3.9 ng/mL; placebo = 14.4 ± 4.3 ng/mL], with about 2/3 of subjects having 25(OH)D level ≤ 15 ng/ml. Baseline sway was similar across groups [vitamin D = 0.311 ± 0.134 cm; placebo = 0.341 ± 0.151 cm]. Following 16 weeks a small decrease in mediolateral sway was seen in the vitamin D group (−0.003 cm) and a small increase in the placebo group (0.015 cm); the between-group difference of −0.021 (95% CI −0.53, 0.012) was not significant (p = 0.208). Mean 25(OH)D levels were 26.2 ± 5.6 ng/mL in the vitamin D group and 13.3 ± 5.1 ng/mL in the placebo group. Mean changes from baseline in body sway of treated patients vs. placebo were not significantly different in patients with 25(OH)D baseline levels ≤ 15 ng/ml (−0.017) vs. > 15 ng/ml (−0.034). When subjects with higher baseline sway (≥0.460 cm; n≈ 30) were analyzed separately, a significant decrease in sway was seen in subjects treated with vitamin D3 vs. placebo (between-group difference −0.200, p = 0.008).

In conclusion, treatment with vitamin D3 8400 IU once-weekly reduced body sway in subjects with elevated basal mediolateral sway, but did not affect sway in subjects with normal basal mediolateral sway. Baseline 25OHD status did not affect the effectiveness of vitamin D3 8400 IU once-weekly on sway.
Conflict of Interest: This study was sponsored by Merck & Co., and all authors were investigators and/or employees.

P291-S

VERTEBRAL COMPRESSION FRACTURES MAY BE SILENT IN OSTEOPOROTIC ELDERLY

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Background and Aim: Spontaneous vertebral fracture is one of the important consequences of senile osteoporosis. Although it may cause morbidity and decline in quality of life, some fractures may not cause pain and can be underdiagnosed if not evaluated by lumbal X-ray. The aim of this study was to determine the frequency of silent vertebral fractures in osteoporotic elderly.

Method: 2422 geriatric patients admitted to our outpatient clinic for comprehensive geriatric assessment were screened and 1428 osteoporotic patients were enrolled in the study. Osteoporosis diagnosis was made according to the results of bone mineral densitometry measures performed by dual-energy X-ray absorptiometry at femoral neck and lumbar spine (L1–4). Lumbar X-ray was performed to each patient to evaluate vertebral compression fractures. Back pain complaint was asked to osteoporotic patients.

Results: Total number of 1428 osteoporotic patients with mean age 72.1 ± 6.5 (29.1% male) were examined in the study. Lumbar X-ray showed vertebral compression fracture in 6.1% of the patients. Activities of daily living evaluated by Barthel's index was significantly impaired in patients with vertebral fracture ($p = 0.021$). Twenty three percent of the osteoporotic patients had back pain complaint. Within patients with vertebral fracture, 78.2% did not have back pain.

Conclusion: Vertebral fractures may be asymptomatic in osteoporotic elderly. Lumbar X-ray should be routine in the assessment of osteoporotic elderly in order to determine silent vertebral fractures and take precautions to prevent further fractures.
Conflict of Interest: None declared

P292-M

DRUG THERAPY UTILIZATION IMPROVES IN PATIENTS WITH SEVERE FOLLOWING A MULTIFACETED OSTEOPOROSIS EDUCATIONAL INTERVENTION: CANADIAN QUALITY CIRCLE (CQC) PILOT PROJECT

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Background: Despite the consequences of osteoporosis and although there are proven benefits to modern pharmacologic therapies for prevention and treatment of osteoporosis, the management of the disease is less than optimal. Thus, the Quality Circle Project was initiated to improve primary care physicians (PCPs) practices for treating patients with osteoporosis.

Methods: The Pilot enrolled a total of 52 family physicians and involved seven QCs. The project consisted of three QCs phases that included: 1) Training & Baseline Data Collection, 2) Educational Intervention & Follow-Up Data Collection, and 3) Strategy Implementation Session. During the educational intervention QC's met to discuss profiles of the physician's management of osteoporosis and to participate in an osteoporosis workshop. This analysis examined the change in drug therapy usage in patients with severe osteoporosis. Severe osteoporosis was defines as a patient with a t-score < -2.5 and a prior fracture. For each phase, PCPs collected data from different patients via chart reviews and a standardized collection form. A total of 1505 and 1359 patient forms were collected during phase I and II, respectively. All patients were women 55 years and older. A total of 54 and 67 patients were classified as having severe osteoporosis. The generalized estimating equations approach was used to evaluate differences in treatment utilization (risedronate, alendronate, etidronate, raloxifene, hormone replacement therapy) pre and post educational intervention. An exchangeable correlation matrix was used for the analysis. Odds ratios and 95% confidence intervals (CI) were calculated.

Results: A total of 90.7% and 92.5% of patients were on therapy at baseline and follow-up, respectively. Findings indicated that prescriptions increased by 2.0 (95% CI: 1.1, 3.5), 1.2 (95% CI: 0.6, 2.6), and 1.9 (95% CI: 0.4, 8.6) for risedronate, alendronate and raloxifene, respectively. Etidronate (0.3; 95% CI: 0.1, 0.8) and hormone therapy (0.1; 95% CI: 0.01, 1.7) use decreased following the educational intervention.

Conclusion: The utilization of QCs altered PCPs practices for treating patients with severe osteoporosis. More patients were on therapy. As a result of improved care, patients should develop fewer fractures and have superior clinical outcomes.
Conflict of Interest: B Kvern, Procter & Gamble/Sonofi-Aventis, consultant G Ioannidis, None A Hodsman, Eli Lilly, Merck Frosst, NPS, Zelos Therapeutics, Servier, Procter & Gamble/Sonofi-Aventis, Medical advisory boards, speaker fees A Papaioannou, Eli Lilly, Merck Frosst, Amgen, Procter & Gamble/Sonofi-Aventis, Novartis, grants, consultant L Thabane, none A Gafni, none D Johnstone, Employee of Procter & Gamble C Crowley, Employee of Procter & Gamble JD Adachi, Eli Lilly, Merck Frosst, Amgen, Procter & Gamble/Sonofi-Aventis, Novartis, grants, consultant

P293-T

TERIPARATIDE: THE IMPACT ON THE QUALITY OF LIFE

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Osteoporosis is a frequent disease among the elderly and has major consequences in terms of mortality, morbidity and cost. Vertebral fractures are the most common osteoporotic fracture and may result in back pain with functional limitations and diminished quality of life. Teriparatide [rhPTH (1–34)] has been shown to increase bone mass and reduce the risk of vertebral and other osteoporotic fractures. In the present study we are describing a patient affected by a severe postmenopausal osteoporosis, with several vertebral fractures, a fracture of the right hip and multiple peripheral fractures. The patient was an 86-years old female. She referred a surgical menopause when she was 40 years old. She did not performed a HRT after menopause. In addition she smoked 10 cig/die and she referred an intolerance to milk. The familiar anamnesis showed a family history of osteoporosis (mother, grandmother and mother's sister with femoral fractures). She also had an ischemic heart disease, hiatal hernia, pulmonary emphysema, hypothyroidism, immobility for 2 years.

We had the first contact with the patient in the mid 2005. She asked us an osteoporotic consultation at home, because she was not able to walk by herself due to the presence of multiple fractures with severe back pain.

At the baseline she had high levels of serum BAP with a secondary hyperparathyroidism. We gave her a treatment with Calcium (1000 mg/day) and vitamin D3 (800 IU/day) to normalize the values. After 2 months we started the subcutaneous injection of 20mcg/day of Teriparatide and Calcium and Vitamin D oral supplementation. We did not observed an increase of the BAP during Teriparatide treatment. However the patient showed a positive general improvement in quality of life with a considerable reduction of the severe back pain. The severity of back pain has been measured using questionnaire with a score of the improvement: 2 = high; 1 = moderate; 0 = no improvement. The reduction of back pain was by 97%. In addition an improvement of the motorial function and tone of mood, was very high at the end of the therapy. Finally no further fractures were occurred during teriparatide treatment. The patient currently is able to walk by herself again, without auxilium. These findings suggest a benefit of teriparatide on quality of life.
Conflict of Interest: None declared

P294-S

CHANGES IN BONE MINERAL DENSITY DURING ANTI-TNF APLHA THERAPY IN RHEUMATOID ARTHRITIS PATIENTS

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Background: secondary osteoporosis related to increased pro-inflammatory cytokines activity (IL-1, TNF alpha), medication (corticosteroids, methotrexate) and impaired exercise, has been well recognized as a common complication of rheumatoid arthritis (RA).

Aims: to assess both generalized (spine and hip) and local (hand) changes in bone mineral density (BMD) and bone metabolism markers in RA patients (pts) receiving anti-TNF alpha therapy.

Methods: 52 pts (43 women, 9 men; mean age of 37.9 years, mean disease duration of 4.5 years) with active RA, receiving biological therapy (infliximab 35 pts, adalimumab 12 pts, etanercept 5 pts), were enrolled in this prospective 1 year study. Assessments included BMD (spine and hip dual X ray absorptiometry, hands dual X ray radiogrammetry) at baseline and 1 year; bone metabolism markers (alkaline phosphatase, serum calcium, osteocalcin levels) at baseline, 3, 6, 12 months; inflammatory (ESR, CRP levels) and immune (rheumatoid factor) parameters and disease activity score (DAS28) at baseline, month 6 and 12. Patients were not allowed to take any anti-resorptive therapy (bisphosphonates, selective estrogen receptor modulators, etc).

Results: both spine and hip bone loss is arrested, while no significant impact upon hand BMD is reported during anti-TNFalpha therapy; statistically significant increase in axial BMD was demonstrated after 1 year in all patients with initial decreased BMD (T-score, Z-score $p < 0.01$); statistically significant increase in osteocalcin level ($p < 0.01$), but no change in alkaline phosphatase and

serum calcium was reported. Statistically significant negative correlation between axial BMD and RA activity was reported at baseline and after one year of therapy ($r = -0.72$, $p < 0.01$; $r = -0.78$, $p < 0.01$).

Conclusion: changes in axial BMD after one year of biological therapy in patients with active RA are related to decrease in pro-inflammatory cytokines levels and RA activity.

Conflict of Interest: None declared

P295-M

POST-MENOPAUSAL OSTEOPOROSIS PATIENTS' PREFERENCES FOR ADMINISTRATION OF PARATHYROID HORMONE TREATMENT

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Background/aims: To design a scientifically valid study to quantify patient preferences for administration features of parathyroid hormone (PTH) treatment.

Methods: A technique which is commonly applied to elicit patients' preference within other therapeutic areas (e.g. diabetes, oncology and haematology) is the discrete-choice experiment (DCE). To our knowledge, a DCE has never before been applied to elicit patients' preferences for features of osteoporosis treatment. In this study a DCE will be applied to quantify preferences for the administration of PTH-treatment from a patient perspective. A DCE is a questionnaire-based stated-preference method in which patients are asked to choose among hypothetical alternatives. By varying the alternatives and repeating the DCE, the patient preferences for different PTH treatment features can be elicited by analysing the patient responses in an advanced statistical model. The initial step, when designing a DCE, is to identify the relevant attributes for the administration of PTH treatment from a patient perspective. A comprehensive list of potential attributes has been prepared based on a literature review. To avoid a cognitive overload of the responding patient, a maximum of five to six attributes will be selected for the final version of the questionnaire. The final identification of the attributes included in the questionnaire will be validated by clinical experts who have in-depth knowledge about administration of PTH-treatment. Furthermore, the resulting questionnaire will be pre-tested using one-on-one verbal-protocol interviews with a sample of PMO patients. After identifying the relevant attributes of PTH-administration, a DCE questionnaire will be developed and data will be collected in five European countries. In total, 500 European PMO patients will be surveyed in order to elicit their preferences for administration features of PTH.

Results: The list of potential attributes includes attributes linked to device design, functionality, ease of administration and clarity.

Conclusion: DCE are the conceptually correct method for eliciting valid patient preferences for PTH administration features. The best-practice procedures for developing and testing the stated-preference instrument will be followed. Possible limitations of this approach include chance of hypothetical bias and measurement error from incomplete understanding of attribute definitions and inattention to trade-off tasks.

Conflict of Interest: M. Asmussen, employed as Health Economics Manager by Nycomed L. H. Hyldstrup, Nycomed, employed as Medical Advisor by Nycomed

P296-T

PROXIMAL HUMERUS FRACTURES IN PATIENTS BETWEEN 50 AND 65 YEARS CAUSE INFERIOR SUBJECTIVE OUTCOME. STRONGER INDICATION FOR ACTIVE SURGICAL TREATMENT?

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Introduction: New surgical methods seem to give us tools to surgically fix proximal humerus fractures in an osteoporotic skeleton using minimal invasive, angle stable screws and plates. Some authors have reported favourable results but also technical failure with the new devices. The aim of the present study was to review the functional data, bone mineral density and radiographic results of a consecutive fracture series before these new methods were merging on the market. We were interested to gather basic data of how the conservatively treated proximal humeral fracture fared in the group that we initially believe to be able to improve the treatment for, patients between 50 and 75 years. Methods and Patients: All patients between 50 and 75 years visiting our department with a proximal humeral fracture between May 2003 and September 2004 were offered a DEXA scan. Patients were referred to their GP for discussion of preventive action or active treatment of osteoporosis if present. One year after the fracture a validated outcome instrument was sent to all patients. The DASH consists of a 30-item disability/symptom scale, scored 0 (no symptoms) to 100. Results: In total 84 patients were identified having a low-energy proximal humeral fracture and a short enquiry was sent to the patients together with an invitation to participate in an osteoporosis screening program. 18 patients were operated and the rest were conservatively treated. 23 patients did not reply and were not followed. 60 patients were referred to a DEXA scan whereof 54 fulfilled the examination. 8 patients had a normal scan, 29 osteopenia and 17 osteoporosis. 7 patients had previously prefracture been screened for osteoporosis. 10 patients were on medication for osteoporosis, 8 Ca VitD, one on bisphosphonates and one on estrogen. The mean Dash score after one year was 29 and the median 28, with 8 patients between 20 and 30 and 20 patients above 30. In the patients with both a DEXA scan and DASH no correlation was found. Discussion: In our material, the majority being treated conservatively, it was found that this younger active group suffer from either osteopenia or osteoporosis and only 8/60 patients had a normal scan. The functional result showed a sustained substantial impact on patient well-being a year after fracture.

Conflict of Interest: J Åstrand, MSD, Speakers Bureau

P297-S

GENDER-RELATED DIFFERENCES IN THE FEMUR GEOMETRY MEASUREMENTS AND THEIR RELATIONSHIP TO BONE MINERAL DENSITY

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Objective: To assess the relation among the BMD, the proximal femur geometry and subjects' demographic characteristics and to compare the groups by defined T score at the femur total.

Method and Materials: The demographic characteristics such as age, weight and height of 750 subjects were recorded. After BMD (gr/cm^2) of proximal femur (neck, trochanter and total femur) was measured by dual-energy X-ray absorptiometry (DEXA), according the T scores at the femur total the subjects were divided into 3 groups: osteoporotic group, osteopenic group and normal group. The femur geometric measurements [(hip axis length (HAL), femoral neck width (FNW) and neck shaft angle (NSA)] were manually measured in the DEXA analysis scans.

Results: We found significant correlations between the proximal femur BMD and the geometric measurements in both genders ($p < 0.05$), except the FNW. All the demographic characteristics were highly related to the BMD at the femur total and trochanter in both genders. While the weight and height values were strongly related to HAL and FNW, no substantial correlations were found with age. Comparison of the groups revealed that all the geometric parameters were longest in the osteoporotic group with a significant difference between the groups for NSA and HAL. Comparison of the genders showed that, both of the BMD values and the femur geometric parameters of the men were significantly higher comparing to that of women. When corrected the impact of the height and weight, these differences between the genders still persisted ($p < 0.05$). Stepwise regression analyses showed that the geometric parameters were independently associated with proximal femur BMD.

Discussion: The present study showed that the longest the femur geometric parameters were in the osteoporotic subjects and men. As the geometric measurements may be the predictive factors of the fracture risk, it may be considered that men have higher risk for fracture in our country.

Conflict of Interest: None declared

P298-M

DIGITAL X-RAY RADIOGRAMMETRY: COMPARISON OF MEASUREMENT IN THE DOMINANT AND THE NON-DOMINANT HAND. RESULTS FROM THE COPENHAGEN CITY HEART STUDY

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Digital X-ray radiogrammetry (DXR) combines the principles of classical radiogrammetry with digital image analysis, and allows an estimation of BMD (DXR-BMD) from an automated reading of a standard hand radiograph captured in PA-projection, with several hundred measurements of the cortical shell of the metacarpals. A high-resolution 300 dpi flatbed scanner digitises the radiograph. The software automatically locates three regions of interest in the three middle metacarpals. It is not possible for the operator to modify the regions. Assuming an elliptic shape of the shaft of the metacarpals, the bone volume per area is calculated. Using the information of a constant amount of mineral per mm3 of bone, the volume per area can be converted into an estimate of the bone mineral density. BMD measured by DXA is traditionally reported to be higher in the dominant arm. This study aimed to examine if this is the case when BMD is estimated by DXR. The study is based on 1300 postmenopausal women from the third Copenhagen City Heart Study. 23 women, who reported equally use of both hands, were excluded. All the radiographs were obtained at standardized conditions. DXR-BMD was obtained by using the X-posure sys-

tem'' (Pronosco-Sectra A/S, Herlev, Denmark). 1209 women were right-handed and 68 left-handed. DXR-BMD in the dominant and non-dominant hand were strongly correlated with a correlation coefficient of 0.939 ($p = 1.0*10-22$). Mean DXR-BMD in the dominant hand was 0.518 g/cm^2, and 0.508 g/cm^2 in the non-dominant hand. The mean difference between the dominant and non-dominant hand was 0.01024 g/cm^2 (SD 0.023; $p = 1.0*10-22$). 67.7 % of the women had a positive difference, signifying that DXR-BMD was higher in the dominant hand. The last 32.3 % had a higher DXR-BMD in the non-dominant hand. Using regression and curve estimation, the differences between the dominant and non-dominant hand were plotted as a function of DXR-BMD in the dominant hand, revealing a positive slope (alpha = 0.83, p~0.0). In conclusion, DXR-BMD in the dominant and in the non-dominant hand correlate highly. DXR-BMD is greater in the dominant hand, but the difference is small. This difference seems to enlarge with increasing BMD in the dominant hand, and could be explained by a greater degree of lateralisation. A more pronounced divergence between BMD in the dominant and non-dominant hand may first be observed when the dominant hand is used in particular and thus is considerably stronger than the other hand.
Conflict of Interest: L. Hyldstrup, Eli-Lilly Denmark, MSD, Novartis, Novo-Nordisk and Nycomed, Consultant

P299-T

NON-INVASIVE MEASUREMENT OF BONE STRONTIUM DURING LONG-TERM TREATMENT WITH STRONTIUM RANELATE

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BACKGROUND: We have developed a non-invasive DPA method (distal forearm immersed in water) for measurement of bone strontium content, using ^{133}Ba and ^{241}Am as radioactive sources. It was previously shown that the ''overestimation'' of BMD by DXA brought about by the higher atomic number of Sr than Ca amounted to 10 % on a molar basis (1) and that the DPA method rendered accurate and precise measurements of the relative Sr content (Sr/(Ca + Sr) in monkey bones (2). We here present humans bone Sr data with the DPA method for the first time. The material comprised 32 female osteoporotic patients from the SOTI and TROPOS studies (two Danish centres) who volunteered to participate in a 3 year open prolonged study of the effect on bone Sr during standard medication with Sr ranelate, one sub-group having received active treatment for 8 years. Trial name: EXTENSION). Simultaneous DXA scanning was done at the same measuring site.

RESULTS: The rise in bone Sr content was most marked during the first 2 years in accordance with the biopsy findings of Boivin et al.(3) Maximum values after 8 years was around 1 %. Pronounced increments in BMD (DXA) were found.

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Conflict of Interest: Olaf Bärenholdt, IRIS Servier, Research Support Niels Kolthoff, IRIS Servier, Research Support Stig Pors Nielsen, IRIS Servier, Consultant, Research Support

P300-S

PERSISTENCE IN OSTEOPOROSIS TREATMENT IN ELDERLY AFTER HIP FRACTURE

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Objectives: To assess patients persistence in osteoporosis (Op) treatments after hip fracture and investigate factors associated with discontinuation.

Methods: A prospective cohort study was conducted on 428 patients, aged ≥ 70 years, admitted to an Orthopaedic Unit with a hip fracture and discharged alive after surgical repair. Op treatments prescribed at the time of hospital discharge were collected from medical record review. A telephone interview at six months after discharge evaluated whether patients were currently taking drugs prescribed. Non-persistence was defined when subjects ceased therapy within six months. Variables considered as potential risk factors for non-persistence, recorded during hospital stay, at discharge and 6 months, were: the Op treatment prescribed, living situation, pre-morbid and six months ambulation ability, the number of drugs other than those for Op prescribed, the ASA score, the presence/absence of dementia, the number of Active Clinical Issues (ACIs) at discharge and being referred to an Osteoporosis Centre (pre-planned visit).

Results: Of 428 subjects enrolled, 155 were excluded for different reasons: 89 died within six months, 28 had missing data and 38 were discharged without an Op treatment. 273 subjects were considered for the analysis: 46 (16.8%) received bisphosphonates (BSPs) plus calcium and cholecalciferol, the others (227, 83.3%) calcium and vitamin D metabolites (Ca + Vit. D). At six months only 104 patients (38.1%) were currently taking the drugs prescribed. In univariate analysis type of Op therapy ($p < 0.001$), presence of dementia ($p < 0.001$), a higher ASA score ($p < 0.001$), > 6 drugs prescribed ($p < 0.001$), ≥3 ACIs at discharge ($p = 0.005$), ambulation disability at six months ($p < 0.001$) and the absence of a pre-planned visit at discharge ($p < 0.001$) were significantly related with discontinuation of treatment. In multivariate model, a number of drugs ≤ 6 (OR 2.01, 95% CI 1.08–3.72, $p = 0.027$) and Op treatment (BSPs and/or Ca + Vit. D) prescribed at discharge (OR 2.70, 95% CI 1.24–5.87, $p = 0.012$) remained the only significant predictors of persistence.

Conclusions: After hip fracture, prescription of Op drugs is very low; poor functional status and a high number of drugs are risk factors of discontinuation. BSPs prescription was poor; however, when prescribed, patients persistence improved. Intervention aimed to improve therapeutic management of elderly after hip fracture are needed to reduce fracture risk.
Conflict of Interest: None declared

P301-M

DOES AN OSTEOPOROSIS AND FALLS CASE-FINDING PROGRAMME IN A UK PRIMARY CARE SETTING LEAD TO LASTING IMPROVEMENTS IN MEASURED STANDARDS OF CARE WHEN COMPARED TO USUAL PRACTICE?

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Background/aims: This study evaluates the persisting documented benefits of a three year case-finding programme in UK primary care.

Methods: A case-finding programme aimed at community dwelling over 65 year old females at risk of osteoporosis was run between 2000 and 2003 in 13 practices. Over 75 year olds of both sexes at risk of falls were also included. Training and support in systematic care was provided along with mentoring and computer-based data collection tools populated with standard coded clinical concepts. Three years after project closure database queries measured performance against some indicators of optimal care in 82,252 patients served by the project compared with that in 474,019 patients receiving normal care.

Results: Overall high risk patients were under-identified and under-treated. Only 1 in 3 of the expected prevalence of over 75 year old females with a fracture since age 45 were identified and only 1 in 3 of those had evidence of interventions for bone health. However, compared to patients receiving standard care those in practices that had received training and education were more likely to receive guideline care. They were more likely to be co-prescribed calcium and vitamin D3 with their osteoporosis treatment (OR 1.50, 95% CI 1.59–1.69). Females >75 years with prior fracture since age 45 were more likely to have had recent osteoporosis treatment or assessment (OR 4.15, 95% CI 3.35–5.13) and females 65–74 with prior fracture more likely to have had a DXA scan (OR 6.10, 95% CI 3.88–9.61). Similarly high risk fallers >75 years were more likely to be referred to a falls clinic (OR 4.35, 95% CI 3.03–6.23), have had an osteoporosis assessment (OR 13.63, 95% CI 8.30–21.88) and patients > 75 years were with a prior fracture since age 45 were more likely to have had a falls assessment (OR 9.37, 95% CI 7.04–12.49). All results were significant ($p < 0.0001$). On the other hand females 65–74 yrs with a fracture since age 45 and osteoporosis were no more likely to have recent treatment or assessment (OR 1.10, 95% CI 0.52–0.34. There were no significant differences in the demography or performance in other clinical areas between the two groups of practices.

Conclusion: In nearly all respects practices who had received initial training and practical support in the management of osteoporotic fracture risk continued to deliver higher standards of documented care in the long term.
Conflict of Interest: J Bayly Advisory Board representation and speakers' bureau for MSD, Roche, Shire, Proctor and Gamble, Sanofi Aventis, Servier, Pfizer, Eli Lilly and Novartis Other authors – no affiliations to declare

P302-T

ANALGESIC EFFICACY OF CALCITONIN FOR VERTEBRAL FRACTURE PAIN

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Background: Fractures, especially vertebral fractures, are a common complication of osteoporosis, leading to significant pain.

Aim: To compare the pain release induced by osteoporotic vertebral fracture, through teriparatide and teriparatide plus calcitonin.

Methods: We performed a study to compare the analgesic effect of 20 mcg teriparatide versus 20 mcg teriparatide plus metered dose intranasal spray 200IU/activation calcitonin in two groups between postmenopausal women undergoing osteoporosis with vertebral fracture. A 10-point visual analog pain scale (1 = least to 10 = most painful) and a four-point pain grade (grade 1 = least to grade 4 = most painful) were used to measure the pain perception.

Results: The mean pain scores for the teriparatide and teriparatide plus calcitonin were 2.3 ± 1.1 and 8.5 ± 1.1, respectively ($P < 0.05$), while the pain grades for teriparatide and teriparatide plus calcitonin were 1.5 ± 0.3 and 3.5 ± 0.4, respectively ($P < 0.05$). In teriparatide group, analgesics were requested, but in teriparatide plus calcitonin group no analgesics were requested ($P < 0.001$).

Conclusion: Using teriparatide is more expensive than other osteoporosis treatment. Studies show that taking a bisphosphonate with hormone replacement therapy (HRT), results in increased bone mass when compared to taking either a bisphosphonate or estrogen alone. Besides, calcitonin is better for osteoporotic vertebral fracture pain release than HRT. However, a larger investigation will be needed to achieve more significant case number.

Conflict of Interest: None declared

P303-S

RECOGNIZING OSTEOPOROSIS AND ITS CONSEQUENCES IN QUÉBEC (ROCQ): THE CARE GAP FOLLOWING A FRAGLITY FRACTURE

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The objective of this analysis was to evaluate the diagnostic and treatment rates of osteoporosis six months following a fragility fracture in women 50 years and over participating in the ROCQ programme, an ongoing patient health-management programme. At phase 1, women with fragility and traumatic fractures were recruited at a cast or outpatient clinic or by mail using a list of names provided by the Quebec provincial health administrative database. Upon receipt of an authorization form, the patients were contacted by phone to answer a short questionnaire that identified their fracture circumstances. Six months following the fracture, women were contacted again by phone (phase 2) to determine the diagnostic (informed of osteoporosis and/or BMD measurement with diagnosis of osteoporosis) and treatment (bisphosphonates, raloxifene, nasal calcitonin or teriparatide) rates of osteoporosis.

After 25 months, 2,697 women (mean age: 65.6 years) completed phase 1 or a refusal questionnaire. A total of 2,166 (80%) sustained a fragility fracture and 531 (20%) sustained a traumatic fracture. To date, 1,295 participants with a fragility fracture have completed phase 2 questionnaires. One-third of the participants reported a previous fracture after 40 years of age. Of those not treated for osteoporosis at phase 1 (1,051), 18% initiated pharmacological therapy within the six-month period following their fracture. At phase 2, only 25% of participants either received a diagnosis of osteoporosis or were on treatment despite 74% consulting a physician during the six to eight months between phases 1 and 2. Women over 65 years of age and those who had a BMD measurement between phases 1 and 2 with a diagnosis of osteoporosis were significantly more likely to be treated ($p < 0.05$). Education level, history of fracture, and level of knowledge were not significantly associated with a higher probability of initiating treatment.

Despite the availability of diagnostic modalities, effective treatments, and adequate health care assessments, there is a substantial care gap in the management of osteoporosis. The decision to initiate treatment is primarily influenced by the BMD measurement result and not by fracture type. The proportion of fragility fractures is higher than expected and the management of osteoporosis is suboptimal.

Conflict of Interest: L. Bessette, Merck Frosst, sanofi-aventis, P&G Pharmaceuticals, Eli Lilly, Novartis Pharma, Amgen Canada, Wyeth and Zelos Therapeutics; Grant/Research support, Consultant. J. P. Brown, Merck Frosst, sanofi-aventis, P&G Pharmaceuticals, Eli Lilly, Novartis Pharma, Amgen Canada, Wyeth and Zelos Therapeutics; Grant/Research support, Consultant. M. Beaulieu, Merck Frosst Canada, staff. M. Baranci, sanofi-aventis Canada, staff. S. Jean, none. K. S. Davison, Merck Frosst, P&G pharmaceuticals, sanofi-aventis, GSK; Consultant. L. G. Ste-Marie, Merck Frosst, sanofi-aventis, P&G Pharmaceuticals, Eli Lilly, Pfizer, Servier, Astra-Zeneca, Genzyme Canada, Hoffmann-La Roche, NPS Allelix, Novartis Pharma, and Zelos Therapeutics; Grant/Research support, Consultant.

P304-M

USE OF GENOMIC PROFILING FOR UNDERSTANDING THE BISPHOSPHONATES MODE OF ACTION AT THE MOLECULAR LEVEL

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Bisphosphonates are the most widely used class of drugs for the treatment of conditions associated with excessive bone resorption, such as osteoporosis. In particular nitrogen-containing bisphosphonates (NBPs) have been proven to be therapeutically effective as antiresorptive drugs. The action of NBPs seems to involve the block of protein prenylation of prosurvival proteins, such as ras, in osteoclasts thus leading to apoptosis. However, these effects may involve different mechanisms and a complete view of molecular targets of NBPs is lacking at present. To this aim, we used the yeast Saccharomyces cerevisiae as a model organism to identify gene targets of NBPs, using a genome-wide, high-throughput screening of yeast heterozygotes, in which one copy of each gene has been deleted and substituted with unique "molecular barcodes", a short stretch of 20bp (TAG) which defines each mutant. Strains carrying the deletion of genes encoding for the targets of these drugs will grow slowly and will be out-competed from the population. The 5936 heterozygous deletion mutants library 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, for about 20 generations. Subsequently, the molecular TAGs were amplified by PCR and discriminated on microarrays containing complementary oligonucleotides for each TAG.

Among the deletion mutants that displayed a slower growth rate in the presence of the drugs (Risedronate and Ibandronate), compared to the untreated strains, we found the strain carrying the deletion for Farnesyl Pyrophosphate Synthetase gene, a well-known target of N-BPs. That finding demonstrated the reliability of the strategy we used and the conservation of the biological processes between yeasts and humans. Moreover, the three different drugs used seem to affect a different set of strains, suggesting that each of the N-BPs used activate specific targets within the cells. Among the strains that displayed drug-induced haploinsufficiency, the most interesting seems to be AHA1, which is a co-activator of HSP90, an important chaperone involved in the activation of signal transduction pathways. The use of this yeast competition assay and genome profiling in drug-induced haploinsufficiency will provide new information on the molecular mechanism of action of this class of pharmaceutical compounds and may help in designing safer, more effective and selective drugs.

Conflict of Interest: None declared

P305-T

COMBINED VERTEBRAL STABILIZATION BY MEANS OF CEMENT-AUGMENTED POSTERIOR INSTRUMENTATION AND BALLOON-KYPHOPLASTY IN OSTEOPOROTIC BURST FRACTURES

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INTRODUCTION: Stabilization of osteoporotic vertebral fractures (type A3) comprises a major challenge. Balloon-kyphoplasty as a single procedure does not address the posterior wall fragment and thus cannot restore axial stability. Classic-type posterior instrumentation tends to fail due to implant loosening. We therefore prefer combined vertebral stabilization by means of cement-augmented bi-level posterior instrumentation and single-level kyphoplasty.

METHODS: Inclusion criteria for this prospective trial were: A3-fractures of Th11–L5; integrity of adjacent discs (MRI); t-score < −2.5 (DEXA). Initial reduction and cement-augmentation was performed by (percutaneous) kyphoplasty (Kyphon) using PMMA-cement. Final reduction was achieved by bi-level instrumentation of the adjacent vertebrae with PMMA screw-augmentation. Both conventional open (USS II; Synthes) and percutaneous (Sextant; Medtronic) techniques were applied for instrumentation. The following data were acquired: subjective pain rating (Visual Analogue Scale-VAS); bisegmental endplate-angle (plain X-rays). Patients were subject to full weight-bearing on day 1. Follow-up was performed on day 1; week 6; and months 3, 6, and 12.

RESULTS: 16 patients with 64 augmented pedicle screws were included. Average patient age was 68 (64 to 78). Average t-score was −2.7 (−3.1 to −2.5). In 6/64 pedicle screws, leakage of cement was noted. Direction of leakage was anterior or lateral for 5, and epidural for 1 case. There was no extrusion of cement during kyphoplasty procedures. All 16 patients experienced marked pain-relief as expressed on the VAS. Average correction of bisegmental endplate-angle was 8.6°. During follow-up, no significant loss of correction was noted. There was no case of implant loosening or cut-out of pedicle screws.

DISCUSSION: Combined cement-augmented instrumentation and kyphoplasty is efficient for stabilization of osteoporotic burst fractures. The typical shortcomings of conventional instrumentation (implant loosening; cut-out of screws) can be avoided. With the fixator providing sufficient axial stability for the posterior spinal wall, this technique allows for far anterior placement of the cement during kyphoplasty, thus adding to its safety. It can be performed percutaneously, additionally fitting elderly patients needs. However, verification of disc-integrity is necessary, as this technique does not address disc space.
Conflict of Interest: None declared

P306-S

LOCALIZATION OF STRONTIUM, BY X-RAY MICROANALYSIS CARTOGRAPHY, IN BONE BIOPSIES OF POSTMENOPAUSAL OSTEOPOROTIC WOMEN TREATED FOR 3 YEARS WITH STRONTIUM RANELATE

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Strontium ranelate (Protelos®) is a new effective and well tolerated treatment for postmenopausal osteoporosis (PMOP). It provides early and sustained vertebral and nonvertebral (including hip) antifracture efficacy (Meunier et al. N Engl J Med 2004, 350: 459–68, Reginster et al. J Clin Endocrinol Metab 2005, 90: 2816–22). In PMOP women, the distribution of strontium (Sr) in bone tissue has only been reported after focal X-ray microanalysis (Boivin et al. Calcif. Tissue Int. 2006, 78 suppl.1: S36–7). However, a global cartography on the entire bone biopsies has never been yet performed.

Cartography, a time consuming approach (1 month full time per sample), was performed on bone samples to evaluate the global distribution of calcium (Ca), phosphorus (P) and Sr in both cortical and trabecular bone tissue on all the surface of samples (analysis on a thickness of 1 μm), and to measure the percentage of bone tissue volume containing Sr. Cartography was performed on four bone biopsies from PMOP women treated for 36 months with strontium ranelate at 2g/day (phase III clinical trials). PMOP women received daily calcium and vitamin D supplements according to their needs.

Global cartography showed that the volume covered by Sr represented 25 to 50 % of the total bone volume, up to 57% of trabecular volume and up to 46% of cortical volume. This was in perfect agreement with the focal distributions of Sr already reported in women (Boivin et al. Calcif. Tissue Int. 2006, 78 suppl.1: S36–7). In the two types of bone, trabecular and cortical, Sr was taken up by bone tissue and heterogeneously distributed. On three of these biopsies, the bone formed under treatment was easily identified by the tetracycline labeling carried out at baseline and Sr was exclusively located in the bone formed after this baseline tetracycline labeling. Furthermore, this bone area was characterized by a slightly lower degree of mineralization as expected in a bone tissue recently formed. However, the degree of mineralization of bone is preserved at the level of the entire biopsy (Boivin et al. Calcif. Tissue Int. 2006, 78 suppl.1: S36–7).

To conclude, cartography of strontium in bone tissue after 36 months of treatment with strontium ranelate, demonstrated the presence of strontium only in tissue resulting from a formation activity during the period of treatment.
Conflict of Interest: G. Boivin Grant/Research Support Servier PJ. Meunier Consultant Servier PD Delmas Consultant – Grant/Research Support Servier

P307-M

ALENDRONATE WITH OR WITHOUT CALCIUM SUPPLEMENTATION WAS SIGNIFICANTLY MORE EFFECTIVE THAN CALCIUM SUPPLEMENTATION ALONE IN IMPROVEMENT OF BONE MINERAL DENSITY AND BONE TURNOVER MARKERS, WITH NO DIFFERENCE IN UPPER-GI TOLERABILITY

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BACKGROUND: The bisphosphonate alendronate and supplemental calcium are each recommended for treatment of osteoporosis in postmenopausal women. However, no trial has directly compared alendronate to supplemental calcium or examined the effect of calcium supplementation on alendronate treatment.

METHODS: This 24-month, randomized, double blind, multicenter trial enrolled healthy, community-dwelling, postmenopausal women with low bone mineral density (BMD). Patients who maintained daily dietary intake of at least 800 mg calcium received daily 400 IU vitamin D and (2: 2: 1) alendronate 10 mg + calcium placebo, alendronate 10 mg + elemental calcium 1000 mg (in the form of calcium carbonate), or alendronate placebo + calcium 1000 mg. Endpoints included BMD at lumbar spine, trochanter, and femoral neck; bone turnover markers (BTMs) BSAP and NTX; and adverse experiences.

RESULTS: Randomized patients (N = 701) were an average of 20.4 years postmenopausal. After 24 months, increases in lumbar spine BMD differed significantly between patients receiving calcium alone (0.8%) and either alendronate alone (5.6%) or alendronate + calcium (6.0%) ($p < 0.001$). Significant differences were also seen at the trochanter and femoral neck ($p < 0.001$). BTMs were significantly lower with alendronate-containing treatments than calcium alone ($p < 0.001$). Addition of calcium supplementation to alendronate did not significantly increase BMD compared with alendronate alone ($p = 0.29$ to 0.97) but did result in a statistically significant, though small, additional reduction in urinary NTX. Adverse experiences, including those in the upper gastrointestinal tract, were similar among treatment groups.

CONCLUSIONS: In postmenopausal women with a daily intake of ≥800 mg calcium and 400 IU vitamin D, 24-month treatment with alendronate 10 mg daily with or without calcium 1000 mg resulted in significantly greater increases in BMD and reduction of bone turnover than treatment with 1000 mg supplemental calcium alone. Addition of supplemental calcium to alendronate treatment had no effect on BMD and resulted in a small though statistically significant additional reduction in NTX.
Conflict of Interest: Drs. Bonnick and Broy have received grant support from and was a consultant to Merck & Co., Inc. F Kaiser, C Teutsch, E Rosenberg, P DeLucca and M Melton are employees of Merck & Co. Inc., and may own stock options.

P308-T

WOMEN WITH POSTMENOPAUSAL OSTEOPOROSIS OR OSTEOPENIA PREVIOUSLY RECEIVING WEEKLY ALENDRONATE OR RISEDRONATE ARE MORE SATISFIED WITH MONTHLY IBANDRONATE

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Ibandronate is a highly potent aminobisphosphonate that is available as a monthly 150mg oral treatment that may improve patient convenience and compliance compared with daily or weekly oral regimens. CURRENT was an open-label, multicentre study designed to evaluate patient satisfaction in women with postmenopausal osteoporosis or osteopenia switched from weekly bisphosphonate therapy with alendronate or risedronate to monthly oral ibandronate 150mg. Patients were divided into two groups on the basis of pretreatment expectations using a Candidate Identification Questionnaire (CIQ) that addressed preference for weekly versus monthly dosing, gastrointestinal intolerance and missed doses of their current bisphosphonate. Patients who answered 'yes' to any of the three items (Y group) were compared with those who answered 'no' to all three (N group). At baseline and 6 months, patient satisfaction with treatment was assessed using the Osteoporosis Patient Satisfaction Questionnaire (OPSAT-Q), a validated instrument addressing side effects, medication convenience, quality of life and satisfaction. The last three domains were normalised on a scale of 0–100. OPSAT-Q scores were converted to Composite Satisfaction Scores (CSS; scale of 0–100). The intent-to treat (ITT) population consisted of 1,678 patients, 1,347 in the Y group and 329 in the N group. While CSS scores were high at baseline (79.9 in the Y group and 80.1 in the N group), satisfaction improved considerably between baseline and month 6 in both groups (+9.2 in the Y group and +7.9 in the N group) after switching to monthly ibandronate. For the entire ITT cohort, convenience, overall satisfaction and quality of life domains improved by 15.6, 12.0 and 9.2 points, respectively, and the adverse side effect score decreased by 0.9 points. Increased satisfaction after switching was reported by the majority of patients in clinically relevant subgroups of the overall cohort according to BMD status, time on medication, history of fragility fractures, age, stomach upset or missed doses with weekly bisphosphonates. Monthly ibandronate was generally well tolerated. These data indicate that switching from weekly oral bisphosphonates to monthly oral ibandronate increases patient satisfaction with treatment even in those patients who reported preferring weekly dosing at the study start. Increased patient satisfaction is likely to improve compliance and persistence with treatment and thereby clinical outcome.
Conflict of Interest: SL Bonnick, F. Hoffmann-La Roche Ltd, GlaxoSmithKline, Consultant M Martens, Eli Lilly, F. Hoffmann-La Roche Ltd, Grant/Research support M Martens, Merck and Proctor and Gamble, Consultant R Civitelli, Procter & Gamble; Hoffmann-La Roche; Eli Lilly & Co.; Novartis, Grant/Research support R Civitelli, Roche; GSK; Merck, Amgen Stock Shareholder (less than $20,000), Eli Lilly; Merck, Wyeth, Consultant KE Friend, F. Hoffmann-La Roche Ltd employee S Silverman, Lilly, Merck, Procter & Gamble, Roche, Speaker's Bureau S Silverman, Merck, Procter & Gamble, Wyeth, Roche,

Novartis, Consultant S Silverman, Novartis, Lilly, Wyeth, Roche, Procter & Gamble, Merck, Research Support

P309-S

RISEDRONATE IS SAFE AND EFFECTIVE IN MEN WITH OSTEOPOROSIS IN A 2-YEAR, DOUBLE-BLIND, RANDOMIZED, PLACEBO-CONTROLLED, MULTICENTER STUDY

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The objectives of this 2-year, double-blind, randomized, placebo-controlled, parallel group, multicenter study were to determine the efficacy and safety of risedronate 35 mg once a week compared to placebo in men with osteoporosis.

Methods: 284 men (95% Caucasian) between the ages of 36 and 84 years, inclusive, who had osteoporosis (lumbar spine T-score £ −2.5 and femoral neck T-score £ −1 SD or lumbar spine T-score £ −1 and femoral neck T-score £ −2 SD) were randomized (2: 1) to either risedronate 35 mg once a week (N = 191) or placebo (N = 93) and received daily supplementation of calcium (1000 mg) and vitamin D (400–500 IU) for 2 years. Efficacy was assessed by percent change from baseline in lumbar spine and total proximal femur, femoral neck, and femoral trochanter bone mineral density (BMD) and bone turnover markers (BTMs) including type I collagen C-telopeptide (CTX), type I collagen N-telopeptide/creatinine (NTX/cr), and bone-specific alkaline phosphatase (BAP). BMD was measured at Months 6, 12, 24, and endpoint (last observation carried forward); BTMs were measured at Months 3, 6, 12, 24, and endpoint.

Results: The primary endpoint showed a statistically significant difference between risedronate and placebo groups in mean percent change from Baseline to endpoint in lumbar spine BMD [4.53% (95% CI: 3.46%, 5.60%)]. The risedronate group had statistically significant increases compared to placebo in mean percent change from Baseline for lumbar spine BMD at Months 6, 12, and 24, total proximal femur and femoral trochanter BMD at Months 12, 24, and endpoint, and femoral neck BMD at Month 24 and endpoint. The mean percent change values for all BTMs (CTX, NTX/cr, and BAP) were statistically significantly reduced in the risedronate group compared to baseline and to placebo at all time points measured. The 2 treatment groups were comparable in overall percentages of patients with adverse events (AEs) (73% placebo, 70% risedronate), serious AEs (16% placebo, 15% risedronate), moderate-to-severe upper GI AEs (4% placebo, 3% risedronate), and overall musculoskeletal AEs (11% placebo, 12% risedronate).

Conclusions: In this 2-year study, risedronate 35 mg once a week was safe and effective for the treatment of osteoporosis in men.

Conflict of Interest: S Boonen, Procter & Gamble, consultant PD Delmas, Procter & Gamble, consultant D Wenderoth, Procter & Gamble, employee KJ Stoner, Procter & Gamble, employee R Eusebio, Procter & Gamble, employee E S Orwoll, Procter & Gamble, consultant

P310-M

BONE TURNOVER MARKER REDUCTIONS AFTER 24 MONTHS OF TREATMENT WITH RISEDRONATE: RESULTS FROM THE RISEDRONATE MALE OSTEOPOROSIS STUDY

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Objective: A 2-year, double-blind, randomized, placebo-controlled, parallel-group, multicenter study evaluating the efficacy and safety of 35 mg risedronate once a week in men with osteoporosis compared to placebo was conducted.

Method: The major inclusion criteria were that patients had to be at least 30 years of age and have a T-score of ≤ -2.5 at the lumbar spine and ≤ -1 SD at the femoral neck or T-score ≤ -1 at the lumbar spine and ≤ -2 SD at the femoral neck. Patients were randomized in a 2: 1 ratio to receive either 35 mg risedronate once a week (N = 191) or placebo (N = 93). The patients also took daily supplementation of calcium (1000 mg) and vitamin D (400–500 IU). The majority (95%) of patients were Caucasian. The bone resorption markers, serum Type-1 collagen C-telopeptide (CTX) and urine Type-1 collagen N telopeptide/creatinine (NTX) and the bone formation marker, serum bone alkaline phosphatase (BAP) were measured at Months 3, 6, 12, and 24.

Results: At the earliest time point tested (Month 3), risedronate demonstrated statistically significant decreases from baseline in all 3 bone turnover markers. The reductions at 3 months were 58.1% (-63.6, -52.7%; $p < 0.001$) for serum CTX, 33.3% (-38.6, -28.0; $p < 0.0001$) for urinary NTX, and 24.5 % (-27.1, -22.0; $p < 0.0001$) for BAP (least square means). The bone marker decreases observed with risedronate were significantly greater than those seen in the placebo group at all time points and the differences between risedronate- and placebo-treated patients were of similar magnitude throughout the study.

Conclusion: The BTM results in the male study were similar to that observed in previous trials in women with osteoporosis treated with risedronate. In addition, the data from this 2-year study showed that 35 mg risedronate once a week had a safety profile similar to placebo and was effective for the treatment of osteoporosis in men (reported previously). Approval of 35 mg risedronate for the treatment of osteoporosis in men at high risk of fractures has been granted by the European Authorities.

Conflict of Interest: S. Boonen, Procter & Gamble, consultant P. Garnerro, Procter & Gamble, consultant PD. Delmas, Procter & Gamble, consultant R. Eusebio, Procter & Gamble, employee CY. Guo, Procter & Gamble, employee

P311-T

WORK-UP FOR SECONDARY CAUSES OF OSTEOPOROSIS IN POSTMENOPAUSAL WOMEN. PRELIMINARY RESULTS

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The prevalence of secondary conditions that contribute to low bone mass in postmenopausal women with osteoporosis (OP) is unknown.

Objectives: To analyse the prevalence of contributing conditions for bone loss in postmenopausal women with OP, as well as the clinical characteristics and the impact of these disorders in the severity of the disease.

Methods: This cross-sectional prospective study included 86 postmenopausal women with OP with a mean age 64,6 ± 9,4 years (44–85) 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-ray, laboratory testing 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 in all patients before treatment.

Results: The mean age of menopause was 47,6 ± 5,4 years (29–57), the mean BMI was 25,3 ± 3,4 Kg/m^2; 54% had previous fragility fractures, 34% had vertebral fractures, 40% had family history of fractures; 57% had vitamin D insufficiency (25OHD < 20 ng/ml), 38% had increased PTH levels (> 65 pg/ml), 28% had hypercalciuria (> 250 mg/24h) and 37% had increased NTX urinary levels (> 65 nM/mM). PTH levels were not related to any of the other analysed variables (25-OH D, creatinine, age, BMD). Lumbar and femoral BMD were similar in patients with or without secondary conditions. Except for a higher prevalence of fragility fractures in women with vitamin D insufficiency (63,4% vs 38,7%, p = 0,038), there were no significant differences in skeletal fractures between patients with or without associated metabolic conditions (increased serum PTH or urinary NTX levels and hypercalciuria).

Conclusions: Metabolic disorders such as vitamin D insufficiency, increased serum PTH levels and hypercalciuria are common in postmenopausal women with OP. Women with vitamin D insufficiency have a higher prevalence of fractures. The influence of these secondary conditions on treatment efficacy is unknown.

Conflict of Interest: None declared

P312-S

THE IDENTIFICATION OF PATIENTS IN NEED OF TREATMENT FOR OSTEOPOROSIS IS IMPROVED BY USING VERTEBRAL FRACTURE ASSESSMENT AT THE TIME OF DXA SCANNING

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Vertebral fractures are the most common type of osteoporotic fracture. They affect about 25% of women over the age of 50. The presence of one osteoporotic vertebral fracture is a major risk for further fractures and up to two-thirds of patients with vertebral fractures can be asymptomatic. Vertebral fractures can be diagnosed by obtaining a lateral image of the spine using a DXA scanner. This technique is called Vertebral Fracture Assessment (VFA).

We aimed to determine the effects of age and BMD on the prevalence, severity, type and site of vertebral fractures and to determine the number of patients with osteoporotic vertebral fractures who would not have met treatment criteria based on BMD measurement alone.

300 randomly selected postmenopausal women who had VFA scans performed at the time of DXA scanning using an iDXA® scanner were included in the study. VFA images were assessed by a blinded investigator (PH) and fractures were graded based on the semiquantitative system of Genant and Wu.

Of the 300 patients, 106 (35.3%) had at least 1 vertebral fracture. Those who had fractures were significantly older and had significantly lower BMD. Based on NICE (National Institute for Health and Clinical Excellence) guidelines from the U.K, 38 patients aged 65–74 met criteria for bisphosphonate therapy without VFA and 58 met criteria with VFA. 21 patients could potentially be treated with bisphosphonates under 65 years of age but when VFA was included the number suitable for treatment increased to 60. 15 patients met criteria for treatment with teriparatide without VFA and 25 met criteria for treatment with teriparatide with VFA (Table).

Future fracture risk determination is dependent upon accurate determining of current clinical status. Given that large numbers of vertebral fractures are asymptomatic some form of screening for vertebral fracture should be undertaken. VFA is a simple method of achieving this and will significantly improve the appropriate treatment of osteoporosis.

Table: Number of patients treated based on NICE guidelines

Treatment based on age	Without VFA	With VFA
Bisphosphonate 65–74 years old	38	58
Bisphosphonate < 65 years old	21	60
Teriparatide	15	25

Conflict of Interest: None declared

P313-M

STRONTIUM RANELATE EFFECTS ON OSTEOBLASTIC DIFFERENTIATION ARE ASSOCIATED WITH PGE2-DEPENDENT GROWTH FACTOR PRODUCTION

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Strontium ranelate (SR) is a new treatment for osteoporosis that has both antiresorptive and anabolic effects. SR increases osteoblastic differentiation and prostaglandin (PG) E2 production in murine marrow stromal cells (MSCs). To investigate the hypothesis that strontium ranelate exerts some of its anabolic effects on osteoblasts via PG production and growth factors produced downstream, we examined the ability of SR to stimulate osteoblastic differentiation and mineralization in MSC cultures from cyclooxygenase-2 (COX-2) knockout (KO) and wild type (WT) mice. COX-2 KO MSCs have a marked decrease in PG production relative to MSCs from COX-2 WT mice. MSCs from 7–8 wk old WT and KO mice were cultured up to 21 days with SR (1 and 3 mM). Alkaline phosphatase (ALP), osteocalcin (OCN), IGF-1 and VEGF mRNA expression were determined by real time PCR. Mineralization was assessed by alizarin red staining. After 14 and 21 days of culture, SR significantly and dose-dependently increased ALP and OCN mRNA expression, respectively, in WT cultures. These effects were associated with an increase in IGF-1 and VEGF mRNA expression by 1.4–1.5-fold ($p < 0.01$) and 1.4–1.7-fold ($p < 0.01$) respectively, after 7 days in SR treated cultures. There was also, a dose-dependent increase in PGE2 production, which occurred after a treatment with SR for 7 days. In MSCs from COX-2 KO mice, ALP and OCN mRNA levels were decreased 50% ($p < 0.05$) and 60% ($p < 0.01$), respectively, compared to WT MSCs, and there was no increase in ALP and OCN mRNA expression with SR. No increase in IGF-1 and VEGF mRNA expression or in PGE2 production was induced by SR in COX-2 KO mice. Osteoblast mineralization was dose-dependently increased in WT cultures after a treatment with SR for 21 days. Mineralization was decreased in COX-2 KO cultures compared to WT cultures, and mineralization induced by SR was decreased in the COX-2 KO cultures.

To summarize, SR increased osteoblastic differentiation and mineralization in a COX-2 dependent manner in murine MSCs, as well as PGE2 production, IGF-1 and VEGF mRNA expression. Coupled with our previous data, this study indicates that several SR effects on bone formation involve the local production and action of PG on osteoblasts, and that SR-induced PG may increase downstream production of growth factors that could eventually stimulate bone formation.

Conflict of Interest: None declared

P314-T

DISSOCIATION BETWEEN BONE FORMATION AND BONE RESORPTION EVIDENCED BY CHANGES IN BIOCHEMICAL MARKERS OF BONE TURNOVER IN PATIENTS TREATED WITH STRONTIUM RANELATE

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Objective: To assess the effects on bone turnover of a 2g/day treatment with strontium ranelate in postmenopausal osteoporosis

Materials and methods: Strontium ranelate is a new oral antiosteoporotic drug to reduce vertebral and hip fracture risks, found in pre-clinical studies to both stimulate bone formation and decrease bone resorption, rebalancing bone turnover toward formation. During phase 3 program, markers of bone turnover were assessed in order to clinically assess the dual mode of action of strontium ranelate. From phase 3 SOTI study, it was already demonstrated that serum concentrations of serum bone alkaline phosphatase isoenzyme (bALP) using an immunoradiometric assay (IRMA) was significantly higher and C-terminal telopeptides of type I collagen (CTX) were significantly lower in strontium ranelate group than in placebo group as measured every 6 months over 3-year treatment period. In TROPOS study (n = 4932 patients, ITT population) both markers were also assessed every 6 months over 3 years. In addition and in both studies, serum procollagen type I carboxy-terminal propeptide (PICP), a marker of bone formation, was measured using a radioimmunoassay (RIA).

Results : In TROPOS study , results showed a significant increase in bALP and PICP serum concentrations while serum concentrations of CTX were significantly lower in the strontium ranelate than in placebo group at each time visit from baseline values over 3 years. In SOTI study, PICP serum concentrations showed similar changes as those measured in TROPOS study.

Conclusion: Serum bone markers changes obtained in two different studies clinically confirm the dual mode of action of strontium ranelate, already evidenced in non clinical studies, both increasing bone formation and decreasing bone resorption.

Conflict of Interest: None declared

P315-S

THE IMPACT OF SUB OPTIMAL COMPLIANCE AND PERSISTENCE WITH BISPHOSPHONATE THERAPY ON POST MENOPAUSAL OSTEOPOROTIC FRACTURE RISK

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Background: Recent studies report sub optimal levels of compliance and persistence with bisphosphonate treatments in clinical practice and this is likely to be compromising the reduction of post menopausal osteoporotic fracture (PMO) risk observed in clinical trials.

Methods: A systematic search of bibliographic sources including MEDLINE, EMBASE (Jan 1990 – Sept 2006) and the Cochrane Library databases was performed to identify primary research studies evaluating the impact of compliance and persistence with bisphosphonates on PMO fracture risk in clinical practice. The results were supplemented by hand searching journal supplements from relevant conference proceedings and using a search engine to identify posted abstracts or posters from conferences available on the internet. The four main search criteria were: bisphosphonates; osteoporosis / osteopenia in postmenopausal women; all types of fracture; compliance and persistence.

Results: The search identified five original studies all based on retrospective observational data sources. Two were specific to bisphosphonates (daily etidronate, daily and weekly alendronate and risedronate). A USA study tracking 35,537 women reported that patients compliant (MPR > 80%) with treatment for 24 months experienced significantly fewer fractures compared to non compliant patients (8.5% v 10.7%, $p < 0.001$, RRR 21%). In those who persisted (refill gap < 30days) fracture rates were also significantly reduced (7.7% v 10.3%, $p < 0.001$, RRR 29.3%). In a nested case control study performed in the Netherlands, persistence for 12 months (refill gap < 50% of previous Rx length) reduced fracture risk by 26% ($p < 0.05$) and for 24 months by 32 % ($p < 0.05$). Three studies, non-specific to bisphosphonates, reported that poor compliance with any osteoporosis medication significantly increased the risk of osteoporotic fracture. In addition, several preliminary reports of bisphosphonate specific studies confirm that the risk of an osteoporotic fracture can be reduced by approximately 25% if patients continue treatment, as well as decreasing the risk of hospitalization and resource use.

Conclusions: The evidence indicates that sub optimal compliance and persistence compromises bisphosphonate effectiveness and increases the risk of PMO fracture. However, more research is needed to strengthen the evidence that delineates the relationship between fracture risk, compliance and persistence in clinical practice.

Conflict of Interest: W. Cowell, Roche Products Ltd., Employee N. Lynch, GlaxoSmithKline, Employee H. Middelhoven, F. Hoffmann-La Roche AG, Employee M. Hunjan, GlaxoSmithKline UK Ltd., Employee

P316-M

PROGRESSIVE EFFICACY IMPROVEMENTS WITH QUARTERLY INTRAVENOUS IBANDRONATE INJECTIONS: THE DIVA STUDY LONG TERM EXTENSION

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In the DIVA study in women with postmenopausal osteoporosis, intravenous (i.v.) ibandronate injections (2mg every 2 months [q2mo] or 3mg every 3 months [q3mo]) achieved superior efficacy ($p < 0.001$)[1, 2] to an established daily oral ibandronate regimen (2.5mg)[3] for lumbar spine bone mineral density (BMD; 6.4% and 6.3% vs 4.8%, respectively, at 2 years). BMD is an important component of bone strength along with the rate of bone turnover and the structure and material composition of bone. The efficacy and safety of both i.v. ibandronate regimens are being further investigated in a 3-year long term extension (LTE) of the DIVA study. This is a multinational, open label, parallel group extension study. Women who had completed 2 years in DIVA and whose compliance was ≥75% in the last year of DIVA were eligible. Patients continued to receive the i.v. ibandronate regimen that they had received in DIVA, either 2mg q2mo or 3mg q3mo, or were re-randomised from daily oral ibandronate. All patients also received calcium and vitamin D supplements. Data from the first year of the LTE have been analysed. A post-hoc, pooled analysis will also be conducted on the subgroup of patients who received i.v. ibandronate throughout the 2 years of the DIVA study and then continued on this treatment into the DIVA study LTE. Of the 1,386 patients who received ibandronate in DIVA, 781 were enrolled into DIVA LTE. Of these, 758 comprised the ITT population (2mg q2mo, n = 365; 3mg q3mo, n = 393, ITT). Compared with BMD gains at the 2-year endpoint of DIVA, additional mean gains in lumbar spine BMD of 0.92% and 0.95% were seen with the 2mg and 3mg regimens after the first extension year of the DIVA study LTE. Additional gains in BMD were also observed at the total hip (0.48% and 0.13%, respectively). Reductions in serum CTX were maintained from the 2-year endpoint of DIVA; median reductions in peak levels at month 6 of DIVA LTE were 81.7% (2mg q2mo) and 85.7% (3mg q3mo) and median increases in trough levels at month 12 were 18.3% (2mg q2mo) and 9.81% (3mg q3mo). In conclusion, further increases in lumbar spine BMD were observed in patients who received an additional year of 2mg q2mo or 3mg q3mo i.v. ibandronate in DIVA LTE and the substantial reductions in bone turnover seen in DIVA continued to be maintained.

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Conflict of Interest: E Czwerinski, F. Hoffmann-La Roche Ltd, GlaxoSmithKline, Amgen, Eli Lilly, Merc, Novartis, Organon, Pfizer, Servier grant/research support B Langdahl, F. Hoffmann-La Roche Ltd, GlaxoSmithKline, grant/research support R Grant, F. Hoffmann-La Roche Ltd employee C Neate, F. Hoffmann-La Roche Ltd employee

P317-T

COMBINED RALOXIFENE TO TERIPARATIDE TREATMENT PROMPTLY IMPROVES BMD IN MULTIFRACTURED PATIENTS NON-RESPONDER TO ANTIRESORPTIVES

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Background and aim: Most data about Teriparatide come from studies on patients naïve to antiresorptives (AR). Addition of alendronate to PTH seems to reduce the bone forming effect of PTH, while Raloxifene plus Teriparatide combination seems to increase the effect on BMD. Few data are available about Teriparatide in patients already on AR, particularly those that fractured again while on treatment (Non-Responder patients, NR). Aim of the study is to evaluate the efficacy of combination therapy with teriparatide and raloxifene vs. teriparatide alone, in multifractured patients non-responders to previous AR. Materials and Methods: The study was a randomized 6-months clinical trial, including 31 non-obese, non-smokers, female patients, ≥ 70 years-old, with BMD T-score < −2.5. Each patient had 2 vertebral fractures (according to semi-quantitative method of Genant et al.), the second one happened while being on AR. Seventeen patients received combination therapy with daily 20 mcg s.c. teriparatide and daily oral 60 mg raloxifene (group 1) and 14 patients received daily 20 mcg s.c. teriparatide alone (group 2). All patients were evaluated, by DEXA, for BMD at lumbar spine and T-score at baseline and after 6-months of treatment. Differences and mean increases in BMD and T-score at baseline and after 6 months between the two groups were compared by Student T test; a $p < 0.05$ was considered statistically significant. Results: Safety was good in all groups. BMD and T-score at baseline and after 6 months are showed in tab 1. While patients treated with teriparatide alone showed only a slight increase in BMD and T-score, those treated with combination therapy experienced a significant increase in BMD and T-score at 6 months vs. baseline ($p < 0.05$). Conclusion: Among patients treated with AR, those treated with alendronate seem to suffer a delay in BMD increase likely due to the suppressive effect of this molecule. NR patients have a greater urgency to increase bone mass. Earlier data suggest that combining Teriparitide and alendronate might be counterproductive. This report suggests that combination of raloxifene and teriparatide is well tolerated in multifractured NR patients and increases BMD more rapidly than teriparatide alone.

Table: Tab 1- BMD and T score baseline and after 6 months

	BMD (t 0)	T-score (t 0)	BMD (t 6)	T score (t 6)
Group 1	0.65 ± 0.08	−3.31 ± 0.58	0.71 ± 0.08*	−2.82 ± 0.67*
Group 2	0.71 ± 0.08	-3.10 ± 0.60	0.74 ± 0.07†	−2.5 ± 1.4†

*p < 0.05 vs. baseline; † p n.s. vs. baseline;

Conflict of Interest: None declared

P318-S

TERIPARATIDE AFTER ANTIRESORPTIVE

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Background: Most data about Teriparatide come from studies on patients naïve to antiresorptives, few data come from studies on patients that used antiresorptive therapy prior teriparatide (TPTD). Aim: to evaluate the increase of T-score, ALP, VAS, spine fracture after 18 months of TPTD, particularly how increase data in patients treated with alendronate, risedronate or raloxifene before TPTD.

Patients, methods: We observed effects of a 18 month teriparatide treatment on BMD, ALP, Pain , semi-quantitative vertebral fracture index (Genant) and Spine deformity index (SDI) in women that completed 18-month treatment with TPTD. The study included 216 women (age 44–89 yrs, mean 71,7) in postmenopausal period (> 5 yrs), in 6 Osteoporosis centre in Meridional Italy. All women received a daily injection 20 mcg of teriparatide associated with a dose of 500 mg of calcium and 400 U of vitamin D; in 115 women changes in T-score at the lumbar spine were measured by DEXA; mean T-score before TPTD was −3.6; in 101 women changes in T-score at the heel were measured by US mean T-score before TPTD was –4.35. Serum ALP was determined. The VAS was administrated. Baseline characteristics and semi-quantitative vertebral fracture index before treatment with TPTD was mean 2, 25, and SDI mean 4, 3; besides we evaluated changes in T score, VAS, ALP, semi-quantitative vertebral fracture index and SDI distinguished patients in 3 groups: those treated with alendronate, risedronate or raloxifene before TPTD compare this data before and after TPTD treatment in each groups

Results: T-score increased significantly in TPTD treated. ALP increased significantly: the mean increase after 18 months of treatment was 56%. The mean of decrease of pain was -41% (VAS), Fracture index and SDI did not change. Interestingly while pain decreased in 3group treated with TPTD, ALP e Tscore increase more in group treated before TPTD with Raloxifene (+76%) than group treated with Alendronate (+23%) and Residronate (+12%) and ALP increase more in group treated before with raloxifene (+137%) than patients treated before with alendronate (+57%) and residronate (+44%).

Conclusion: Improvement in BMD, ALP, pain VAS was very important, there are not change in semi-quantitative vertebral fracture index or SDI, but in the group treated with raloxifene was very increase BMD e ALP than patients treated with residronate and alendronate

Conflict of Interest: None declared

P319-M

EFFECTS OF IBANDRONATE, DAILY OR MONTHLY ADMINISTERED, ON BONE QUALITY AND REMODELLING IN ORCHIDECTOMIZED RATS

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Ibandronate (IB) is a new biphosphonate which is being used in the treatment of postmenopausal and corticosteroid osteoporosis, but there are few dates about its usefulness in the prevention and treatment of osteoporosis due to androgen lack. On the other hand, the possibility of its monthly administration, instead of daily, presents an added advantage.

The aim of this work was to study the effects produced by the lack of androgens on several factors related to bone quality in male rats, and the ability of IB, daily (d) or monthly (m) administered, to prevent the effects produced by orchidectomy. Forty, 9 month-old, male Wistar rats were operated (sham-operated or orchidectomized). The following groups were studied: SHAM (n = 10): sham-operated rats, treated with placebo and sacrificed 20 weeks after surgery. OQX (n = 10): orchidectomized rats treated with placebo 20 weeks and sacrificed 20 weeks after surgery (OQX + IBd and OQX + IBm): orchidectomized rats treated with 1 μg/Kg/day or 28 μg/Kg/28 days, subcutaneous injection for 20 weeks.

After sacrifice, bone mineral density (BMD) was determined by DEXA in situ, in the lumbar spine and in the whole left femur. Computerized microtomography (μCT) in femur by Skyscan 1172, serum tartrate resistant acid phosphatase (5b isoenzyme) and osteocalcin were performed.

OQX group presented values of lumbar and femoral BMD lower than SHAM group. Both treatments, IBd and IBm, prevented the loss of BMD due to orchidectomy. Results from μCT showed a decrease in BV/TV, trabecular number, trabecular pattern factor and degree of anisotropy, and an increase in the trabecular separation without differences in their thickness. A redistribution of the model structure from plates to rods was also observed. Ibandronate treatment, both daily and monthly, prevented all these changes. Also, the general increase in bone remodelling due to orchidectomy was not observed in ibandronate treated rats. The above results suggest that monthly treatment with ibandronate is so effective as daily treatment in order to prevent changes in bone quality due to androgen lack in rats.

Conflict of Interest: None declared

P320-T

RISEDRONATE AND ALENDRONATE FOR THE REDUCTION OF HIP AND NONVERTEBRAL FRACTURES, A RETROSPECTIVE COHORT STUDY

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Objective: To compare the onset of fracture reduction between therapies, we conducted a retrospective cohort study to assess the 6-month and 12-month incidence of nonvertebral fractures and hip fractures in cohorts of female patients (over 65 years) newly treated with risedronate and alendronate.

Methods: Both risedronate and alendronate therapies have been shown to reduce the incidence of nonvertebral fractures in randomized, placebo-controlled clinical trials. Further analyses of these data suggested that risedronate may reduce fractures temporally earlier than alendronate. Patients, identified within 2 pooled datasets of health services utilization, were new users of once-a-week dosing of risedronate (n = 12,215) or of alendronate (n = 21,615). Two fracture outcomes were identified: patients with a composite (hip, wrist, humerus, clavicle, pelvis, leg) of nonvertebral fractures (n = 376 and 507 through 6 and 12 months, respectively) and hip fractures (n = 73 and 109 through 6 and 12 months, respectively). Cox proportional hazard modeling was used to compare the incidence of fractures between cohorts. In addition, sensitivity analyses were performed.

Results: A greater percentage of the risedronate cohort had baseline risk factors for fracture than the alendronate cohort. After statistical adjustment for these differences, the risedronate cohort had a lower incidence of nonvertebral fractures [19% (p-value = 0.05) at 6 months and 18% (p-value = 0.03) at 12 months] and of hip fractures [46% (p-value = 0.02) at 6 months and 43% (p-value = 0.01) at 12 months] than did the alendronate cohort. As with all cohort studies, the interpretation of the results may be limited by the non-randomized nature of the study design. However, these results do not appear to be from baseline differences in fracture risk between cohorts and are consistent with the results of analyses of clinical trials.

Conclusion: These results suggest that during the period studied, more patients on risedronate are protected from fracture than patients on alendronate.

Conflict of Interest: P. D. Delmas: Procter & Gamble, consultant S. L. Silverman: Procter & Gamble, consultant N. B. Watts: Procter & Gamble, consultant J. L. Lange: Procter & Gamble, employee R. Lindsay: Procter & Gamble, consultant

P321-S

SECONDARY FRACTURE PREVENTION MEASURES IN ORTHOPEDIC WARDS IN BELGIUM

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Objective: In-hospital diagnosis of osteoporosis in fracture patients in the orthopedic wards is lower than 10% and the prescription rate of treatments is less than 5%. The present report describes the efficiency of a secondary prevention program in Fracture patients in Orthopedic Wards (FORWARD) in a Belgian hospital care setting.

Methods: Orthopedic surgeons willing to participate in the program were requested to refer their patients with clinical fractures for bone densitometry and an osteoporosis specialist's advice.

Results: In 36 hospitals data were collected about 4116 fracture patients. Females represented 73,5% of the population. Fracture prevalence increased until the age of 80 to 85 years, with mean age of 78 year in women and 74 year in men. Most of the fracture cases were hospitalized (88%) and the main fracture type included in the program was hip fracture (45%). Previous clinical fractures were reported in 21% of the patients. 9% had previous DXA examination or concomitant osteoporosis treatments and were therefore excluded for DXA referral. Appointments for DXA examination were made in 66% (n = 2718) of the patients and results were obtained from 53% (n = 2181). The diagnostic classification was as follows: osteoporosis 56%, osteopenia 33% and normal bone density 11%. Nearly all cases were referred for diagnostic confirmation of the problem by an osteoporosis specialist, mainly rheumatologists and physiotherapists. Final clinical diagnosis of osteoporosis was accepted in 39% of the cases. Treatment with calcium and vitamin D was started in 1303 patients (31%), with bisphosphonates in 888 patients (21%) and with SERMs or others drugs in 108 patients (< 3%). No data about compliance to these treatments were obtained in the present project.

Conclusion: The active referral by orthopedic surgeons of the fracture patients in the orthopedic ward to DXA units and osteoporosis specialists results in the identification of osteoporosis in 39% of the patients. Implementing effective measures and treatments for (secondary) fracture prevention in this high risk population could lead to cost-savings in the short term. Initiatives to promote the patient flow needs to be elaborated and maintained by an active local care organisation.

Conflict of Interest: None declared

P322-M

3D FEMUR GEOMETRY FROM 2D DXA SCANS: BONE VOLUME ESTIMATION

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Dual energy x-ray absorptiometry is a primary diagnostic tool for assessing skeletal fragility. Bone geometry, spatial distribution and micro-architecture also play important roles in bone strength. We evaluated a new method to measure bone volume from femur DXA scans, comparing the calculated virtual measurements to known volumetric measurements in animal femur samples.

Materials and methods: A total of 25 proximal pig femurs (mean weight 78 kg and age 5 months) were evaluated. The pig femurs were scanned with a narrow angle fan beam DXA device (GE Lunar Prodigy), using standard femur scan mode. Virtual bone volume was derived with special software by summing the individual 0,25mm slice volumes obtained from each cross sectional area, and assuming a variable elliptical geometry. True bone volume was calculated for two sections, femoral neck (FN) and femoral head (FH), sliced from the pig femur, with the cut axis perpendicular to the FN-axis. The Cavalieri method of fluid displacement was used to calculate the volume of these sections. The DXA scans and the Cavalieri measurements were repeated three times to determine in vitro reproducibility. Measurements were compared by a paired sample T-test and the relationship was studied by linear regression equation and Pearson's bivariate correlation. Accuracy error was calculated as $(1 - r^2)$, reported as a percentage. The measurement reproducibility was shown as the coefficient of variation (CV) and results were expressed as a percentage.

Results: There was a strong correlation between the virtual DXA 3–D and the Cavalieri 3-D measurements at the FN ($r^2 = 0.938$, $p < 0.0001$) and for the FN + FH ($r^2 = 0.928$, $p < 0.0001$). The paired t-test did not show significant differences between the measurements. Using the Cavalieri measurements as reference, the accuracy error for calculated volume was 12% at FN and 13.8% for FN + FH. The CV for FN and FN + FH calculated volume was 5.66% and 4.93% respectively. The true volume CV for FN and FN + FH was 3.61% and 1.7%.

Conclusions: Even assuming a fixed relationship between geometrical shape and animal specimen, our results showed a very good correlation between measured 3-D volume and the 3-D volume calculated from a DXA-scan. This

technique may offer a non-invasive and economical method to assess spatial bone distribution in critical areas of the skeleton.
Conflict of Interest: None declared

P323-T

CAN DXA ACCURATELY MEASURE ABDOMINAL FAT? COMPARISON WITH MRI AND ANTHROPOMETRIC ASSESSMENT

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Abdominal fat (AF) is associated with an increased risk for metabolic and cardiovascular disorders. Direct measurement of fat mass by magnetic resonance imaging (MRI) is accurate but expensive, complex, and relatively inaccessible. MRI pixel values for given tissues may vary from slice to slice or between individuals as a result of heterogeneity in the magnetic field. We evaluated the assessment of AF with Dual-Energy X-ray Absorptiometry (DXA) and compared the results with those obtained by MRI.

A total of 29 subjects (age 19.9 ± 2.0 years; BMI range: 16.9–29.7) had DXA total body assessment, MRI total fat measurement, and anthropometric measurements [waist circumference (WC), weight, height]. DXA AF (g) in the android region (between the ribs and pelvis) was measured with a Lunar Prodigy (GE Healthcare) equipment. MRI Excite11 (GE Healthcare) total fat measurement in the L3 slice was converted from volume to mass using the density of 0.92 kg/l for adipose tissue. Results were analyzed by Pearson's correlation.

DXA AF showed a significant correlation with MRI AF in the L3 slice ($R = 0.965$, $p < 0.001$; $R = 0.953$ in subjects with BMI < 25; $R = 0.916$ in subjects with BMI > 25). There was also a positive correlation between WC and DXA AF ($R = 0.836$, $p < 0.001$), and between WC and MRI total fat mass in L3 slice ($R = 0.825$; $p < 0.001$). DXA AF showed a positive correlation with MRI visceral fat mass in L3 slice ($R = 0.676$; $p < 0.001$).

DXA regional body composition analysis is a reliable method for measurement of abdominal fat in a rapid and comfortable manner.
Conflict of Interest: None declared

P324-S

DXA BODY COMPOSITION CHANGES IN PATIENTS WITH ANOREXIA NERVOSA AFTER WEIGHT GAIN

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Anorexia nervosa is the third most common chronic disease in adolescent girls and can lead to severe bone loss with a low bone turnover state. Anorexia nervosa is a condition of weight loss by self-imposed caloric restriction. With the use of total body DXA measurements it is possible to follow changes in bone mineral and body composition. We evaluated the impact of changes in fat and lean mass on bone mass in anorexic patients followed for one year.

A total of 36 female patients (18.5 ± 4 years) participated in the study. Twenty-seven subjects suffered from irregular menses or even amenorrhoea. All subjects had a baseline total body DXA measurement. Thirteen subjects also had a follow-up measurement after 11,5 ± 4,3 months of treatment, on average. Total and regional body composition was measured by a GE Lunar Prodigy DXA device, which calculates fat mass and %fat in the abdominal (android) and hip (gynoid) regions, and the android/gynoid %fat ratio. In addition, total body bone mineral content (BMC), lean mass (g), fat mass (g) and total mass (kg) were measured.

Baseline measurements showed a positive correlation between BMC and lean mass ($r = 0.613$; $p < 0.0001$) and between BMC and total mass ($r = 0.587$; $p < 0.0001$). At follow-up, there were significant correlations between the percent change in BMC and percent weight change ($r = 0.801$; $p = 0.002$) and between % BMC change and the % change in fat mass ($r = 0.876$; $p < 0.0001$). However, no correlation was found between % BMC change and the % change in lean mass ($r = 0.209$; $p = NS$). When we analyzed the changes in regional fat, the % BMC change showed a significant correlation with android fat mass ($r = 0.759$; $p < 0.004$).

We may conclude, from this small sample, that the changes in BMC in these anorexic patients are modified especially by changes in fat mass. The main advantage of DXA is that changes in both fat and lean mass can be monitored. Scale weight indicates the overall weight change, but without specific differentiation of fat and lean mass in various regions of interest. DXA may be a valuable tool to evaluate total and regional changes in fat and lean tissue, as well as BMC, in anorexia nervosa patients.
Conflict of Interest: None declared

P325-M

FOREARM BMD AND AGE, NOT FALLS OR PREVIOUS FRACTURE IDENTIFIES FRACTURE RISK

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Forearm bone density (BMD) scanning may be useful to identify women at risk of fracture. Treatment is most effective if BMD is low. We have investigated whether scanning women > 50y will identify those with subsequent fracture and the value of risk factors.

1479 community recruited women (63.9 ± 9.6y) had forearm scanning between 1999–2002. If BMD was < 0.34g/cm^2 (Jones and Davie 1998) the patient's doctor was informed and treatment suggested. After 5.0 ± 1.5y, 1088 responded to a questionnaire requesting information, including fracture, since scanning. Women with no fracture were compared with those with hip, wrist or ankle fracture. Women with other fracture sites (187) and DON'T KNOW (78) were excluded. Multivariate analysis, ANOVA and Chi square were calculated. Women were divided into those under and over 70y at scanning. Initial BMD was not low in ankle fracture (< 70y 0.43 ± 0.09; > 70y0.38 ± 0.03) but although low (TABLE) in wrist fracture (< 70y) was above 0.34. Hip fracture was rare < 70y. After 70y wrist and hip fracture women had low BMD. Previous fracture or fall in the previous year was uncommon in hip fracture. Bisphosphonate use was low, only 27% in hip fracture > 70y. In a multivariate analysis neither falls nor previous fracture were significant in determining fracture at either wrist or hip, only age and BMD remaining significant. Very low BMD was not a feature of women < 70y developing wrist fracture over the next 5y. Scanning in this age group will not identify many women who merit treatment, low BMD being unlikely even in wrist fracture. Women with ankle fractures did not have low initial BMD. Women > 70yr with either wrist or hip fracture had low BMD. Few had previous fracture. Wrist, but not hip, fracture patients had more falls in the previous year. As most hip fractures occurred in women > 70y and as falls are as frequent in non-fracture women, targetting by previous fall or fracture will overlook most future fracture patients. Greater use of BMD measurements in women > 70y is indicated to identify those requiring treatment.

Table:

# SITE	AGE < 70Y			AGE > 70Y		
	none	wrist	hip	none	wrist	hip
N	593	19	2	184	12	11
BMD A	0.452	0.408*	0.457	0.376	0.311*	0.331*
% < 0.34	3.7	15.8*	0	28.3	83.3*	54.5
%prev B	–	42	0	–	3	7
%fall C	8	0	0	13.4	40	16.7

*$p < 0.05$ cf no # (A) mean g/cm^2 (B) wrist# (C) in last y

Conflict of Interest: None declared

P326-T

COMMUNITY BONE DENSITOMETRY: INFLUENCE ON TREATMENT INITIATION AND COMPLIANCE?

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With aging, fractures cause substantial morbidity and mortality, placing a burden on limited health resources. Identifying those individuals most at risk of fracture by forearm bone mineral density (BMD) measurement (which relates to WHO defined osteoporosis in the femoral neck) is quick and inexpensive.

During 1999–2002 1769 community recruited women > 50y had forearm BMD measured and were categorised as having osteoporosis (BMD < 0.34 g/cm^2), osteopenia (BMD 0.34–0.419 g/cm^2) or normal BMD (> 0.419 g/cm^2). After scanning, treatment with bisphosphonate (BP) was suggested to the General Practitioner for women with osteoporosis. All women were been re-investigated 5.02 ± 1.47y later to ascertain whether a BP was started and the degree of compliance. Comparisons were made using ANOVA or Chi squared.

Of 1769 women originally scanned, 1479 (age at scan 63.9 ± 9.6y, mean ± SD) were still alive, and asked to complete a questionnaire. Those replying (1088 age 63.6 ± 9.4y) were younger than non-respondents (73.9 ± 11.9y; $p < 0.001$).

Women were divided into 3 BMD groups: 15.4% (age 73.5 ± 7.2y) were osteoporotic, 29.7% (66.6 ± 8.6y), osteopenic and 54.9% (59.2 ± 7.3y) had normal BMD. In the lowest BMD gp 73.4% had used calcium, 72.1% BP, 17.8% HRT; in the middle gp 54.7%, 35.7%, 26.9%, and in the highest 31.0%, 5.3%, 44.1%, at any time. BP use was more likely with a previous fracture history ($p < 0.01$). A first degree relative with wrist or hip fracture did not affect compliance.

In a subgroup of 469 women initiation and duration of BP therapy was investigated. Of 66 women with osteoporosis, 6.1% started before and 62.1%

(47.0% within 1y) after the scan; 31.8% had never used a BP. At follow up, 53% of those already on or starting BP continued (36% of whole osteoporosis gp). Women with new fractures ($p < 0.01$) were more likely to continue BP. Neither a history of maternal hip fracture nor age at starting BP affected compliance. Median duration of BP use after scanning was 4.0y (inter-quartile range, 1.75–5.0). Of 248 women with normal BMD, 3.6% started BP (77.8% continuing).

Forearm scanning identifies the elderly at the greatest risk of osteoporosis and increases the proportion taking treatment. Many patients never start treatment, and only half continue. A previous fracture history increased compliance, but hip fracture history in the family had no effect. Identification of patients with osteoporosis should be connected with means of encouraging adherence.

Conflict of Interest: None declared

P327-S

FIRST RESULTS OF THE SABRE STUDY: THE EFFECTS OF RISEDRONATE SODIUM ON BONE METABOLISM IN POSTMENOPAUSAL WOMEN RECEIVING ADJUVANT ANASTROZOLE FOR EARLY BREAST CANCER

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In postmenopausal women, aromatase inhibitors have been associated with increased bone turnover and reduction in bone mineral density (BMD). The Study of Anastrozole with the Bisphosphonate RisedronatE (SABRE; NCT00082277) is an ongoing, multicentre, Phase III/IV study evaluating the effects of the bisphosphonate, risedronate sodium, on BMD and bone turnover in postmenopausal women receiving the aromatase inhibitor anastrozole for hormone receptor-positive early breast cancer (EBC). Lumbar spine and hip T-scores calculated from baseline BMD were used to stratify patients according to risk of fracture as follows: higher risk (HR) = T-score < -2.0 and/or history of fracture; moderate risk (MR) = T-score < -1.0 but $>/= -2.0$; and lower risk (LR) = T-score $>/= -1.0$. HR patients received oral open-label anastrozole (1 mg/day) plus risedronate (35 mg/week). MR patients were randomised in a double-blind manner to receive anastrozole plus either risedronate or placebo. LR patients received open-label anastrozole only. All patients received calcium and vitamin D supplements. Bone turnover was assessed at baseline, 3 and 6 months by measuring serum procollagen type I amino terminal peptide (PINP) and bone alkaline phosphatase (bone ALP) as markers of bone formation, and serum C terminal crosslinking telopeptide of type I collagen (sCTX) as a marker of bone resorption. Here we present the planned, blinded interim analysis of bone markers from baseline to 6 months, with p-values based on log-transformed mean changes. Overall, 234 patients received treatment. Changes in bone turnover markers from baseline to 6 months are shown in Table 1. LR patients ($n = 42$) experienced a significant increase in sCTX ($p = 0.05$), but no other significant changes. HR patients experienced significant decreases in all bone markers ($p < 0.0001$; $n = 38$). In the MR group ($n = 144$), all markers were significantly reduced in patients receiving anastrozole plus risedronate compared with those receiving anastrozole plus placebo ($p < 0.0001$; $n = 77$ in each group). We have shown for the first time that risedronate reduces bone turnover in anastrozole-treated postmenopausal women with hormone receptor-positive EBC and a pre-existing moderate or higher risk of fracture. SABRE was funded by AstraZeneca and Aventis.

Table: Median change from baseline to 6 months (%)

	N	PINP	bone ALP	sCTX
LR	42	−5.1	−1.3	9.0
MRplacebo	77	−15.3	−5.9	−2.5
MRrisedronate	77	−54.1	−26.4	−50.5
HR	38	−44.0	−22.6	−40.2

Conflict of Interest: G Clack, AstraZeneca, Employee, Shareholder R Eastell, AstraZeneca, Grant Research Support, Consultant R Hannon, AstraZeneca, Other (post supported by funding from AstraZeneca) J Mackey, AstraZeneca, Consultant, Speaking Honoraria J Mackey, Sanofi-Aventis, Consultant, Speaking Honoraria C Van Poznak, Amgen, Consultant C Van Poznak, Berlex, Consultant C Van Poznak, Novartis, Consultant C Van Poznak, Roche, Consultant

P328-M

SECONDARY PREVENTION OF OSTEOPOROTIC FRACTURES – AN INTERDISCIPLINARY PILOT PROJECT IN HANUSCH-HOSPITAL, VIENNA (PARTIAL RESULTS)

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Introduction: Every year about 16.500 people sustain a hip fracture in Austria. With a fracture rate of 19.7 hip fractures for 10.000 citizens over 65 years of age, Austria is in the fore of the European countries (EU average is 13.5 fractures). Patients with fractures are in high risk of further osteoporotic fractures and yet they do not receive appropriate attention and therapy. Methodology: The pilot project's aim is the development and test of interdisciplinary cooperation of diagnosis and therapy planning between the accident surgery ward, the osteoporosis section of the internal ward, and GPs. The patients are registered in three groups: Group 1: women over 50 years of age with forearm fractures treated on an outpatients basis; Group 2: hip fractures of stationary women and men over the age of 50; Group 3: preoperative osteoporosis information for planned joint renewal operations. All registered patients are controlled again after 3, 6, and 12 months. Two osteoporosis specialist nurses take a key role in the handling of the project, being responsible for registration of fracture patients, information about the project, obtaining patient's permission, osteoporosis- and accident anamnesis, screening of the nutritious and cognitive status of stationary patients. Results: (from the hip fracture group 12/2005–06/2006): 221 patients (76 % women, 24 % men). The average age of female fracture patients is 83.3 years (54–100 years, SD ± 8,99 years); that of average male fracture patients is 76.3 years (43–97 years; SD ± 10,39 years). Average duration of stay: 17.5 days (8–41 days; SD± 5,4) irrelevant of age and sex; it depends primarily on the post operative process and comorbidities. Mortality rate for the first 6 months after the fracture (7.7 %; 13 women, average age: 87.9 years; 4 men, average age: 88.6 years). Indoor mortality rate: 1.4 % (n = 3). Mobility before fracture: 37.6 % without support, 23 % walking stick, 18 % crutches, walking frame, wheel chair, no data for 20%. Anamnesis with patient not possible: 36.7 % (dementia, language problems) Osteoporosis anamnestically known: 20.3 % (88.9 % basic therapy with calcium/vitamin D3, thereof 42.3 % therapy with a bisphosphonate)Discussion: The high age of fracture patients and the high rate of cognitive deficiencies begs the question of a safe bone-specific therapy. The usage of oral bisphosphonates is not sufficiently guaranteed (compliance data is currently being collected).

Conflict of Interest: None declared

P329-T

SECONDARY PREVENTION OF OSTEOPOROTIC FRACTURES: PROBLEMS IN OSTEOPOROSIS TREATMENT IN REAL LIFE. (PARTIAL RESULTS)

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Introduction: A large evidence base now exists for treatment interventions that will reduce fracture risk. However, the key area of practice now is how to get this evidence base into clinical practice. Fracture care often represents the first opportunity for clinical management of osteoporosis; however, many patients do not receive any evaluation after a fracture.

Methodology: Within one year all inpatients of an accident surgery with hip fracture sustained spontaneously or from a fall no greater than standing height ward and all female outpatients over 50 years with forearm fractures were identified and diagnosed/treated possible osteoporosis.

Results: (from the hip fracture group 12/2005–06/2006): 221 patients (76 % women, 24 % men). The average age of female fracture patients is 83.3 years (54–100 years, SD ± 8,99 years); that of average male fracture patients is 76.3 years (43–97 years; SD ± 10,39 years). Anamnesis with patient not possible: 36.7 % (cognitive impairment, dementia)

Osteoporosis anamnestically known: 20.3 % (women: 22.6%; men: 13.2%). 38.6% (women: 41.5%; men: 30.4%) already sustained an osteoporotic fracture without any therapeutic consequences.

Many other risk factors are investigable (immobility, medicamentous treatment with cortisone, ...).

25-OH-vitamin D lower than 50nmol/l: 78.7 %

Discussion: Effective treatments are available to reduce fracture risk in patients with osteoporosis. Prioritisation of assessment and treatment for those patients at highest risk of fracture will ensure the optimal utilisation of healthcare resources. The provision of vitamin D is insufficient.

Conflict of Interest: None declared

P330-S

THE EFFECT OF AMYLIN(1–8) ON BONE IN OVARIECTOMIZED RATS

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Background: The 37 amino acid peptide amylin is known to be involved in bone development via actions on osteoclasts and osteoblasts. The aminoterminal fragment amylin(1–8) has been shown to increase bone volume in male mice, when administered systemically for four weeks. However reports on any effects in disease models of osteoporosis are not available.

Aim: To evaluate the potential of small amylin mimetics in osteoporosis treatment, we investigated the bone anabolic effect of amylin(1–8) in ovariectomized rats, a well established model of postmenopausal osteoporosis.

Method: 120 six month old Sprague-Dawley female rats were randomized to eight groups (n = 15). Six groups underwent surgical ovariectomy and two groups were sham-operated. Ten weeks after surgery one sham and one OVX group were sacrificed serving as baseline. Three groups were once daily injected s.c. with amylin(1–8) in three different doses (5, 20 and 100 nmol/kg/day). Furthermore one group received vehicle and one group received PTH(1–34) (20 nmol/kg/day) serving as a positive control. After eight weeks of administration all groups were sacrificed.

Results: Bone mineral density (BMD) of femoral neck, total femur and thoracic vertebrae was significantly lower in vehicle-treated OVX compared to sham at both time points. No effect of amylin(1–8) administration was seen compared to vehicle-treated OVX. PTH(1–34) increased BMD significantly compared to both vehicle-treated OVX and sham. The vehicle-treated OVX group, the sham group and the group receiving the highest dose of amylin(1–8) were further assessed by histomorphometry (n = 5). Histomorphometric analysis of the right tibia showed no significant effect of treatment with amylin(1–8). Bone volume was significantly lower in OVX compared to sham confirming the BMD measurements.

Conclusion: We did not find any significant effects of Amylin(1–8) on BMD or histomorphometric indices in ovariectomized rats. Thus amylin(1–8) does not seem to be a viable principle in the treatment of postmenopausal osteoporosis.
Conflict of Interest: M. Ellegaard, Zealand Pharma, Grant Research Support M. Stahlhut, Zealand Pharma, Grant Research Support C. Thorkildsen, Zealand Pharma, Grant Research Support J. S. Petersen, Zealand Pharma, Grant Research Support, Shareholder

P331-M

CO-PRESCRIPTION OF CALCIUM &/OR VITAMIN D WITH ONCE WEEKLY BISPHOSPHONATE THERAPY: FINDINGS FROM A FRENCH LONGITUDINAL PATIENT DATABASE

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Objectives: Calcium and Vitamin D (Vit-D) supplementation are recommended as co-medication to antiresorptive therapy. The objectives of this study were to analyze general practitioners (GPs) co-prescription habits of Calcium and/or Vit-D in patients taking once a week bisphosphonate therapy.

Methods: The French Thales longitudinal database, comprising patient records from a representative sample of 1,200 GPs was used. These patient data, collected daily, were extrapolated to represent 56,000 GPs Data collected during 1st June to 31st August 2004 from patients taking either weekly alendronate 70mg or risedronate 35mg formed the basis of the analysis. Summary statistics of Calcium and/or Vit-D co-medication with once a week bisphosphonate therapy were performed.

Results: Of the 369,828 patients included in the analysis, 44% (162,171 patients) received a prescription for either Calcium and/or Vit-D in addition to their once a week bisphosphonate therapy. The majority (87%) of the patients receiving such co-medication took Calcium and Vit-D in addition to their bisphosphonate.

Conclusions: Over half of the patients (56%) treated with once a week bisphosphonate therapy did not receive any supplementation with either Calcium or Vit-D. New approaches towards helping osteoporotic patients adhere to recommended guidelines of taking Calcium and Vit-D supplementation in addition to their bisphosphonate are required.
Conflict of Interest: P. Fardellone: Procter & Gamble, Consultant B. Mann: Procter & Gamble, employee

P332-T

A NEW COMBINATION PACKAGING FOR OSTEOPOROSIS TREATMENT; PATIENT PREFERENCE AND EXPECTED ADHERENCE TO THE THERAPY

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Objective: Calcium and Vitamin D (Vit D) supplementation are recommended as comedications to antiresorptive therapy. Combination packaging may facilitate the correct administration of bisphosphonates (BP) plus Calcium and Vit D supplementation. The aim of this study was to evaluate patient perception of compliance, convenience and completeness of a new packaging of risedronate 35 mg plus Calcium and Vit D supplementation.

Method: A combined packaging was developed containing four weekly boxes with each weekly box containing a blister pack with one 35 mg risedronate tablet, 6 sachets of calcium 1000 mg and Vit D 880 UI and a patient information leaflet. A quantitative patient research study was conducted with 200 women, in three cities in France during October 2006. Participants were 55 years or older (mean age 67 years; sd = 9) and divided into two groups: Group 1 (N = 100) had a diagnosis of osteoporosis (OP) and were BP users either with or without supplementation; Group 2 (N = 100) was included in the study regardless of OP diagnosis and were receiving no treatment for OP. All participants gave consent to participate to face-to-face interviews using a standard questionnaire.

Results: The study showed that 60% of patients found the product practical to use. 63% and 67% found it easy to use and easy to remember when compared to separate packaging. Patients believed that the combined packaging would help them to take their BP regularly (66%) and correctly (67%) and to take Calcium and Vit D supplementation more regularly and correctly (68%) than the separate packaging. 70% of patients believed that the combination packaging would help them not to forget to take Calcium and Vit D supplementation. When exposed to the combination packaging only, 80% of patients considered the combination packaging to be a complete OP therapy.

Conclusion: Patients found the combined packaging more practical and more convenient to use. They believed it would help them taking their prescribed OP therapy correctly and more regularly. This study provides evidence that the new combined packaging of risedronate 35 mg plus Calcium and Vit D provides patients with a tool to better adhere to the recommended OP therapy and to optimize the effectiveness of their treatment.
Conflict of Interest: P. Fardellone: Procter & Gamble, consultant B. Mann: Procter & Gamble, employee

P333-S

A CONVENIENT NEW APPROACH TO SIMPLIFY OSTEOPOROSIS TREATMENT REGIMEN FOR RISEDRONATE PLUS CALCIUM AND VITAMIN D

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Objectives: Calcium and Vitamin D (Vit-D) supplementation are recommended as co-medication to antiresorptive therapy. Convenient combination packaging can facilitate appropriate intake of Bisphosphonates (BP) plus calcium and Vit-D supplementation. The objectives of this study were to evaluate patient understanding of dosing instructions and patient preference for two types of packaging of risedronate 35 mg, calcium and Vit-D supplementation.

Methods: A combined package was developed containing four weekly boxes with each weekly box containing a blister pack with one 35 mg risedronate tablet, 6 sachets of calcium 1000 mg and Vit-D 880 IU and a patient information leaflet. A quantitative patient research study was conducted on women aged 55 years and older in five cities within France. Participants were BP users with diagnosed osteoporosis and Non-BP users regardless of osteoporosis diagnosis. All participants gave consent to participate in face-to-face interviews, using a standard questionnaire. The participants were presented the combined and separate packages once. The between-packaging comparison of participant understanding of the dosing instructions was analyzed using generalized estimating equation methodology and patient preference was analyzed using a cumulative logit model.

Results: The study included 200 women. The combined packaging resulted in a significantly higher proportion of questions being answered correctly (80.3%) compared to the separate pack (70.7%) ($p = 0.0004$). 72% of participants preferred the combined pack ($p < 0.0001$). The main reasons for preference were: ''practicality of a weekly box'' (51.7%), ''less likely to forget/to get wrong'' (51.7%), ''having 2 products in the same box'' (33.6%) and ''clarity of the schedule'' (30.8%).

Conclusion: This patient study provides evidence that the combined pack of risedronate plus Calcium plus Vit-D is preferred over separate packs and improves overall understanding of dosing instructions. Combined packaging offers a convenient combination osteoporosis therapy, potentially improving treatment compliance and providing the maximum efficacy of an osteoporosis therapy.
Conflict of Interest: P. Fardellone: Procter & Gamble, consultant A. Varbanov: Procter & Gamble, employee

P334-M

PREDICTING VERTEBRAL FRACTURES: SMALL VERTEBRAL DEFORMITIES INCREASE THE RISK OF SUBSEQUENT VERTEBRAL FRACTURE AT ONE YEAR – EARLY AND EFFECTIVE FRACTURE RISK REDUCTION WITH RISEDRONATE

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In a previous study1, 70% of the women with symptomatic early vertebral deformity (with 5–10% vertebral height reduction) progressed into new vertebral fracture within 1 year. In this research, we evaluated the risk of developing a new vertebral fracture at Month 12 for vertebrae with various degrees of height reduction (< 10%, 10–20%) at baseline utilizing data from two large phase III randomized, double-blind, placebo-controlled clinical trials. The treatment benefit with risedronate was also examined.

Postmenopausal women from the risedronate VERT-MN and VERT-NA trials with evaluable radiographs at baseline and Month 12 were included in the analysis. Baseline degree of vertebral height reduction (< 10%, 10–20%) was defined by the maximum percent reduction in anterior, midpoint or posterior height for each vertebrae, separately. Prevalent fracture status and new vertebral fractures were assessed using quantitative and semi-quantitative methods. Discrepancies between the two methods were adjudicated by an expert radiologist. Vertebrae with greater than a 20% height reduction or prevalent fracture at baseline were excluded from the analysis. The relative odds for a new fracture at month 12 in vertebrae with 10–20% vs < 10% reduction at baseline was calculated using the Generalized Estimating Equations (GEE) method. The treatment benefit of risedronate was examined using the same model.

1912 postmenopausal women (953 placebo, 959 risedronate 5mg/d) had at least one vertebrae at baseline with a height reduction less than 20%. Average age was 69 years and mean lumbar spine T-score was -2.5 SD. All patients received 1000 mg calcium daily and if baseline levels were low, 500 IU of vitamin D. For the patients in the placebo group, 1.4% of vertebrae had a new fracture at Month 12. Vertebrae with 10–20% height reduction at baseline were 54% more prone to fracture ($p = 0.03$) relative to vertebrae with < 10% reduction. The risk of a new vertebral fracture did not differ significantly ($p = 0.79$) between vertebrae with different degrees of height reduction for risedronate patients. Irrespective of the degree of height reduction at baseline, risedronate demonstrated a 63% to 73% vertebral fracture risk reduction at month 12 ($p < 0.02$).

One year of treatment with risedronate appears to reduce vertebral fracture risk irrespective of the degree of baseline vertebral height reduction. (1 Ceccarelli M, et al Osteoporosis Int 2005)

Conflict of Interest: D. Felsenberg: Procter & Gamble Consultant C. Recknor: Procter & Gamble Consultant X. Zhou: Procter & Gamble employee

P335-T

SEQUENTIAL TREATMENT WITH FULL-LENGTH PARATHYROID PTH(1–84) OF POSTMENOPAUSAL WOMEN WITH PRIMARY OSTEOPOROSIS

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Background: PTH therapy decreases the risk of vertebral fractures and increases Bone Mineral Density (BMD). When PTH(1–84) is followed by antiresorptive treatment the increase in BMD is maintained. Whether the osteoporotic women will benefit from another course of PTH (1–84) after the initial course and the follow-up treatment with a bisphosphonate remains to be proven.

Design: The PEAK study (Preotact after a brEAK) 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 initially treated with 12 months of full length parathyroid hormone (PTH 1–84) followed by 12 months of treatment with risedronate followed by either 12 months treatment with PTH (1–84) or risedronate.

The 3 consecutive treatment phases (the subjects will be randomised after period II):

Trial Period I: 12 months PTH (1–84)

Trial Period II: 12 months risedronate

Trial Period III: 12 months either risedronate or PTH(1–84)

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 included in the trial. At the end of the trial bone biopsies will be obtained.

Results to be achieved: After the first year the increase in lumbar spine BMD after PTH(1–84) treatment in bisphosphonate naïve patients will be compared to patients pre-treated with bisphosphonates or raloxifen. Further data on the changes in serum and urinary calcium in postmenopausal women with osteoporosis will be gathered. After two years of treatment the results from PaTH trial (PTH(1–84) followed by alendronate) can be compared with the results from PEAK using risedronate in place of alendronate. At the end of the trial results will show whether a second course of PTH (1–84) increases lumbar spine BMD more than continued treatment with risedronate.

Conflict of Interest: D. Felsenberg, Amgen, Aventis, Chugai, Glaxo, Lilly, MSD, Novartis, Nycomed, Organon, P&G, Pfizer, Roche, Schering, Servier, Teva, Wolff, Wyeth, Grant/Research Support Amgen, P&G, Glaxo, Lilly, MSD, Novartis, Nycomed, P&G, Roche, Servier, Consultant D. Hosking, MSD, Novartis, Nycomed, Pfeizer, Roche/GSK, Consultant.N, MSD, Novartis, Nycomd, Roche/GSK; lecture fee. Eli Lilly, MSD, Novartis, Nycomed, Grant support. P. Delmas, Nycomed, Consultant R. Rizzoli, Nycomed, Consultant M. L. Brandi, Nycomed, Consultant M. Diaz-Curiel, Nycomed, Consultant L. H. Hyldstrup, Employed by Nycomed

P336-S

IN VIVO ASSESSMENT OF TRABECULAR AND CORTICAL BONE MICROARCHITECTURE BY HIGH-RESOLUTION COMPUTED TOMOGRAPHY SPECIFIES THE NATURE OF BMD CHANGES FROM PEAK BONE MASS TO THE MENOPAUSE

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Many fractures occur among women with mildly impaired BMD. This argues for alterations in bone structure, which remain poorly understood. We evaluated microarchitecture at distal radius and tibia by high-resolution computed tomography (XtremeCT, Scanco) in 125 young females (YF, mean age ± SD, 20.4 ± 0.6 yrs) and 105 of their mothers, including 54 pre- (PreM, 48.1 ± 3.2 yrs) and 51 post-menopausal (PostM, 53.3 ± 3.2 yrs; 5.0 ± 3.4 YSM) women. BMD was evaluated by DXA at radius (Rad.) and hip. To avoid biases due to shared genetic effects, comparisons were made between PreM and PostM and the respectively unrelated YF.

BMD decreased 9% at femur neck ($p < 0.001$) and 5% at total hip ($p = 0.04$) in PreM vs YF, but Rad.BMD did not. In PostM, hip BMD was further decreased and ultradistal Rad.BMD was 4–5% lower compared to both PreM ($p = 0.01$) and YF ($p = 0.04$). Explaining the absence of early Rad.BMD loss, Rad. cortical density (D.comp) increased between YF and PreM (+7%, $p < 0.0001$), then partially decreased in PostM ($p = 0.01$ vs PreM). Rad. cortical thickness (C.Th), but not cross-sectional area (CSA), also tended to increase between YF and preM (+7%), then decreased in PostM (-8%, $p = 0.03$ vs PreM). Moreover, Rad. trabecular microarchitecture in PreM was not significantly different from YF nor PostM. However, trabecular density (BV/TV) and number (TbN) were lower, and spacing (TbSp) higher, in PostM compared to YF ($p = 0.02$–< 0.001), suggesting a more progressive loss of trabecular structure. In contrast, paralleling the early hip BMD changes, tibia trabecular BV/TV and thickness (TbTh) were significantly lower in PreM than YF (−12% and −8%, respectively, $p = 0.01$), whereas cortical structure and CSA were unchanged. After menopause, both cortical and trabecular microarchitecture declined (D.comp, −5%, C.Th, −12%, both $p < 0.001$, TbN, −8%, $p = 0.02$, BV/TV, −8%, $p = 0.07$, TbSp, +10%, $p = 0.03$, vs preM), while CSA increased compared to YF (+9%, $p < 0.01$). In general, BMD measurements were correlated with parameters of cortical and trabecular microarchitecture at both radius and tibia ($R2 <$ or $= 0.53$).

In conclusions, before the menopause, BMD declines at hip, reflecting loss of trabecular microarchitecture at weight bearing sites, whereas BMD is stable at radius, where some cortical gain occurs. After the menopause, BMD and structural bone parameters decrease at both sites. Alterations in cortical and trabecular microarchitecture at radius in early menopause may contribute to Colles' fractures.

Conflict of Interest: S. Ferrari, MSD, Speakers Bureau and consultant S. Ferrari, AMGEN, Research Grant and consultant; S. Ferrari, P&G, consultant S. Ferrari, Eli Lilly, consultant

P337-M

EFFECT OF PROPRANOLOL ON THE GLUCOCORTICOID-INDUCED BONE CHANGES IN YOUNG RATS

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Recent investigations revealed the role of the sympathetic nervous system in the regulation of bone growth and remodeling. Propranolol, a nonselective beta-

adrenergic receptor antagonist, was reported to counteract bone loss induced by estrogen deficiency or mechanical unloading in rodents.

Glucocorticoid therapy is the most common cause of secondary osteoporosis. The aim of the present study was to investigate the effect of propranolol on development of changes induced by administration of prednisolone in the skeletal system of young rats.

The experiments were carried out on 6-week-old male Wistar rats, divided into 4 groups (n = 8): I – control rats, II – rats receiving prednisolone 21-hemisuccinate sodium salt (7 mg/kg s.c.), III – rats receiving propranolol hydrochloride (10 mg/kg p.o.), IV – rats receiving both prednisolone and propranolol at the above doses. The animals were sacrificed after 28 days of daily drug administration. 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), as well as mechanical properties of the femur were examined.

Although in young rats prednisolone did not significantly affect bone growth and mineralization, it impaired histomorphometric parameters (group II). In comparison with control rats, significant decreases in the width of osteoid, trabeculae and epiphyseal cartilage were observed.

Propranolol improved bone mineralization and slightly increased width of trabeculae in rats of group III. Propranolol counteracted the histomorphometric changes induced by prednisolone (rats of group IV). However, long bones of rats receiving both drugs were smaller, lighter and weaker than those of the other groups.

Concluding, propranolol seemed to advantageously affect the cancellous bone of prednisolone-treated rats, but at the same time to augment some unfavourable changes caused by prednisolone in the cortical bone.

Conflict of Interest: None declared

P338-T

ORAL IBANDRONATE IMPROVES HIP STRUCTURAL PARAMETERS IN THE BONE STUDY

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Daily and intermittent oral ibandronate increased spine and femur bone mineral density (BMD) and reduced vertebral fracture incidence (3-year fracture risk reduction: 62% and 50%, respectively) in the BONE study.[1] However, bone strength also depends on bone geometry; therefore Hip Structural Analysis (HSA) was used to retrospectively analyse the femur DXA scans from BONE participants. In the BONE study, women with postmenopausal osteoporosis (PMO) were randomised to daily oral ibandronate (2.5mg), intermittent oral ibandronate (20mg every other day for 12 days every 3 months) or placebo. Daily calcium and vitamin D supplements were provided. DXA scans at baseline and study end were processed at Synarc with HSA software developed at Johns Hopkins University. HSA results from women recruited at European sites will be presented and include: BMD; cross-sectional area (CSA), which confers axial load resistance; section modulus, which indicates ability to withstand bending loads; and buckling ratio. These parameters were assessed at narrow neck, intertrochanteric and shaft regions. ANCOVA was used to compare treatment groups with age and baseline values as covariates. This report includes only Hologic scans since, before unblinding, a technical error was found in applying HSA measures in approximately 10% of measurements obtained with Lunar DPX scanners. Data will be presented from a total of 833 women with paired measurements. There were no statistically significant differences between treatment groups in age, height or weight at baseline. Average follow up was 2.98 years (range: 1.42–3.26). Hip structural parameters generally remained stable or deteriorated in the placebo group but improved significantly after ibandronate treatment ($p < 0.05$) for all parameters assessed. Thus, compared with placebo, treatment with oral ibandronate over 3 years appears to improve resistance to axial and bending loads at the intertrochanter and shaft, and resistance to buckling failure in all three regions. These findings support those obtained for BMD and vertebral fracture incidence in the BONE study.

1. Chesnut CH, et al, J Bone Miner Res 2004;19: 1241–9.

Conflict of Interest: T Fuerst, F. Hoffmann-La Roche Ltd, GlaxoSmithKline grant/research support TJ Beck, F. Hoffmann-La Roche Ltd, GlaxoSmithKline, Amgen, Merck and NPS consultancy fees HK Genant, Synarc, GSK, Roche, Lilly, Novartis, Merck, Servier, Amgen, Wyeth, Aventis, P&G, Hologic, Lunar grant/ research support, consultancy fees

P339-S

GAINS IN BMD WITH ONCE WEEKLY RISEDRONATE. TWO YEARS RESULTS

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Introduction: Risedronate has been used in the treatment of postmenopausal osteoporotic women with success; it provided an effective protection against a decrease in bone mineral density (BMD). Weekly administration of oral bisphosphonates is a strong patient preference.

Objectives: To examine the effects of Risedronate once weekly on vertebral and total hip BMD change in postmenopausal osteoporotic women and upper gastrointestinal tolerability over 24 months.

Material and methods: Twenty seven postmenopausal women aged 44–79, mean 63.9 years with low bone mass BMD total hip and/or L1–L4 lumbar spine T-score between −1.0 and −3.5 without contraindication to be treated with Risedronate. BMD was measured by Dual-Energy X-ray Absorptiometry (DXA) two times: before treatment and after 2 years. Risedronate 35 mg once weekly was given to all patients 24 months. Tolerability was assessed by adverse experience reporting.

Results: After 2 years treatment, BMD at the lumbar spine increased with 2.5%. Gain in BMD at lumbar spine was from 0.841 ± 0.014 g/cm^2 to 0.884 ± 0.014 g/cm^2. In total hip the gain in BMD was from 0.839 ± 0.068 g/cm^2 to 0.848 ± 0.068 g/cm^2, a difference of 1.1%. No significant incidence of upper gastrointestinal adverse events.

Conclusion: Risedronate 35 mg once weekly had a good gastrointestinal tolerability. A significant improvement was found both lumbar spine and total hip BMD after two years of treatment. Risedronate 35 mg once weekly increases BMD at the lumbar spine and proximal femur in postmenopausal osteoporosis.

Conflict of Interest: None declared

P340-M

CIRCULATING OSTEOCLAST PRECURSOR CELL POPULATIONS AND THEIR CYTOKINE RECEPTORS ARE NOT TARGETS FOR ALENDRONATE ACTION IN POSTMENOPAUSAL OSTEOPOROSIS

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Circulating osteoclast precursor cells reside in the monocyte (CD14+) fraction of peripheral blood. Monocytes express key surface proteins including: the macrophage colony stimulating factor receptor (M-CSFR); the adhesion molecule receptor (CD11b) and the type II receptor for tumour necrosis factor (TNFRII), which are involved in osteoclast differentiation. The effect of the bisphosphonate, alendronate, on osteoclast precursors *in vivo* is currently unknown. The aim of the study was to determine the effect of alendronate therapy on osteoclast precursors in a) 15 healthy postmenopausal women, ages 55 to 70 (mean 61) b) 15 untreated osteoporotic women, ages 66 to 83 (mean 71) c) 15 osteoporotic women taking alendronate therapy, ages 55 to 81 (mean 68). Peripheral blood was drawn and the monocyte population was characterised using fluorescent labelled antihuman antibodies, CD14-FITC Ab, (Diaclone), M-CSFR-PE Ab (R & D Systems), αCD11b-APC Ab and αTNFRII- PE Ab (BD Pharmingen). Crosslinked C-telopeptides of type I collagen (CTX) were measured by ELISA (Nordic Bioscience Diagnostics). The mean fluorescence intensity (MFI) compared for each receptor and the percentage of receptor positive CD14+ cells was determined by flow cytometry (Becton Dickinson FACScalibur^{TM}). The median (range) for each parameter is shown in the table below. There were no significant differences between groups in the expression of M-CSFR, CD11b and TNFRII by CD14+ cells or in the percentage of cells co-expressing CD14+ and each of the receptors. Serum CTX was significantly lower (by 90%), in women treated with alendronate, compared to healthy controls or untreated osteoporotic women. We conclude that osteoclast precursors and the osteoclastogenic cytokine receptors, M-CSFR, CD11b and TNFRII are not targets for the action of alendronate therapy.

Table:

	Healthy Control Group	Untreated Osteoporotic Group	Alendronate Treated Group
MFI M-CSFR	23.4 (4.4–38.9)	23.2 (0.0–49.5)	22.2 (5.89–84.3)
MFI CD11b	268.8 (21.2–437.2)	258.8(111.2–510.6)	286.8 (4.4–505.3)
MFI TNFRII	16.2 (12.6–38.9)	17.4 (4.1–95.4)	31.7 (2.7–58.2)
% CD14+/ M-CSFR+ Cells	6.6 (5.1–12.3)	3.1 (1.7–12.4)	5.5 (1.4–12.2)
% CD14+/ Cd11b+ Cells	6.8 (3.2–11.1)	5.2 (0.0–16.2)	5.0 (0.0–11.6)
% CD14+/ TNFRII+ Cells	8.4 (5.3–12.6)	4.7 (0.9–12.5)	2.9 (0.9–19.2)
CTX (ng/ml)	0.78 (0.37–0.83)	0.59 (0.15–1.20)	0.06 (0.00–0.21)*

*$p < 0.01$ by ANOVA

Conflict of Interest: None declared

P341-T

INSUFFICIENCY SUBTROCHANTERIC FRACTURES IN PATIENTS ON ALENDRONATE THERAPY: A CAUTION

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Introduction: Subtrochanteric fractures of the femur are unusual injuries and usually occur after high-energy trauma or in very osteoporotic patients. Lately, we have been seeing in our departments a series of subtrochanteric fractures occurring in postmenopausal women who had sustained low-energy trauma. These patients were all on alendronate therapy for 2 to 5 years. Alendronate is an anti-resorptive agent that has gained widespread use in the treatment of osteoporosis. Whilst numerous studies have shown that it is a relatively safe drug for long-term consumption, some have expressed concerns that prolonged effect of this drug may lead to deleterious effect on bone strength. This is due to its powerful suppression of the bone modelling. By highlighting this problem of subtrochanteric fractures, we hope to draw attention to a possible association between long-term bone modelling suppression and increased fragility of the bone due to alendronate therapy and also to define the characteristics of the susceptible group of patients.

Method: Surgical log books of all surgeons over a 10-month period in two orthopedic departments were examined to identify cases of low-energy subtrochanteric fractures. The injury mechanisms, fracture characteristics, bone densities and drug histories of these patients were examined.

Results: 13 cases of subtrochanteric fractures were studied. Of these 9 were on long-term alendronate and 4 were not. Patients treated with alendronate were younger (average 67 years old), more active, and sustained fractures in the metaphyseal–diaphyseal junction with very minimal or no trauma. Five patients had prodromal pain in the affected hip in the months preceding the fall. Three patients demonstrated a stress reaction in the cortex in the contralateral femur.

Conclusion: This study suggests that prolonged suppression of bone remodeling with alendronate may cause a new form of insufficiency fracture of the femur. We believe this finding is an important one and spells the need for caution in the long-term use of alendronate in the treatment of osteoporosis.

Conflict of Interest: None declared

P342-S

STRUCTURAL PARAMETERS OF DISTAL RADIUS AND IPSILATERAL ULNA SHOW GOOD CORRELATION

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Introduction: Structural parameters, e, g, trabecular thickness and separation at the distal radius have shown high predictive value for load to failure in mechanical testing. Other mechanical tests have shown good correlation to implant failure during cyclic loading. In conclusion these parameters could be used to predict implant failure after distal radius fracture. However, in a fracture situation direct measurement of the location is no longer possible due to artefacts of the different fragments. In this situation parameters of intact ipsilateral ulna might be used to replace values of the fractured radius. The aim of our study was to determine the correlation of the structural parameters of cancellous and trabecular bone between radius and ipsilateral ulna.

Methods: 164 human cadaveric forearms were measured with high-resolution pQCT. The measurements were performed using a new generation of in-vivo 3D-pQCT (Scanco Medical, m. Twenty percentμSwitzerland) providing an isotropic nominal resolution of 93 of the forearm (radius and ulna) were measured und divided into five subsequent regions of interest. Direct 3D morphometry was used to compute bone volume (BV), bone volume density (BV/TV), trabecular thickness (Tb.Th), number (Tb.N), separation (Tb.Sp) and cortical thickness (C.Th) for both bones separately. Paired correlation between each radial and corresponding ulnar parameter was calculated after testing for normal distribution. The correlations were stratified by the five regions from ultradistal to proximal.

Results: All radial parameters showed significant correlation to the corresponding ulnar parameter at each region of interest (all correlation $p < 0.001$). Whereas parameters of the cortex showed best correlation at the most proximal region (BVcortex $r = 0.94$, cortical thickness $r = 0.84$), trabecular parameters correlated best in the most distal region (BVcancellous $r = 0.89$, Tb.N $r = 0.79$). The trabecular microstructure was in all regions significantly stronger at the ulnar side, e, g, Tb.Thulna = 0, 25 vs. Tb.Thradius = 0.23, resulting in higher bone volume density (BV/TVulna = 23.9% vs. BV/TVradius = 15.5%).

Conclusion: The ipsilateral ulna might be utilized to estimate microstructural parameters at the distal radius, especially in case of distal radius fracture. Further prospective research could help to find out whether radius and ulna underlie the same adaptation processes to loading and remodeling.

Conflict of Interest: None declared

P343-M

EFFECT OF FULL-LENGTH PARATHYROID HORMONE PTH(1–84) IN REDUCING NEW VERTEBRAL FRACTURES IN OSTEOPOROSIS PATIENTS WITH AND WITHOUT A MATERNAL HISTORY OF OSTEOPOROSIS

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Background: Osteoporosis patients at high risk of fracture have benefited from the arrival of the potent anabolic human recombinant parathyroid hormones. Information about previous fragility fractures and information about low BMD help to identify patients at high risk for fracture. In addition other clinical risk factors exist. Factors readily accessible by primary care physicians are important as they may help general practitioners to identify patients at risk of fragility fractures. One of these clinical risk factors is family history of osteoporosis. It is not known whether patients identified by having a maternal family history of osteoporotic fracture will respond to therapy.

Methods: In this exploratory analysis, new vertebral fracture incidence was studies in subjects without a prevalent vertebral fracture as a function of maternal family history of osteoporosis in TOP (Treatment of Osteoporosis with PTH[1–84]) clinical study, designed to determine the effectiveness of PTH(1–84) in preventing vertebral fractures in osteoporotic woman during 18 months of treatment. A total of 2532 subjects were enrolled: placebo ($n = 1246$); 100 microgram per day PTH(1–84) ($n = 1286$). Subjects with BMD T-score of below or equal to −2.5 at the lumbar spine, femoral neck, or total hip or 1–4 prevalent vertebral fractures were included. This analysis included only those subjects without a prevalent vertebral fracture.

Results: 727 subjects had a maternal family history of osteoporosis and 1329 did not have a maternal family history of osteoporosis. In subjects with a maternal family history of osteoporosis, PTH reduced the risk of new vertebral fractures by 74% (95%CI: −7% to −93%) from 11 to 3 incident vertebral fractures. In subjects without a history of maternal family osteoporosis, PTH(1–84) reduced the risk of new vertebral fractures by 61% (95% CI: +22% to −88%) from 10 to 4 incident vertebral fractures.

Conclusion. This analysis suggests that it may be possible to identify at risk patients based on low BMD and maternal risk factor who benefit from treatment with PTH(1–84).

Conflict of Interest: Dr. Carlos Gómez Alonso, Nycomed, consultation Dr. Minerva Rodríguez-García, Nycomed, consultation Ms. Hanna Greisen, International Medical Affairs, Nycomed Denmark

P344-T

QUANTITATIVE ULTRASOUND IN THE MONITORING THE EFFECTS ON BONE OF TERIPARATIDE AND ANTIRESORPTIVE DRUGS

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There is a growing conviction that BMD by DXA. Moreover, is not able to register all the positive changes induced . In the last decade there has been a]hPTH (1–34)[in bone by teriparatide growing interest in the assessment of bone status using quantitative ultrasound (QUS) techniques.

This study aimed to evaluate whether teriparatide influences QUS parameters and to compare the changes of QUS with that of BMD. Sixty postmenopausal women (aged 71.1 ± 6.8 yrs) with established osteoporosis and under treatment with antiresorptive drugs, after a run-in phase during which they received only calcium (1000 mg) and vitamin D (400 IU), were randomly assigned to a 18-month treatment with either once daily 20 μg teriparatide ($n = 30$) or to continue the previous antiresorptive treatment ($n = 30$). At the end of the 18 month study period the teriparatide patients received bisphosphonates.

At baseline and at 2–6 months intervals we measured QUS at calcaneus, by Achilles-GE, Lunar (speed of sound: SoS, broadband ultrasound attenuation: BUA and Stiffness: S) and at phalanxes, by Bone Profiler-IGEA (amplitude dependent speed of sound: AD-SoS, bone transmission time: BTT, fast wave amplitude: FWA). BMD at right hand (BMD-H), at lumbar spine and at femur was also assessed. (Prodigy, GE-Lunar).

In the teriparatide group AD-SoS decreased at month 6 (-1.7%, $p < 0.05$) and thereafter it tended to increase (0.7% and 0.8% at month 12 and 18, respectively). BTT decreased significantly ($p < 0.01$) at all time points, whereas FWA significantly ($p < 0.01$) increased by 13.1 %, 15.7% and 13.6% at month 6, 12, and 18 respectively. At month 18 in teriparatide group BMD-H was reduced (-3.0% $p < 0.01$) whereas lumbar spine BMD (BMD-LS) and femoral neck BMD (BMD-FN) were increased (9%, $p < 0.01$ and 4.2%, $p < 0.01$, respectively). In the control group both BMD-FN and total hip BMD (BMD-FT) did not show any significant changes during the study period. A 6 month bisphosphonate treat-

ment following teriparatide induced a significant increase in BMD-FN and stabilization in BMD-LS, BMD-H and QUS parameters.

Our study shows that in women with established osteoporosis a 18-month teriparatide treatment increased BMD at axial skeleton but significantly decreased BMD-H. Moreover, teriparatide determined important changes in BTT and FWA, two parameters obtained from the analysis of ultrasonographic trace at phalanxes, which could be considered in monitoring for the effect of teriparatide on bone.

Conflict of Interest: None declared

P345-S

GOOD PERSISTENCE AND ADHERENCE WITH ORAL BISPHOSPHONATES REDUCE FRACTURE RATE IN PATIENTS WITH OSTEOPOROTIC FRACTURES

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Background / aims: In clinical trials oral bisphosphonates have been shown to reduce the risk of fractures in patients with osteoporosis. It can be assumed that the effectiveness of oral bisphosphonates depends on persistence and / or adherence with therapy. We therefore investigated the influence of persistence and adherence with oral bisphosphonates on fracture risk in a naturalistic setting.

Methods: Claims data from a large German sickness fund (about 1.5 million lives insured) were considered. Within the period 2000 to 2004, patients with a defined index prescription of a bisphosphonate (no prescription of bisphosphonates within 180 days before, observation time at least 360 days before and 180 days after index prescription) were included. Fracture rates within 180, 360, and 720 days after the index prescription were compared between persistent and non-persistent patients. In an extended Cox regression model applying multiple event analysis, the influence of adherence was analysed. Persistence was defined as the duration of continuous therapy, and adherence was measured in terms of the medication possession ratio (MPR; proportion of days supplied with medication).

Results: We identified 4.451 patients with an index prescription. In patients with a fracture before the index prescription, fracture rates were reduced by 29% ($p = 0.025$) comparing persistent and non-persistent patients within 180 days after the index prescription and by 45% ($p < 0.001$) within 360 days. Within 720 days a 9% ($p = 0.752$) reduction was observed. In patients without a fracture before the index prescription, low incident fracture rates made it difficult to see a significant effect. However, within 720 days after the index prescription, a non-significant reduction of 11% ($p = 0.709$) in fracture rates was observed in persistent patients. The extended Cox regression model showed that good adherence (MPR ^{3} 0.8) reduced the fracture risk by about 39% (hazard ratio 0.61, 95% CI 0.47–0.78; $p < 0.01$).

Conclusion: In patients with osteoporosis-related fractures, good persistence and adherence with oral bisphosphonates reduce fracture risk significantly. The effect can be observed within 180 day after therapy onset. In patients without a known fracture, a longer period of continuous oral bisphosphonate therapy may be needed until a significant fracture reduction can be observed.

Conflict of Interest: IGES Institute for Health Care Research, Research Support

P346-M

KYPHOPLASTY PERSISTENTLY REDUCES PAIN IN PATIENTS WITH OSTEOPOROTIC VERTEBRAL FRACTURES – 3 YEAR OUTCOME OF A PROSPECTIVE CONTROLLED COHORT STUDY

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Introduction: Recently we have shown reduction of pain and improvement of morphological parameters after kyphoplasty in patients with painful osteoporotic vertebral fractures compared to a conservatively treated control group. To evaluate long-term effectiveness of the kyphoplasty procedure we reassessed the patients of this trial in a 3 year follow-up.

Methods: Kyphoplasty was performed in 40 of 60 consecutive patients with primary osteoporosis and painful vertebral fractures, 20 patients served as controls. All patients received a pharmacological osteoporosis treatment (1000mg calcium, 1000IU vitamin D3, oral aminobisphosphonate), pain medication and physiotherapy. Pain (visual analog scale (VAS), range 0–100), mobility (EVOS score 0–100) and radiomorphological parameters and incident fractures were assessed at baseline, after 12 and after 36 months.

Results: After kyphoplasty the pain score (VAS) improved from 73.8 to 55.6 during the first year and to 54.0 after 36 months. The pain score of the conservatively treated control group changed from 66.4 to 64.0 during the follow-up period. Mobility scores for the kyphoplasty cohort improved from 43.8 to 54.8 and remained elevated compared to conservative controls (improved from 39.8 to 43.6) for at least 3 years. Remarkably, the fracture incidence of the kyphoplasty cohort was significantly reduced throughout the 3 year study period compared to controls ($p < 0.05$). Clinically asymptomatic cement leakages were observed in 9,7 % of the vertebral bodies, which is comparable to previous reports. No complications of neurological, embolic or cardiovascular symptoms occurred after kyphoplasty.

Conclusions: Kyphoplasty is a safe method for a sustained pain reduction in patients with painful osteoporotic vertebral fractures in addition to medical treatment, if performed in appropriately selected patients by an interdisciplinary team. Significantly reduced pain and thus improved mobility throughout the follow up period of three years appear to contribute to the reduced fracture incidence after balloon kyphoplasty.

Conflict of Interest: None declared

P347-T

EFFECTS OF TERIPARATIDE TREATMENT IN OSTEOPOROTIC PATIENT WITH PREVIOUS CANCER PATHOLOGY

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A 56 y.o. male osteoporotic patient, at age 34 was diagnosed a right hand sarcoma epitelioide requiring amputation of the 2° e 3°ray followed by chemotherapy (definitive resolution). Patient suffered hepatopathy during chemotherapy, with altered hepatic functionality which was pharmacologically treated. In 2002, patient suffered fractures at the 5°, 8°, 9°right ribs and at the 6°left rib, resolved without intervention. In 09/2003, patient reported back-pain, due to a vertebral fracture in T12, treated with an orthopedic corset without pharmacological intervention. In 10/2003, the T12 fracture further worsened and patient underwent surgery for spinal stabilization, with no osteoporosis therapy. Few months later, the back-pain first resumed, then become chronic, likely for the mobilization of the vertebral fusion. In 02/2006, it was required a revision with a larger vertebral fusion (no pharmacol. therapy). After few months, patient suffered again back-pain, sciatalgia and spondilo listesi at L2–L3, L3–L4 with stenosis of the canal between L5 and S1. That led to laminectomia of L5 and stabilization of L3-S1, linked to the previous spine fusion system. Patient still used an orthopedic corset and no osteoporosis drug. Despite the surgical procedures, patient still suffered from severe back-pain and kyphosys. In 05/2006, an x-ray after a new acute back-pain episode showed a new fragility T9 fracture. After evaluation of plasma, hepatic and bone markers, the patient began the treatment with teriparatide. At the same time T8, T9 were stabilized by kyphoplasty. The treatment was interrupted few days/month to monitor the safety of treatment on hepatic and bone metabolism. Six months after the treatment started, patient was put on a rehabilitation hydro kinesis treatment and become able to quit the orthopedic corset. He was able to seat in a standing postura, stating a back-pain reduction by 85%. Plasma markers did not indicate any safety concern. This case confirms how teriparatide can reduce fracture related back-pain and ameliorate osteoporotic patient's life. Teriparatide might help in mobilization of vertebral fusions due to poor bone conditions possibly in relation with the activation of bone repair mechanisms. This case suggests that in very severe impaired osteoporotic patients at high risk of new multiple fractures with a previous remote oncology history, teriparatide might have a positive benefit/risk ratio. Lacking teriparatide alternatives of similar efficacy, we suggest larger controlled efficacy and safety studies.

Conflict of Interest: None declared

P348-S

CLINICAL IMPORTANCE OF ACCURACY-ERROR AND REFERENCE VALUES IN DXA – A NATIONWIDE STUDY

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Background: DXA is essential in the diagnosis of osteoporosis. In virtually all clinics, rigorous quality control is performed on each machine. There is, however, no mandatory national or international cross-calibration program. Moreover, different sets of reference values are applied at individual clinics. The clinical impact of these variations is unknown.

Aim: To evaluate the accuracy error on DXA-scans at a national level, compare the applied reference ranges, and evaluate the clinical impact of these two factors (i.e. the prevalence of osteoporosis in a clinical cohort of patients referred from general practice).

Patients and methods: European Spine Phantom (ESP) was circulated between 8 clinics and scanned 10 times on each of 16 scanners following reposition. Data on the reference values used in clinical setting were collected from the clinics. Data on a cohort of 745 consecutive patients referred from general practice with suspected osteoporosis to a single center were used as clinical reference. The accuracy errors and the variation in diagnostic threshold (T = -2.5) were assessed. Moreover, the number of patients in the cohort that would have been diagnosed with osteoporosis in each clinic was calculated.

Results: A total of 8 clinics with 16 scanners (primarily Hologic), covering 50% of the clinical scans performed in the country, participated in the study. The variation between scanners ranged from 2.5 to 18.7%, 1.3 to 11.4 %, and 2.3 to 8.4% at BMD levels of 0.5, 1.0 and 1.5 g/cm^2, respectively. The accuracy error at T = −2.5 varied from –0.03 to 0.10 g/cm^2). Similarly, the cut-off value for osteoporosis used in the clinics varied from 0.71 to 0.81 g/cm^2. The number of patients in the cohort that would be diagnosed with osteoporosis at the different clinic varied from 113 to 240.

Conclusion: Our data demonstrate a considerable variation in accuracy error between DXA-scanners and an unacceptable wide range in the applied reference ranges. This translates into an unacceptable diagnostic variation. We propose that these problems should be solved by a national or international quality assurance program and implementation of validated reference values. Our study is ongoing and national data will be presented.

Conflict of Interest: K. Brixen, Merck Sharp & Dohme, Grant/Research/Support, Eli Lilly, Consultant, Speakers Bureau, Novartis, Consultant, Speakers Bureau, Servier, Consultant, Speakers Bureau

P349-M

SERUM SRANKL LEVELS IN OSTEOPOROTIC PATIENTS USING A SIGNAL AMPLIFIED ELISA

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Background: The role of receptor activator of nuclear factor kB-ligand (RANKL) and its decoy receptor Osteoprotegerin (OPG) in bone remodelling has been well established during recent years. Several studies have been performed to elucidate the value of both molecules as serum markers in bone research. Data about serum levels of both molecules have been collected for multiple myeloma (1), osteoporosis (2) rheumatoid and osteoarthritis (3, 4) and many other pathological conditions. Although the usefulness of the OPG/RANKL system for bone research is undisputed, the very low levels of circulating soluble RANKL (sRANKL), sometimes make the determination of serum levels difficult. Therefore we recently developed a new assay to improve serum analytics of sRANKL.

Methods/Results: The improved assay is based on capturing sRANKL by OPG adsorbed to microtiter plates. Signal amplification is achieved by an enzyme cycling system using the conversion of NADH and NAD and back thus creating a molecule of coloured formazan by each turn of the cycle. To optimise the enhancement system the current sRANKL ELISA had to be redesigned in terms of plate blocking, buffer additives, conjugate and assay procedure. We have been able to increase the sensitivity by a factor of 4, lowering the detection limit to 0.02 pmol/L. Using this assay we determined sRANKL levels in a population of healthy blood donors and of samples from osteoporotic patients. Correlations with OPG, CTX and BMD are presented.

Conclusion: The presented assay, will be a major improvement of the analysis of human sRANKL serum and plasma levels.

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Conflict of Interest: None declared

P350-T

BISPHOSPHONATES STIMULATE LINEAR GROWTH OF FETAL RAT METATARSAL BONES AND PROTECT CHONDROCYTES FROM DEXAMETHASONE-INDUCED APOPTOSIS

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Background: Increasing numbers of children are being treated with bisphosphonates for diseases associated with low bone mineral density and increased risk of fractures, such as osteogenesis imperfecta. However, there has been some concern about their use in growing children, especially with regard to effects on longitudinal bone growth.

Aims: The main objective of this study was to establish, whether the commonly used bisphosphonates alendronate and pamidronate affect longitudinal bone growth in vitro. Furthermore, we studied if bisphosphonates could be used in the prevention of glucocorticoid-induced growth retardation.

Methods: Rat metatarsal bones were isolated from day E20 fetuses and cultured in the presence of various concentrations of bisphosphonates. In some experiments, bisphosphonates were added in cultures in combination with dexamethasone (Dexa). After capturing digital images of metatarsal bones at different time points, bone lengths were measured using an image analysis program.

Results: Alendronate and pamidronate were observed to have dose-dependent effects on longitudinal bone growth. At a high concentration (1 mM), both alendronate and pamidronate completely blocked bone growth. However, at clinically more relevant concentrations (0.1 – 10 μM), alendronate and pamidronate slightly stimulated fetal metatarsal bone growth. We have previously shown that Dexa decreases viability and stimulates apoptosis in human chondrocytic cell line HCS-2/8. To address the question if bisphosphonates can counteract the effect of Dexa on chondrocytes, we treated HCS-2/8 cells with Dexa (25 μM) in combination with alendronate (1 μM) or pamidronate (1 μM), and observed a partial rescue of viability and decreased levels of apoptosis when compared to Dexa alone. The underlying molecular mechanisms for these effects need to be further investigated.

Conclusion: Our data suggest that bisphosphonates have anti-apoptotic effects on chondrocytes, improve longitudinal bone growth and may even prevent glucocorticoid-induced growth retardation. Based on our in vitro findings, it could be speculated that bisphosphonates may be effective in preventing glucocorticoid-induced growth retardation in children. Clinical studies are needed to confirm this.

Conflict of Interest: L. Sävendahl, Novo Nordisk A/S, Grant Research Support and Speakers Bureau L. Sävendahl, Pfizer Inc, Grant Research Support and Speakers Bureau L. Sävendahl, Insmed Inc, Speakers Bureau L. Sävendahl, Sandoz AB, Speakers Bureau L. Sävendahl, Ipsen Scandinavia A/S, Speakers Bureau

P351-S

SERUM CALCIUM VALUES AFTER ONE MONTH'S TREATMENT WITH FULL-LENGTH PARATHYROID HORMONE PTH(1–84) OF POSTMENOPAUSAL WOMEN WITH PRIMARY OSTEOPOROSIS

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Background: The human recombinant parathyroid hormones (PTHs) represent a class of potent anabolic agents for the treatment of primary osteoporosis in postmenopausal women. Physiological PTH increases serum calcium. We report the total serum calcium values after one month treatment with PTH(1–84) in the PEAK (Preotact after a brEAK) trial.

Method: 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(1–84). 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. By 1 May 2007 we expect the majority of patients to be enrolled in the study.

Results: By 12/2006 48 patients had reached one months of treatment with PTH(1–84). The majority (83%) of patients had a serum calcium below the upper limit of normal. 17% had mild elevations in serum calcium and none had marked elevations of serum calcium.

Conclusion: In this first 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:

Total s-calcium	< 2.55 mmol/l	2.55–2.68 mmol/l	> 2.68 mmol/l
N	40	8	0
%	83	17	0

Conflict of Interest: D. Felsenberg, Amgen, Aventis, Chugai, Glaxo, Lilly, MSD, Novartis, Nycomed, Organon, P&G, Pfizer, Roche, Schering, Servier, Teva, Wolff, Wyeth, Grant/Research Support Amgen, P&G, Glaxo, Lilly, MSD, Novartis, Nycomed, P&G, Roche, Servier, Consultant D. Hosking, MSD, Novartis, Nycomed, Pfeizer, Roche/GSK, Consultant. MSD, Novartis, Nycomd, Roche/GSK; lecture fee. Eli Lilly, MSD, Novartis, Nycomed, Grant support. P. Delmas, Nycomed, Consultant R. Rizzoli, Nycomed, Consultant ML. Brandi, Nycomed, Consultant M. Diaz-Curiel, Nycomed, Consultant L.H. Hyldstrup, Employed by Nycomed

P352-M

STRONTIUM RANELATE AND CALCIUM EXERT CUMULATIVE EFFECTS ON OSTEOCLASTS BY ACTIVATION OF DIFFERENT INTRACELLULAR SIGNALING PATHWAYS, DOWNSTREAM THE CALCIUM-SENSING RECEPTOR

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We previously demonstrated that both strontium ranelate and calcium stimulate the calcium-sensing receptor (CaR) and induce mature osteoclast (OC) apoptosis, acting through the IP3-signaling pathway (Ca^{2+}) or DAG-PKC-signaling pathway (Sr2+). We first confirmed that PKC is not involved in the Ca^{2+}-induced OC apoptosis and investigated the PKC isozyme implicated in the effects of Sr2+. Ten-day old rabbit mature OC were cultured in presence of Ca^{2+} (24mM) and staurosporine (0.1mM) or calphostine C (1mM). These two PKC antagonists were unable to reduce the Ca^{2+}-induced OC apoptosis, confirming that Ca^{2+} stimulates osteoclast apoptosis in a PKC independent manner. Specific siRNA, designed to down regulate the expression of different PKC isozymes, were transfected into mature OC for 36 h, followed by the addition of Sr2+ (25 mM) for 48 h. Only silencing of the PKCbII gene inhibited by 50% the Sr2+-induced OC apoptosis compared to that observed in OC transfected with scrambled siRNA. It seemed then important to characterize how OC apoptosis is regulated in the simultaneous presence of both Ca^{2+} (15mM) and Sr2+ (6–30mM), situation found under strontium ranelate treatment. The concentrations tested are likely to be reached in the sub-osteoclastic compartment during the bone resorptive process. The effects of the combination of Sr2+ and Ca^{2+} on OC apoptosis and NF-kB nuclear translocation were additive when compared to the effects of each ion alone. Finally, the simultaneous inhibition of the IP3-signaling pathway and DAG-PKC-signaling pathway inhibited the OC apoptosis induced by the combination of Sr2+ and Ca^{2+}. Taken together, our data demonstrate that each ion exerts its own effect on OC through distinct and additive intracellular signaling pathways, downstream the CaR. Under strontium ranelate treatment, Sr2+ could cumulate its own effects to those of Ca^{2+} and lead to a more powerful effect in down-regulating the osteoclastic bone resorption process. The additive effects of Sr2+ and Ca^{2+} may be linked to the increased bone mass and the reduction of fractures observed in strontium ranelate treated patients.

Conflict of Interest: None declared

P353-T

AGING OF CORTICAL BONE IN MEN AND WOMEN. A CROSS-SECTIONAL STUDY OF METACARPAL CORTICAL DIMENSIONS IN 4100 NORMAL SUBJECTS USING DIGITAL X-RAY RADIOGRAMMETRY

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Changes in cortical bone with age influences the risk of several fracture types. It is generally believed that the net loss in cortical bone is larger in women due to a lager endosteal resorption and smaller periosteal gain in women than in men. In this study the cortical dimensions of metacarpal 2–4 were determined in 2615 postmenopausal women and 1507 males aged 40–85 using digital x-ray radiogrammetry (DXR, Sectra-Pronosco, Herlev Denmark). After digitalization of the x-rays, measurements are automatically performed 118 times per cm. The CV% of this technique is 0.35%. In this study average values for the three measured metacarpals are presented. The study is based on data and hand x-rays from a subgroup of men and women participating in the Copenhagen City Heart Study.

For each subject measurements of endosteal and periosteal diameter were performed and the cortical thickness as well as the cross-sectional bone area was calculated. The average annual change in percent was then calculated for each parameter.

In contrast to previous findings only a very small periosteal apposition rate is seen in both sexes, but it is not known whether this is only a characteristic of the metacarpal bones or a general phenomenon related to cortical bone. Measurements of the inner diameter revealed an annual loss of 1.42 % in females, but only the half in males. The outer diameter is 15% larger in males, but no sex difference in the (small) increase with age in the periosteal diameter were found.

Therefore, the age-related changes in the metacarpal bone seem to be caused by endosteal resorption rather than periosteal apposition. The biomechanical consequences of these findings are that lower fracture incidence in males are caused by larger bones since puberty rather than due to longitudinal changes during adult life.

Conflict of Interest: l Hyldstrup, Eli-Lilly Denmark, MSD, Novartis, Novo-Nordisk and Nycomed, Consultant

P354-S

PROFILE OF PATIENTS TREATED WITH TERIPARATIDE. RESULTS FROM THE DANISH PTH DATABASE

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At the introduction of Teriparatide in 2003 a national database was established with the purpose of gaining information on the results obtained in Denmark. The reporting of patients to the database is voluntary and so far 465 patients from 13 different clinics have been included (app. 40% of all patients, who have received this treatment).

Compared to the inclusion criteria of the pivotal trial* the Danish patients chosen for PTH treatment seem to have more severe osteoporosis with a significantly lower T-score in the spine and hip, as well as a higher number of prevalent vertebral fractures. Male patients are significantly more likely to be treatment naïve, while most women has been on antiresorptive treatment before Teriparatide was instituted.

Following treatment with Teriparatide a significant increase in BMD of the lumbar spine was seen, reaching 10,8% at 18 months ($p < 0.0001$), while BMD of total hip reached 4,5% ($p < 0.0001$). The increase in BMD showed no gender difference, neither at the spine nor the hip. Side effects of treatment were few and only few new fractures have been reported during the treatment period.

Conclusion. Using a voluntary national database on PTH-treatment is has been possible to gain information on app. 40% of all treated patients in Denmark. The average patient seems to have more severe osteoporosis than patients in the pivotal trial with consequently even better cost-effectiveness of the treatment. The BMD response in the lumbar spine and total hip matches that of the pivotal study. Only a small number of patients reported new fractures during treatment and the side effects were few and mild.

*) Neer et al. N Engl J Med.2001;344: 1434–41.

Table: Baseline data

	Numbers	Age	Treatment naïve (%)	Spine T-score	Hip T-score	No. of VF
Total	465	69,1	26,7	-2,9	-2,5	4,01
Males	85	67,7	43,5	-3,0	-2,5	3,78
Females	380	69,5	23,0	-2,9	-2,5	4,07

Conflict of Interest: L. Hyldstrup, Eli-Lilly Denmark, MSD, Novartis, Novo-Nordisk, Nycomed, consultant B. Langdahl, Eli-Lilly Denmark, MSD, Novartis, Nycomed J B Jensen, Eli-Lilly Denmark, MSD, Novartis, Nycomed, Sanofi, Servier K. Brixen, Merch Sharp & Dohme, Grant/Research/Support, Eli Lilly, Consultant, Speakers Bureau, Novartis, Consultant, Speakers Bureau, Servier, Consultant, Speakers Bureau P. Schwarz, Lilly, Consultant

P355-M

NO INFLUENCE OF PREVIOUS BISPHOSPHONATE TREATMENT ON TERIAPRATIDE RESPONSE. THE DANISH PTH DATABASE

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Many candidates for PTH-treatment have received bisphosphonates (BP) for shorter or longer periods prior to the initiation of the PTH injections. This may have impact on the outcome of the treatment due to the potential inhibition of the anabolic response to PTH. In the Danish PTH Database information on previous BP treatment was present in 212 cases. Of these 94 never received BP (defined as never or less than 3 months), while 49 patients had stopped BP treatment more than 6 months before PTH and 69 patients were on BP until Teriparatide was prescribed. Patients were treated either with alendronate, risedronate or ethidronate, none received i.v. bisphosphonates. The vast majority received alendronate 70 mg weekly. Subsequently all patients were treated with Teriparatide.

Baseline spine BMD did not differ between groups. In the group that never received BP for more than 3 months, the expected increase in spine BMD was seen, reaching +9,5% at 18 months, while the patients with a free interval of more than 6 months before PTH treatment rose to +8,0% and the group treated with BP until the start of PTH treatment had an increase of 9,0%. The increase was significant in all 3 groups ($p < 0.0001$), but the difference between the groups did not reach statistical significance at any time point. The present study does therefore only partly confirm previous findings, with a tendency towards a smaller BMD increase in the lumbar spine at 6 months, but no difference after 12 and 18 months. Consequently, it does not seem necessary to include a treatment free interval for BP-treated patients before PTH treatment is commenced.

Table: Baseline and follow up data

BP treatment duration	No	Age	male/female	BMD spine start	BMD spine 18 months
< 3 months ever	94	69,6	17/77	0,774	0,847
> 6 months earlier	49	70,2	9/40	0,775	0,836
At treatment start	69	70,1	11/58	0,743	0,810

Conflict of Interest: L. Hyldstrup, Eli-Lilly Denmark, MSD, Novartis, Novo-Nordisk, Nycomed, consultant B. Langdahl, Eli-Lilly Denmark, MSD, Novartis, Nycomed J B Jensen, Eli-Lilly Denmark, MSD, Novartis, Nycomed, Sanofi, Servier K. Brixen, Merck Sharp & Dohme, Grant/Research/Support, Eli Lilly, Consultant, Speakers Bureau, Novartis, Consultant, Speakers Bureau, Servier, Consultant, Speakers Bureau P. Schwarz, Lilly, Consultant

P356-T

COMPARISON OF VERTEBRAL AREA, BMC AND BMD IN OLDER WOMEN UTILIZING THE REGULAR–LATERAL OR MORPHOMETRIC SCANS BY iDXA

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Newer bone densitometers have the option for the lateral spine to be evaluated for BMC/BMD and for morphometric shapes, the later commonly known as LVA (Lateral Vertebral Assessment) and used to detect vertebral compression and/or deterioration in vertebral shape. The LVA scans generated by iDXA can also produce BMC and areal BMD results. Our objective was to compare the BMC/BMD values generated by LVA to those generated by the regular–lateral scans. Our hypothesis was that if the BMC/BMD values from both scans were comparable, there would be no need to perform both scans and only LVA scans could be sufficient. This preliminary study included 37 healthy, Caucasian, postmenopausal women aged 56.5 (3.7) (mean (SD)) y, whose lateral and LVA scans were performed consecutively in the same scanning session by iDXA (GE Medical System, Madison, WI), software version enCORE 10.50.086 and analyzed by the same operator. The BMC values (g) for regular–lateral and LVA scans, respectively were: B2–B3 = 5.27(1.47) vs. 5.46 (1.57); B2–B4 = 8.17(2.29) vs. 8.47(2.30); and B3–B4 = 5.59(1.75) vs. 5.85(1.86). With the same respect, the BMD values (g/cm^2) were: B2–B3 = 0.611(0.15) vs. 0.619 (0.15); B2–B4 = 0.646(0.15) vs. 0.653(0.15); and B3–B4 = 0.659(0.18) vs. 0.669(0.17). All respective values were highly and significantly correlated ($p \leq 0.0001$), with Pearson's r for BMC of 0.811 for B2–B3; 0.870 for B2–B4; and 0.885 for B3–B4. The r's for BMD were 0.955 for B2–B3; 0.965 for B2–B4; and 0.953 for B3–B4. Similar good correlations were obtained between regular–lateral and LVA scans for the vertebral areas. Based on these preliminary results, we conclude that LVA scans could be utilized for the assessment of both bone mass (BMC/BMD) and morphometric changes and by that save time and reduce the radiation exposure to subjects. However, studies with more subjects and more extensive analyses are necessary for the final conclusions. Funded by USDA/CSREES/NRI #2004-05287.

Conflict of Interest: None declared

P357-S

APPROPRIATE BONE MINERAL DENSITY TESTING FOLLOWING A MULTIFACETED OSTEOPOROSIS EDUCATIONAL INTERVENTION: CANADIAN QUALITY CIRCLE (CQC) NATIONAL PROJECT

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Background: The use of Quality Circles (QCs) involving small groups of people from similar work environments who meet to identify and analyze problems and to recommend solutions regarding these work related problems an integrated disease management process, was utilized to improve primary care physicians' (PCPs) management of osteoporosis in accordance with the Osteoporosis Canada 2002 Guidelines.

Methods: The project consists of five phases: wave I data collection, 1st educational intervention, wave II data collection, 2nd educational intervention, and wave III data collection. During the educational intervention QC's met to discuss profiles of the physician's management of osteoporosis and to participate in an osteoporosis workshop. This interim analysis (wave I & II) examined the change in appropriate BMD testing. Guidelines suggest that BMD testing should be conducted in patients with one or more major or 2 or more minor risk factors for future fracture (high risk). A total of 340 (wave I) and 301 (wave II) FPs formed 34 QC. For each wave, PCPs collected data from different patients via chart reviews and a standardized collection form. A total of 8376 (wave I) and 7354 (wave II) patient records were selected at random and analyzed. All patients were women 55 years and older. The generalized estimating equations approach was used to evaluate differences in appropriate utilization of BMD testing pre and post educational intervention. An exchangeable correlation matrix was used for the analysis. Odds ratios and 95% confidence intervals (CI) were calculated.

Results: Univariate results indicated that high risk patients were 1.4 (95% CI: 1.2, 1.7) more likely to have a BMD test following the educational intervention as compared with low risk patients. The likelihood of a BMD test increased if the patient was ≥ 65 years (OR 1.2; 95% CI: 1.1, 1.4), was menopausal before the age of 45 years (OR 1.6; 95% CI: 1.2, 2.1), or had other major risk factors (OR 1.5; 95% CI: 1.2, 1.9). Patients with prior fractures were less likely to have a BMD test following the educational intervention as compared with patients without a fracture (OR 0.7; 95% CI: 0.5, 0.9).

Conclusions: the use of Quality Circles, an integrated disease management process, is an effective stepwise knowledge translation approach that improves PCPs' utilization of BMD testing in their patients.

Conflict of Interest: B Kvern, Procter Gamble/Sonofi-Aventis, consultant G Ioannidis, None A Hodsman, Eli Lilly, Merck Frosst, NPS, Zelos Therapeutics, Servier, Procter & Gamble/Sonofi-Aventis, Medical advisory boards, speaker fees A Papaioannou, Eli Lilly, Merck Frosst, Amgen, Procter & Gamble/Sonofi-Aventis, Novartis, grants, consultant L Thabane, none A Gafni, none D Johnstone, employee Procter & Gamble C Crowley, employee Procter & Gamble JD Adachi, Eli Lilly, Merck Frosst, Amgen, Procter & Gamble/Sonofi-Aventis, Novartis, grants, consultant

P358-M

COMPERATIVE STUDY BETWEEN HISTOLOGICAL AND RADIOLOGICAL METHODS IN THE DIAGNOSIS OF INVOLUTIONAL OSTEOPOROSIS IN PATIENTS WITH FRACTURE NECK FEMUR

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BACKGROUND: In a prospective cross sectional study of the measurement of

osteoporosis in patients with femoral neck fracture (FNF) using the histological method of diagnosis and the obtained results compared to those from non fractured normal individuals (controls). In this study, we also compare the histological and radiological methods in the measurement of the degree of osteoporosis in those group of patients with FNF.

METHODS: The histological method depend on histomorphometric analysis of bone biopsies taken from the spinous processes or laminae of non fractured control group during surgical treatment of disc prolapse and from neck of femur of patients with FNF during surgical treatment of the fracture. We depend on three indices in histomorphometric analysis these are osteoid seam width, osteoblast surface and osteoid surface.

The radiological method depend on measurement of bone mineral density (B.M.D.) using dual energy X-ray absorptiometry (DEXA) for fractured pa-

tients with the scan done to the contra lateral non fractured hip and lumbar spines.

RESULTS: We found positive histological evidences of osteoporosis in 68%of patients with fracture neck femur and 12.2% in control group and there is moderately strong correlation between histological histomorphometric analysis and DEXA in the diagnosis of involutional osteoporosis in fractured hip patients.

CONCLUSION: 1. DEXA is noninvasive, affordable, easy method for diagnosis of osteoporosis but less efficient than histological histomorphometric method of diagnosis.

2. DEXA can detect up to 88.2% of possible cases of osteoporosis (sensitivity 82.2%) but specificity of this diagnostic tool is 62.5% at T-score < -2 i.e. it is sensitive but less specific.

3. The mean difference in T-score in Femoral Dexa (FDEXA) and Lumber DEXA (LDEXA) is almost zero, so DEXA of one region can reflect the changes in the other region

Conflict of Interest: None declared

P359-T

BIOCHEMICAL MARKERS OF BONE TURNOVER; INITIAL RESPONSE TO PTH 1–34 TREATMENT AND RELATION TO CHANGES IN BMD AND BMC

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Aim: Treatment of osteoporosis with PTH 1–34 is due to its high cost limited to few seriously ill patients. Response to treatment is variable and not all patients seem to benefit from the treatment. In this study we wants to examine if biochemical markers of bone turnover measured in the first 6 months after initiating treatment was related to changes in BMD and BMC after 1 and 1½ year.

Method: A total of 33 patients, 25 women and 8 men, aged 66 and 57 years of age respectively, with severe osteoporosis completing treatment with PTH 1–34 for 18 months were examined. DXA were measured in the hip and lumbar spine at start and after 12 and 18 months. At start and after 1, 3 and 6 months fasting serum samples were examined for both formation and resorption markers. We examined time to significant changes in the markers and the relation between changes in markers and changes in BMD and BMC at 12 and 18 months.

Results: After 1 month there were significant increases in PINP, NTX, BAP and osteocalcin. Significant changes were seen in CTX and TRAP after 3 months, while no changes were seen in ICTP, OPG and RANKL. In the lumbar spine significant higher values of both BMC and BMD were seen after 12 and 18 months treatment while no significant changes were seen in the hip region. Women had significant higher bone turnover than men, but a lower response to treatment.

Conclusion: In spite of highly significant changes in bone markers within the first 3 months there were no relation to changes in area BMD or BMC at the lumbar spine or femoral region after 12 and 18 months. Bone markers are not able to predict BMD and BMC response to PTH 1–34 treatment.

Conflict of Interest: J. B. Jensen, Eli-Lilly, Novartis, Nycomed, Servier, Sanofi-Aventis, advisory committee P. Schwarz, Eli-Lilly, MSD, Nycomed, advisory committee L. Hyldstrup, Eli-Lilly, Nycomed, Novo, advisory committee

P360-S

FRACTURE RISK ASSESSMENT IN ACUTE HOSPITAL ADMISSIONS

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Background/Aims: 1 in 2 women over the age of 50 will have a fracture in their remaining life time. Clinical risk factors such as parental history of hip fracture (under age 75), prior fracture (after age of 50), previous use of systemic steroid, alcohol intake >2 units per day, current smoking and rheumatoid arthritis increases the fracture risk partly independent of BMD (1). The aim of this study was to identify this patient group who were eligible for cost effective intervention with Alendronate (2). The quality of relevant clinical documentation was also examined.

Method: All acute hospital admissions to the Medical Emergency Unit in a large teaching District General Hospital (UK) were reviewed over 5 randomly chosen days in August 2006. Clinical risk factors for increased fracture risk in female (>50 years) assessed. Patients with Abbreviated Mental Test Score $<7/10$, terminally ill, or those discharged home excluded. Initial admission documentation regarding clinical risk factors of fracture, previous diagnosis of osteoporosis and treatment were also examined.

Results: Total admissions = 277. Female above 50 years age = 82 (30%). 52 case notes analysed. In the group analysed, 5 patients were 50–60 years, 18 were 60–70 years, 20 were 70–80 years and 9 were $>$ 80 years of age. Prevalence of risk factors included: parental facture: 2 (3.8%); prior fracture: 13 (25%): past use of systemic steroid: 16 (30.8%): rheumatoid arthritis 5 (9.6%); alcohol 2 (3.8%) and smoking 8(15%). 19 (36%) patients had no risk factor. 9 women qualified for preventive treatment without DXA scan and 26 after scan. It implies that 67% (35/52) of female above age 50 or 13%(35/277) of all admissions needed intervention. 10(19%) patients had a prior diagnosis of osteoporosis and were already on treatment. However, in only 1(2%) admission case note relevant information were documented.

Conclusion: 2/3rd of women (above age 50) admitted to the Medical Emergency Unit do not receive treatment for fracture prevention (2). Documentation of risk factors for osteoporosis is inadequate. Acute medical admissions should be viewed as an opportunity for fracture prevention.

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Conflict of Interest: None declared

P361-M

COMPARISON OF CHANGES IN BONE MINERAL DENSITY AND BONE TURNOVER MARKER WITH ALENDRONATE ONCE-WEEKLY AND BI-WEEKLY IN POSTMENOPAUSAL KOREAN WOMEN

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Introduction : Recently, alendronate 5mg daily administration also showed preventive effect on bone loss not so much as that of alendronate 10mg daily administration. And for the convenience of patients and cost-effectiveness, increasing the dosing interval should be also considered. Therefore, we designed the comparative studies which measuring the changes of bone mineral density and bone turnover marker between alendronate 70mg weekly administration and alendronate 70mg bi-weekly administration for the postmenopausal women in the one of the Women's Health Care Centers in Seoul, Korea.

Materials and Methods : We retrospectively evaluated the postmenopausal women with T score $<$ -2.0 treated with alendronate 70mg weekly or biweekly and compared the changes of bone mineral density and bone turnover marker.

Results : Lumbar bone mineral density was increased by 4.3%, and femoral bone mineral density was also increased by 2.3% in alendronate 70mg weekly group. However, in the group with alendronate 70mg bi-weekly administration, lumbar bone mineral density was decreased by 0.3%, femoral bone mineral density was increased by 1.0%. Comparing the changes of bone mineral density in each groups, statistical significant increase of lumbar and femoral density was observed in the alendronate 70mg weekly group, but that of alendronate 70mg bi-weekly group did not show the statistical differences.($p < 0.05$) Bone turnover markers such as alkaline phosphatase, deoxypyridinolone showed decreasing pattern, but not statistically significant.

Conclusions : Our study suggested alendronate 70mg bi-weekly administration also has preventive effect on bone loss, even though not strongly, but below the physiologic decreseaing rate of bone mass.

Conflict of Interest: None declared

P362-T

LIPID PROFILE CHANGES IN POSTMENOPAUSAL KOREAN WOMEN TREATED WITH ALENDRONATE (10 mg) FOR 2 YEARS

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Background : Bisphosphonate also has an association with cholesterol synthethic process as well as osteoporosis. We planned this study to recognize the effect of alendronate (10mg) on serum lipid level in postmenopausal Korean women.

Methods : We evaluated postmenopausal Korean women aged over 50 who visited the Osteoporosis clinic. The changes of serum lipid levels including total cholesterol, triglyceride, HDL were evaluated.

Results : After 2-year alendronate (10mg) administration, total cholesterol level was decreased by 11.8 ± 3.7mg/dl, and HDL level was increased by 5.2 ± 1.4 mg/dl with compared to the baseline lipid level. Both of the results showed the statistical significances. Changes of triglyceride and fasting blood sugar levels also showed a declined pattern, but not statistically significant.

Conclusions : Alendronate might have a beneficial effect on lipid metabolim to decrease cholesterol and increase HDL.

Figure 1. Serum cholesterol level after 2-year alendronate(10mg) administration

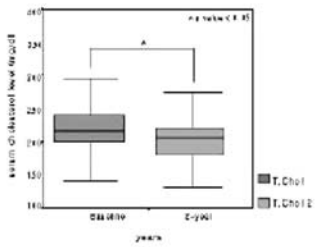
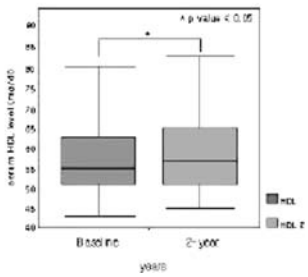


Figure 2. Serum HDL level after 2-year alendronate(10mg) administration



Conflict of Interest: None declared

P363-S

ABSTRACT WITHDRAWN

P364-M

EFFICACY OF HIGH DOSE BLACK COHOSH

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Background: The aim of this study is to assess the efficacy of the high dose combined preparation of black cohosh and St. John's wort, Feramin Q® in menopausal women with climacteric symptoms. And also to assess the effect of Feramin-Q® on bone mineral density.

Methods: A total of 236 postmenopausal women with climacteric symptoms were allocated to either conventional dose (Feramin Q® 2 Tablets/day) group or high dose (Feramin Q® 4 Tablets/day) group. After 3 months, the change of vasomotor symptoms, vaginal dryness, generalized bodyache were assessed. The bone mineral density of lumbar spine and femur neck were measured in 74 postmenopausal women who finished 1 year treatment with Feramin Q®.

Results: There was a substantial improvement in vasomotor symptoms and generalized bodyache in both conventional and high dose group after 3 months treatment. However, the improvement was significantly higher in high dose group including vaginal dryness ($p < 0.05$). There was a increase in bone mineral density in femur neck after 1 year of high dose Feramin-Q® treatment, although it was not significant.

Conclusion: This study suggests that the high dose combined preparation of black cohosh and St. John's wort could be an effective alternative treatment option for menopausal women, especially for who refuse estrogen therapy or who have conditions which are contraindicated to estrogen therapy.

Conflict of Interest: None declared

P365-T

DIFFERENCES IN PHYSICAL PERFORMANCE WHEN COMPARING MEN AGED 70–80 YEARS WITH AND WITHOUT SELF-REPORTED FALLS – BASELINE DATA FROM THE POPULATION-BASED MROS STUDY

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Background: Not only BMD, also muscle strength, balance and a variety of other functional capacities have been reported to diminish with aging and to be associated with falls and fractures. The aim of this study was to investigate if elderly men, who have reported falls the last year, differed in muscle strength, muscle function and level of physical activity compared to non-fallers. If so, this/these trials could be a potential source for intervention as to prevent future falls (and fractures).

Materials and methods: The data are from the population based longitudinal study MrOs-Sweden. The subjects ($n = 3016$) age 70–80 were randomly selected at three sites, Gothenburg, Malmö and Uppsala. Baseline data of self-reported falls were used to compare fallers with non-fallers regarding handgrip strength, timed stand test, 6-metres walking test, 20-centimetres narrow walk test, and physical activity and resting patterns. The fallers were non-significant older than the non-fallers 75.4 versus 75.7 years ($p = 0.06$).

Results: The fallers had in the right hand a 2.6 kg weaker ($p < 0.001$) and in the left hand a 2.1 kg weaker ($p < 0.001$) grip strength, a 1.2 seconds slower timed stands test ($p < 0.001$), a 0.4 seconds slower 6 meters walking test ($p < 0.001$) and 0.2 seconds slower 20 cm narrow walk-test ($p < 0.001$). The daily walking distance for exercise (3.7 km versus 4.0 km, $p = 0.08$) or as part of daily routine (1.4 km versus 1.3 km, $p = 0.16$) did not differ between the fallers or non-fallers but the fallers were sitting (6.1 h versus 6.6 h, $p < 0.01$) and lying (8.2 versus 8.3 h, $p < 0.05$) more each day than the non-fallers.

Summary: Self-reported fallers among elderly Swedish men performed inferior in all physical tests and spend more time sitting and lying than the non-fallers, even if their daily walking distance was no different. As most of the reported traits are possible to target, these could possibly be a strong potential source for intervention as to reduce the fall and fracture risk in elderly men.

Conflict of Interest: None declared

P366-S

VERTEBRAL BODY EVALUATION BY VFA IN CLINICAL PRACTICE

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Introduction: To accurately assess fracture risk in an individual patient the presence of vertebral fracture(s) is at least as important as low bone mineral density (BMD) values. In addition to BMD assessment advanced bone densitometers offer possibility to assess vertebral heights through Vertebral Fracture Assessment (VFA). Both approaches (semiquantitative and quantitative assessments) require exact detection of vertebral body edges. In clinical practice the accurate edge detection may be hampered by the presence of degenerative changes, scoliosis, overlapping of another bone structures (e.g. ribs in the upper part of thoracic spine, which is the most difficult part of vertebral column to evaluate).

Accurate identification of vertebral crush fractures may influence the clinical decisions about the need and type of osteoporosis treatment.

Aim: To evaluate the contribution of VFA fracture detection for decision making in clinical practice, to compare evaluability in men and women separately, to assess the influence ov age, weight, height and Body Mass Index (BMI).

Material and methods: Patients routinely examined on a basis of the outpatient clinic during 14 months (Sep 2005–Nov 2006): 95 males and 96 females with proven or suspected osteoporosis. Examinations were performed by GE Lunar Prodigy scanner, EnCORE sw version 8.6, scan length 38 cm.

Evaulability of individual vertebrae was expressed as a percentage of all vertebrae assessed separately for following regions: T8–L4, T7–L4, T6–L4, T5–L4 and T4–L4.

The influence of sex, age, weight, height (i.e. Body Mass Index, BMI) was assessed.

Results: With increasing age the number of not evaluable vertebrae in both sexes increased., the effect observed as well when larger portions of thoracic vertebral column were involved into analysis. Weight and BMI had no influence on VFA evaluability. In the range of T8–L4 adequate evaluability was comparable in subsets of women (80%) and men (84%).

Conclusion: In the setting of our clinical practice the routine use of VFA left about one fith to one sixth vertebral bodies unevaluable, which prompted the necessity to refer these patients for routine X-Ray study. These proportions increase with advancing age . The evaluability of vertebral bodies if upper levels of thoracic spine were included was even lower.

Conflict of Interest: J. Rosa, Eli Lilly, Consultant

P367-M

CHANGES IN SERUM CTX AFTER 5 YEARS RISEDRONATE TREATMENT IN POSTMENOPAUSAL GREEK WOMEN

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Aim: The aim of this study was to investigate the effect on serum CTX following daily administration of Risedronate for 5 years in early postmenopausal women .

Material and Methods: Forty early postmenopausal women between 48–53 years old (mean 50 years), 6 months–1 year after the menopause, with T score < 2SD on lumbar spine DEXA and without any prior metabolic disorders or fractures were separated into 2 groups: Group A ($n = 30$) received 5mg Risedronate, 1mcg Alphacalcidol and 1000 mg Calcium carbonate daily for 12 months

and 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 1000 mg calcium carbonate thereafter. From 36 months Risedronate was given as 35mg once weekly. Serum and urine bone turnover markers were measured at 0, 6, 12, 24, 36, 48 and 60 month intervals by automated electrochemiluminence assay. No premenopausal values were available for comparison. Two patients in group A discontinued treatment after 24 months.

Results: Group A showed a statistically significant decrease in sCTX (11, 6%, $p < 0.0005$) as early as 6 months after treatment whilst there were no statistically significant changes after the 12month period. In group B sCTX was increased (19.3%, $p < 0.0005$) while the rest of the markers showed a statistically significant decrease for the same period. No values fell below the normal range.

Conclusion: Changes in sCTX, demonstrate that Risedronate effectively decreases bone 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 60 months period provided that vitamin D intake is sufficient. The fact that no values fell bellow normal excludes the untoward presence of frozen bone.

Conflict of Interest: None declared

P368-T

DESPITE OF MANY INFORMATION CAMPAIGNS, OSTEOPOROSIS REMAINS UNDERDIAGNOSED AND UNDERTREATED IN SWITZERLAND

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Background: Osteoporosis is well recognized as a public health problem since population aged 65 yrs and over will increase these next decades. In Switzerland, it is expected that the direct costs of osteoporosis will increase by 30% till 2020. Therefore, it is of major importance to detect and to treat patients at high risk of fracture, particularly those with fragility fractures. To sensitize physicians, many information campaigns have been done these past years.

Method: From March 2005 to March 2006, all patients aged 50 yrs and over, admitted for a low trauma fracture at the Emergency Unit of the South Fribourg Hospital were included in a new fracture pathway. All patients, women (W) and men (M), were informed about osteoporosis and their fracture history and treatment were recorded. Moreover, a DXA (lumbar spine, femoral neck, total hip) assessment was performed (Hologic Discovery).

Results: 244 patients (185 W, 59 M) were included in this project. Of these patients, 93 W (50%) and 36 M (61%) had one or more previous fractures, including 64 W and 20 M with at least a typically OP fractures (vertebrae, forearm, hip or proximal humerus). Among these patients with previous OP fractures, only 16 women (25%) and 2 men (10%) received a treatment against osteoporosis (bisphosphonates), whereas 84% of W and 85% of M and a T-score ≤ -2.

Discussion: Even it has been shown that to treat patients at high risk of fractures is cost-effective, less than 25% of patients with clinical osteoporosis received specific treatment. The development of fracture pathways in hospitals in which fractured patients are taken in charge is crucial.

Conflict of Interest: None declared

P369-S

PRIMARY CARE PHYSICIANS' PREFERENCES TO BISPHOSPHONATE THERAPIES IN PATIENTS WITH SEVERE OSTEOPOROSIS FOLLOWING A MULTIFACETED OSTEOPOROSIS EDUCATIONAL INTERVENTION: CANADIAN QUALITY CIRCLE (CQC) PILOT PROJECT

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Background: We hypothesize that enrolling primary care physicians in Quality Circles (QCs), a multifaceted integrated disease management process strategy, would improve primary care physicians (PCPs) practices for treating severe osteoporosis.

Methods: The Pilot enrolled a total of 52 family physicians and involved seven QCs. The project consisted of three QCs phases that included: 1) Training & Baseline Data Collection, 2) Educational Intervention & Follow-Up Data Collection, and 3) Strategy Implementation Session. During the educational intervention QCs met to discuss profiles of the physician's management of osteoporosis and to participate in an osteoporosis workshop. This analysis examined how PCPs preferences for administering bisphosphonate therapy (risedronate, alendronate, etidronate) to patients with severe osteoporosis changed following the educational intervention. osteoporosis. Severe osteoporosis was defines as a patient with a t-score < -2.5 and a prior fracture. Physician drug therapy preferences were classified as follows: ''given and preferred'', and ''given and not preferred'', and ''not given but preferred''. For each phase, PCPs collected data from different patients via chart reviews and a standardized collection form. A total of 1505 and 1359 patient forms were collected during phase I and II, respectively. All patients were women 55 years and older. The results are summarized using counts (percent).

Results: A total of 24 and 38 patients were on risedronate, 24 and 29 on alendronate, and 19 and 10 on etidronate at baseline and follow-up, respectively. Findings indicated that the ''given and preferred'' preference changed from 45.8% to 63.2% for risedronate, 33.3% to 55.2% for alendronate, and 31.6% to 20.0% for etidronate following the intervention. The ''given and not preferred'' preference changed from 16.7% to 13.2% for risedronate, 25.0% to 13.8% for alendronate, and 63.2% to 80.0% for etidronate. The ''not given but preferred'' preference changed from 37.5% to 23.7% for risedronate, 41.7% to 31.0% for alendronate, and 5.3% to 0.0% for etidronate.

Conclusion: QCs was an effective knowledge transfer technique that improves PCPs preferences to more potent bisphosphonate therapies in patients with severe osteoporosis. The use of potent bisphosphonates may reduce the future fracture risk of these high risk patients.

Conflict of Interest: B Kvern, Procter & Gamble/Sonofi-Aventis, consultant G Ioannidis, None A Hodsman, Eli Lilly, Merck Frosst, NPS, Zelos Therapeutics, Servier, Procter & Gamble/Sonofi-Aventis, Medical advisory boards, speaker fees A Papaioannou, Eli Lilly, Merck Frosst, Amgen, Procter & Gamble/Sonofi-Aventis, Novartis, grants, consultant L Thabane, none A Gafni, none D Johnstone, Employee of Procter & Gamble C Crowley, Employee of Procter & Gamble JD Adachi, Eli Lilly, Merck Frosst, Amgen, Procter & Gamble/Sonofi-Aventis, Novartis, grants, consultant

P370-M

DAILY ORAL AND INTERMITTENT INTRAVENOUS IBANDRONATE HAVE SIMILAR BONE SAFETY PROFILES

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Bone mineral density (BMD), markers of bone turnover, and structure of bone are important components of bone strength. Daily oral ibandronate reduces bone turnover, increases BMD and does not adversely affect newly formed bone in women with postmenopausal osteoporosis (PMO).[1] Intravenous (i.v.) ibandronate injections (2mg every 2 months [q2mo] and 3mg every 3 months [q3mo]), provided improved efficacy to daily oral ibandronate (2.5mg) in the DIVA study.[2, 3] We report here the effects of i.v. ibandronate on bone structure in DIVA participants. Single transiliac bone biopsies were obtained from 109 women after 22 or 23 months' treatment. All biopsies underwent qualitative histology; quantitative histomorphometry was conducted on 89 evaluable samples, of which 87% had detectable tetracycline labelling at both the trabecular surfaces and cortices. All samples showed newly formed bone with normal lamellar structure and no signs of woven bone, cellular toxicity or indicators of osteomalacia. Mineral apposition rate (MAR) was normal and median activation frequency (Ac.f) was lower than that seen in untreated osteoporotic (0.42/year)[4] and healthy (Table) postmenopausal women, and similar to that of healthy premenopausal women (0.13/year).[4] Mineralising surface was comparable to that of healthy premenopausal women but lower than in healthy postmenopausal women (Table).[5] Changes were accompanied by normal bone microarchitecture in all cases. Findings were similar to those obtained with daily oral ibandronate.[1] In women with PMO, intermittent i.v. ibandronate injections do not adversely affect bone architecture and they reduce bone remodelling into the healthy premenopausal range. 1. Recker RR, et al. Osteoporos Int 2004;15: 231–37. 2. Delmas PD, et al. Arthritis Rheum 2006;54: 1838–46. 3. Emkey R, et al. Arthritis Rheum 2005;52: 4060 (Abstract L8). 4. Recker RR, et al. J Bone Miner Res 1988;3: 133–44. 5. Recker RR, et al. J Bone Miner Res 2004;19: 1628–33.

Table: Key histomorphometric parameters (median)

	2.5mg daily (n = 32)	2mg q2mo i.v. (n = 27)	3mg q3mo i.v. (n = 30)	Women[4, 5, *] postmenopause
O.th (μm)	5.00	4.70	5.05	9.58
MAR (μm/day)	0.63[a]	0.56[b]	0.60[c]	0.53
Ac.f (n/year)	0.13[a]	0.06[b]	0.06[c]	0.37
Mineralising surface (%)	1.64	0.61	0.87	6.1

*healthy; O.th = osteoid thickness; n = a29, b22, c27

Conflict of Interest: B Langdahl, F. Hoffmann-La Roche Ltd/GlaxoSmithKline, grant research support and consultant L-G Ste-Marie, F. Hoffmann-La Roche Ltd/GlaxoSmithKline, Merck Frosst, Alliance for better bone health, Procter & Gamble Pharmaceuticals and Sanofi Aventis Pharma Inc., Pfizer Inc., Eli Lilly Inc., AstraZeneca Inc. Genzyme Canada Inc. consultancy I Jonkanski, F. Hoffmann-La Roche Ltd employee C Neate, F. Hoffmann-La Roche Ltd employee RR Recker, F. Hoffmann-La Roche Ltd, GSK, Merck, Lilly, Wyeth, P&G, Amgen, Novartis and NPS Allelix

P371-T

IBANDRONATE IS WELL TOLERATED BY PATIENTS INTOLERANT OF WEEKLY ALENDRONATE OR RISEDRONATE: RESULTS FROM THE PRIOR STUDY

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Oral bisphosphonates are first-line treatment for women with postmenopausal osteoporosis but gastrointestinal (GI) intolerance may be a problem, leading to poor adherence with therapy. Ibandronate is available in both intravenous (i.v.) and oral forms, with extended dosing intervals (3mg every 3 months i.v., 150mg monthly oral) that may improve adherence with therapy. PRIOR is a 1-year, open label study in postmenopausal women with osteoporosis or osteopenia who discontinued daily or weekly alendronate or risedronate due to perceived or actual GI intolerance. Patients are given the option of either monthly oral or quarterly i.v. ibandronate, and are required to be capable of receiving either treatment. Patients are permitted to switch route of administration once during the study for adverse events attributable to study treatment. The proportion of patients who experience a change from baseline in severity or frequency of GI symptoms is being assessed every 3 months by a self-administered GI Experience Survey. Adverse events including clinical vertebral and non-vertebral fractures and laboratory parameters are continually assessed. A total of 545 patients have been enrolled and data from 542 are available for this 6-month analysis. Quarterly i.v. ibandronate was chosen by 396 women (73%) and monthly oral ibandronate by 146 (27%). After 6 months of treatment, 94.9% of patients who chose i.v. treatment and 87.7% who chose monthly oral treatment remained compliant with their chosen therapy. At 4 months, GI tolerance scores improved by at least 10% in 85.6% of patients on i.v. ibandronate and 77.2% of those on oral ibandronate. Overall increases in GI tolerance scores were 36.6% and 28.1%, respectively. A total of 26 patients had switched route of administration by the time of this analysis, 15 from i.v. to oral treatment (for various reasons including flu-like symptoms in two patients and injection-site reactions in three) and 11 from oral to i.v. (all for GI intolerance). Also, 18 i.v. patients and 32 oral patients withdrew. These data suggest that both monthly oral and quarterly i.v. administration of ibandronate are clinically useful alternatives for patients intolerant of weekly oral bisphosphonates. Both ibandronate regimens were well tolerated by women with GI intolerance to daily or weekly risedronate or alendronate. GI symptoms were improved when patients switched to quarterly i.v. or monthly oral ibandronate.

Conflict of Interest: A Laster, Procter & Gamble, Hologic, Grants/Research Support A Laster, Genentech, Lilly, Merck, Procter & Gamble, Roche, GSK, Hologic, Consultant A Laster, Genentech, Lilly, Merck, Procter & Gamble, Roche, GSK, Abbott, Pfizer, Speaker Bureau K Friend, F. Hoffmann-La Roche Ltd employee EM Lewiecki, Merck, Eli Lilly, Novartis, Sanofi Aventis, Amgen, Pfizer, Wyeth, Roche, GSK, Procter & Gamble, Grant/Research Support EM Lewiecki, Merck, Eli Lilly, Novartis, Procter & Gamble, Sanofi Aventis, Roche, GSK, Wyeth, Servier, Amgen, Consultant EM Lewiecki, Procter & Gamble, Shareholder

P372-S

CORRELATION OF PHALANGEAL QUANTITATIVE ULTRASOUND WITH PQCT AND DXA MEASUREMENTS IN HEALTHY POSTMENOPAUSAL

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Background: Quantitative ultrasound (QUS) parameters are associated with material properties of cortical bone and are used to predict the risk of osteoporotic fractures. Peripheral quantitative computed tomography (pQCT) allows the measurements of compartment-specific density and geometry-based parameters of cortical bone. However, the diagnosis of osteoporosis is based on the assessment of bone mineral density measurements with dual X-ray absorptiometry (DXA), but it does not consider the space distribution and inherent material properties of bone tissue. The aim of the study was to examine the hypothesis that pQCT and phalangeal QUS provide additional information to the DXA in the prediction of osteoporotic fracture risks in postmenopausal healthy women.

Method: We investigated the correlation between amplitude-dependent speed of sound (Ad-SoS) using phalangeal QUS (DBM Sonic Bone Profiler. Igea, Carpi, Italy) and trabecular, cortical + subcortical and total bone mineral density (tBMD, cBMD and totBMD) using pQCT of distal radius (XCT 2000. Stratec, Pforzheim, Germany) and bone mineral density (BMD) at the spine (L2–L4) and femoral neck (FN) by DXA (Excell. Norland-Stratec, Fort Atkinson, WI) in 216 postmenopausal healthy Spanish women between 46 and 82 years old and 8.31 ± 6.81 years since menopause (YSM).

Results showed that Ad-SoS measured by phalangeal QUS were significantly correlated with volumetric bone mineral density tBMD, cBMD, and totBMD of distal radius ($r = 0.147$, 0.172 and 0.178 respectively; $p < 0.05$ in all), but not with DXA measurements at L2–L4 and FN ($p > 0.05$). By stepwise regression, being Ad-SOS as dependent variable and tBMD, cBMD, totBMD, L2–L4 BMD, and FN DMD as independent variables, only cBMD ($\beta = 0.156$, $p = 0.0096$) was significant statistically.

Conclusion: Ad-SoS seems to be a useful method to monitoring volumetric bone mineral density in postmenopausal healthy women.

Conflict of Interest: None declared

P373-M

ASSOCIATION BETWEEN BIOCHEMICAL MARKERS OF BONE TURNOVER AND TOTAL SKELETAL UPTAKE OF TECHNETIUM-99M LABELED METHYLENE DIPHOSPHONATE

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Background: The skeletal uptake of 99mTc labeled methylene diphosphonate (99mTc-MDP) is regularly used for producing images of pathological bone uptake. In a clinical context incorporation of 99mTc-MDP reflects bone formation. Quantification of such uptake by calculation of the total skeletal uptake (TSU) of 99mTc-MDP would therefore correlate with biochemical markers of bone turnover. This study was done in an attempt to validate a panel of biochemical markers of bone formation and resorption with TSU of 99mTc-MDP in a sample of postmenopausal women.

Methods: 22 postmenopausal women (52–80 years) who were free of bone diseases or bone-active medications volunteered to participate. The total body bone mineral density (BMD) was measured by DXA. Scintigraphy procedure was performed by injecting 520 MBq of 99mTc-MDP. Whole body images were taken 3 minutes and 5 hours after injection to obtain whole body radioactivity. The TSU of 99mTc MDP after 5 hours was calculated by reducing the urinary loss and the soft tissue uptake from the first image and expressed as a percentage of the radioactivity of the 3 minutes image.

The bone formation markers used were serum bone specific alkaline phosphatase (S-Bone ALP) and four different assays for serum osteocalcin (OC). Bone resorption was assessed by serum tartrate resistant acid phosphatase 5b (S-TRACP5b) and serum C-terminal cross-linked telopeptides of type I collagen (S-CTX). Also three different assays for urinary OC (U-OC) were analysed.

Results: The median TSU of 99mTc MDP was 23 % (range 5 to 48 %). All bone turnover markers were significantly correlated, with the TSU of 99mTc MDP with r-values from 0.52 ($p = 0.013$) to 0.90 ($p < 0.001$). Even though the two bone resorption markers had higher correlations (S-TRACP5b $r = 0.90$ and S-CTX, $r = 0.80$) with TSU of 99mTc MDP, than the bone formation markers (S-Total OC, $r = 0.72$, and S-Bone ALP, $r = 0.66$), the difference was not statistically significant. The TSU of 99mTc MDP did not correlate with age, weight, body mass index or BMD.

Conclusions: Biochemical markers of bone turnover are strongly correlated with the skeletal metabolism as measured by TSU of 99mTc MDP. Even though 99mTc MDP should be incorporated into bone as a formation procedure, markers of bone formation seem not to be more correlated with such uptake than markers of bone resorption. This should be due to the tight coupling between formation and resorption taking place in healthy postmenopausal women.

Conflict of Interest: None declared

P374-T

THE EFFECTS OF LOW-INTENSITY PULSED ULTRASOUND ON PREVENTING THE DISUSE OSTEOPOROSIS IN RATS

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Background: Low intensity pulsed ultrasound (LIPU) is an important and effective physiotherapy modality used frequently in enhancing fracture healing.

But its effects on disuse osteoporosis are not clear. In the present study, we compared the effects of LIPU on immobilization and paralytic disuse models.

Methods: 28 male SD rats were divided into 4 groups (n = 7 each group) randomly. Two groups were performed patellar tenotomy on right legs as immobilization models and received sham-LIPU or LIPU treatment, and two groups were performed neurectomy of right sciatic nerves as paralytic disuse models and received sham-LIPU or LIPU treatment. At 4 weeks post operation, average bone mineral density (aBMD) of both right and left tibia were measured by using quantitative computer tomography (QCT) at the level of epiphysis.

Results: In sham-LIPU groups, the aBMD in operated groups decreased significantly than that of the non-operated groups (Paired t-test $p < 0.01$). In LIPU groups, aBMD did not decrease significantly in tenotomy group ($p > 0.05$). But there was also a difference between operated and non-operated tibia in neurotomy groups ($0.01 < p < 0.05$). Moreover, in neurotomy groups, difference was found in bone loss percentage change between sham-LIPU and LIPU groups (Independent t-test, $p < 0.05$). While, there was no difference in tenotomy groups.

Conclusions: LIPU has an effect on preventing the bone loss in the disuse model without neural injury (Immobilization), while it may not play a significant role in preventing the bone loss in the disuse model with neural injury (paralytic disuse). The results indicated that the innervation in bone may play an important role in the LIPU effects.

Conflict of Interest: None declared

P375-S

BONE MINERAL DENSITY AND BONE TURNOVER IN POSTMENOPAUSAL WOMEN WITH REMISSION CUSHING'S DISEASE

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It is well known that clinical signs of endogenous hypercorticism including secondary osteoporosis rapidly reverse on the base of hormonal remission after radiosurgical treatment of Cushing's disease (CD). The aim of the study was to assess BMD and bone turnover biochemical markers in postmenopausal women with complete CD-remission.

We examined 43 women aged 38–67 years in physiological postmenopause who had a complete long-term hormonal CD-remission for at least 2 years (11 ± 6.8 years in average) after combine treatment with hemiadrenalectomy and radiosurgery. Control group comprised 98 healthy postmenopausal women at the same age and postmenopausal age. BMD at lumbar spine (L2–L4), proximal femur and distal forearm was measured utilizing DEXA. Bone turnover was assessed by serum osteocalcin and CTx.

Our main findings were BMD in postmenopausal CD-patients was higher vs. controls in lumbar spine (1.233 ± 0.17 vs. 1.146 ± 0.20 g/cm^2, $p < 0.05$), trochanter (0.873 ± 0.15 vs. 0.809 ± 0.15 g/cm^2, $p < 0.05$) and total proximal femur (1.067 ± 0.16 vs. 0.949 ± 0.21 g/cm^2, $p < 0.01$). Frequency of normal BMD was significantly higher in spine (93%), trochanter (93%), total proximal femur (93%) and distal forearm (81%) in CD-group vs. control (62%, 76%, 77% and 59% accordingly, $p < 0.05$). It is also revealed a lower frequency of osteopenia in spine (14%), trochanter (7%) and distal forearm (14%) in CD-group vs. control (32%, 23% and 33% accordingly, $p < 0.05$), although incidence of vertebral deformities were more in CD-group (37%) vs. control (18%, $p < 0.05$). We found significantly lower levels of CTx ($p < 0{,}001$) and osteocalcin ($p < 0{,}01$) in CD-patients vs. controls. A maximal BMD gain was observed in patients with CD remission about 4–5 years and BMD progressively decreased in women with CD-remission more than 5 years. BMD in patient with CD-remission more than 20 years was significantly lower ($p < 0.05$) vs. CD-remission 4–9 years.

Our results demonstrate that postmenopausal women with complete CD-remission have a higher BMD and a lower bone turnover than healthy postmenopausal women of the same age and maximal increase in BMD is observed at about 5 years of complete CD-remission.

Conflict of Interest: None declared

P376-M

EFFECTS OF PRIOR ANTIRESORPTIVE THERAPY ON MARKERS OF BONE FORMATION AFTER 6 MONTHS OF TERIPARATIDE TREATMENT IN POSTMENOPAUSAL WOMEN WITH OSTEOPOROSIS: RESULTS FROM THE EUROFORS TRIAL

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The aim of this predefined analysis of the EUROFORS trial was to investigate the effect of a prior predominant antiresorptive (AR) therapy on the changes in biochemical markers of bone formation in women treated with teriparatide (TPTD) for 6 months, following predominant AR use for at least one year. Predominant AR was defined as an AR taken for >12 months, with any other bisphosphonate being taken for <3 months.

Methods: 326 patients had received a predominant AR [alendronate [ALN], risedronate [RIS], etidronate [ETI], non-bisphosphonate [NBP] before starting TPTD, and had serum N-terminal propeptide of type 1 collagen (PINP) measured at baseline and at least one follow up visit. The response was examined in a Multiple Measures Repeated Model (MMRM) including type and duration of predominant prior AR, lag time between stopping prior AR and starting TPTD, age and body mass index.

Results: Mean age (SD) was 69.5 (7.5) years. Median (Q1–Q3) AR therapy duration and lag time were 29 (19–48) months and 28 (15–46) days respectively.

Pre-ALN and pre-RIS groups had lower bone formation markers at baseline vs the other two groups ($p < .05$). After 1 month of TPTD treatment, all subgroups showed significant ($p < .001$) increases from baseline for PINP and BSAP. At month 6, PINP levels in the pre-RIS group were significantly greater vs. pre-ALN and pre-NBP. Type of previous predominant AR drug and visit were associated with changes in all markers at 6 months.

Conclusion: Biochemical markers of bone formation increase after 6 months of TPTD regardless previous treatment with AR. Type of previous AR is associated with the endpoint value, with pre-RIS group showing a greater response than the other groups, though the clinical significance of this is unclear.

Table:. Raw median (25th–75th IQ range) values of P1NP (μg/L)

Predominant AR subgroup (n)	Baseline	1 month	6 months
Pre-ALN (141)	21.2 (12.3–33.6)	57.7 (33.0–81.2)	122.9 (81.6–205.1)
Pre-RIS (74)	25.1 (18.0–34.7)	65.9 (48.2–96.0)	183.4 (117.4–258.2
Pre-ETI (38)	44.8 (30.7–55.7)	71.6 (54.8–100.2)	138.3 (74.0–231.8)
Pre-NBP (73)	36.5 (26.8–49.5)	75.8 (51.3–98.0)	124.9 (73.3–187.5)

*Raloxifen = 32; HT = 33; Calcitonin = 6; Vit D metab = 2 cases

Conflict of Interest: F. Marín, C. Barker, H. Oertel, T. Nickelsen are full-time employees of Eli Lilly.

P377-T

LONG-TERM BENEFICIAL EFFECTS OF STRONTIUM RANELATE ON THE QUALITY OF LIFE IN PATIENTS WITH VERTEBRAL OSTEOPOROSIS (SOTI STUDY)

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Objective: In osteoporotic postmenopausal women with prevalent vertebral fracture, quality of life (QoL) is severely impaired. After 3 years of treatment, in a prespecified analysis, strontium ranelate was demonstrated to prevent QoL deterioration in women with vertebral osteoporosis1. The aim of this study was to assess the long-term effects of strontium ranelate, over 4 years on health-related quality of life (HRQoL).

Materials and methods: 1240 Caucasian postmenopausal osteoporotic women included in the double-blind placebo-controlled SOTI study (618 receiving strontium ranelate 2g/d and 622 receiving a placebo) were followed for 4 years.

HRQoL was assessed every 6 months using both the generic questionnaire SF36 and the specific module QUALIOSTâ (23 items specific for vertebral osteoporosis, with a Global score and two sub-scores : physical and emotional) in patients with a baseline and at least one post baseline assessment for the SF36 and QUALIOSTâ questionnaires. The QUALIOSTâ questionnaire presented an excellent internal reliability and reproducibility and a satisfactory concurrent validity with SF36.

Results: Baseline characteristics were similar between-groups (mean age ± SD: 69.7 ±7.3 years, QUALIOSTâ Total score: 39.3 ± 21.4). After 4 years of treatment, QUALIOSTâ scores were significantly lower at endpoint in the strontium ranelate group than in the placebo group and demonstrated improved QoL compared with a deterioration in the placebo group ($p = 0.02$ for the global score; $p = 0.01$ for the Psychological score and $p = 0.03$ for the

Physical score). Analysis of the QUALIOSTâ item specifically relating to back pain revealed that the number of patients free from back pain was 28% higher in the strontium ranelate group compared with placebo ($p = 0.005$).

Conclusion: Strontium ranelate is the first anti-osteoporotic treatment shown by robust methods to be beneficial for the quality of life of patients with vertebral postmenopausal osteoporosis. This beneficial effect on HRQoL was sustained over 4 years of treatment.

1 ECCEO, Roma, 16–19 March 2005.

Conflict of Interest: None declared

P378-S

MINERAL DENSITY AND BONE TURNOVER IN A PATIENT AFFECTED BY SYSTEMIC LUPUS ERYTHEMATOSUS (SLE) TREATED WITH TERIPARATIDE

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Systemic Lupus Erythematosus (SLE) predominantly affects women and is more common in blacks. Although survival rates have improved, over one half of patients with SLE have permanent damage in one or more organ systems. Arthritis and cutaneous manifestations are most common, but renal, haematological and neurological manifestations contribute largely to morbidity and mortality. Additional musculoskeletal complications of SLE include osteonecrosis and osteoporosis. Osteonecrosis, also called avascular necrosis of bone, occurred in 14 percent of patients. It most commonly affects the hip joints. Osteoporosis occurs in 64% of patients and osteoporosis of the lumbar spine is associated with the highest dosage of prednisone and its cumulative effects. Treatment approaches emphasize the use of a combination of drugs to minimize chronic exposure to corticosteroids. Based on currently available evidences, the first choice for prevention and treatment of glucocorticoid-induced osteoporosis should be an oral bisphosphonate such as alendronate (70 mg/wk) or risedronate sodium (35 mg/wk). In the present study we describe a 55-years-old patient affected by SLE with glucocorticoid-induced osteoporosis. She had a severe osteoporosis with multiple vertebral fractures, who was treated with oral bisphosphonate. After 1 month of treatment, the patient developed an adverse drug reaction, characterized by an epidermal necrosis. She stopped the therapy with the remission of symptoms. She started a treatment with teriparatide [recombinant human PTH (1–34)], 20 mcg/day for 18 months. The bone mass was evaluated by lumbar and femoral neck DXA, by ultrasound and by QCT at baseline and after 12 and 18 month of therapy. An evaluation of bone markers was performed at baseline, 2, 6, 12 and 18 month of therapy. After 2 months of therapy an increased Bone Alkaline Phosphatase was observed and maintained during all the period of treatment. DXA analysis showed an increase of bone mass both at femoral neck and lumbar spine. The same results was observed using the ultrasound measurement. The QCT was performed at baseline and after 12 and 18 month. We observed a significant increase in bone mass (65 mg/m3, 85,5 mg/m3 and 96,2 mg/m3 respectively). In conclusion, teriparatide should be considered as a useful therapy for the treatment of osteoporosis in patients affected by autoimmune disease as SLE.

Conflict of Interest: None declared

P379-M

IMPACT OF BISPHOSPHONATE WASH-OUT PRIOR TO TERIPARATIDE THERAPY IN CLINICAL PRACTICE

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Concurrent use of bisphosphonate therapy reduces the anabolic effect of parathyroid hormone. Consequently, in clinical practice interruption of antiresorptive therapy is generally recommended before the initiation of rhPTH. The relationship between the duration of bisphosphonate wash-out and changes in bone turnover and BMD during subsequent PTH treatment, however, remains unclear.

The study cohort consisted of 37 women and men [age, 64.1 ± 14.4 yrs (mean ± SD)] with primary osteoporosis followed in one single Endocrine Clinic. After cessation of bisphosphonate therapy (oral weekly, n = 30; IV 2-monthly, n = 6) patients were started on teriparatide therapy (20 μg daily) plus calcium and vitamin D supplementation and were followed prospectively over 12 months. Markers of bone turnover (bone-specific alkaline phosphatase [BALP], osteocalcin [OC], aminoterminal propeptide of type I procollagen [PINP], carboxyterminal cross-linked telopeptide of type I collagen [βCTX]) were assessed in fasting serum samples at baseline and at 1, 5, and 12 months. Baseline and endpoint BMD were assessed at the lumbar spine and femoral neck.

Biochemical markers of bone formation increased early in the course of therapy (PINP and OC, $p < 0.001$; BALP, $p = 0.03$) and were followed by an increase in βCTX ($p < 0.001$). After 12 months, the mean increase in BMD was 5.6 ± 7.2% at the lumbar spine ($p < 0.003$) and 5.4% ± 11.3% at the femoral neck ($p = 0.03$). Median duration of prior bisphosphonate therapy (42.9 mts, range 3–114) and median duration of bisphosphonate wash-out time (BWT; 4.6 mts; range 0–78) was comparable between oral or IV treated patients (p = ns). BWT correlated only with PINP before starting rhPTH ($r = 0.47$, $p = 0.01$). No relationship was observed between BWT and change in biochemical markers or change in BMD. Patients with positive treatment responses (ΔPINP ≥10 μg/l or ΔLSBMD ≥3%) had similar median BWT as compared to patients with negative responses (p = ns).

Conclusions: This study confirms that beneficial effects of teriparatide on intermediate bone endpoints can be translated into clinical practice with less constringent methodological circumstances as in RCTs. Furthermore, as bisphosphonate wash-out does not appear to influence the treatment effect, teriparatide therapy can be started without prior bisphosphonate wash-out.

Conflict of Interest: None declared

P380-T

THE EFFECT OF PRIOR BISPHOSPHONATE EXPOSURE ON THE EARLY TREATMENT RESPONSE TO TERIPARATIDE IN CLINICAL PRACTICE

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Background: Teriparatide (TP), PTH 1–34, is an anabolic bone agent which is effective at preventing osteoporotic fractures. In the UK, Teriparatide can now only be prescribed to those patients who fail to respond to prolonged treatment with bisphosphonates (BP). However, it has been demonstrated that prior BP exposure blunts TP's anabolic effect. We audited the response to TP achieved in patients attending our osteoporosis clinic to assess the affect of prior BP exposure in clinical practice.

Method: All patients started on TP in our hospital are entered into a database. This contains full medical and drug history as well as BMD and laboratory data. We identified all patients who had at least 12 months treatment with Teriparatide. We divided patients into 2 groups depending on whether or not they had prior BP exposure and compared the response to treatment using P1NP and BMD.

Results: 46 patients had been treated for at least 12 months, 32 with prior BP exposure (mean age 71) and 14 without (mean age 69). There was no significant difference between the groups at baseline apart from there being significantly more prior fractures in those patients treated with BP. The mean duration of BP exposure in those treated with BP was 67.1 (sd 34.8) months and the BP had been discontinued a mean of 0.7 (sd 1.7) months previously. P1NP increased significantly at 3 and 6 months in both groups in response to Teriparatide. However, those without previous BP treatment had a higher baseline P1NP (49 vrs 30ug/l, $p = < 0.01$) and this remained higher at 3 months (109 vrs 76ug/l, $p = 0.14$) and 6 months (183 vrs 132 ug/l, $p = 0.13$) although the difference was not significant. In the prior BP and BP naïve groups the change in BMD was similar at the spine (8.2% vrs 8.1%, $p = 0.93$). Patients without prior BP exposure had a small reduction in BMD at the hip compared to a small increase seen in BP treated patients (-0.67% vrs 1.98%, $p = 0.06$).

Conclusion: This study demonstrated a trend towards a smaller, but still significant, increase in P1NP in response to TP in patients with prior BP exposure. Although this suggests a blunting of the anabolic effects of TP, in our clinic population this is not resulting in a reduction in BMD gain. At both the spine and hip the BMD response to TP was similar, if not better, in those with prior BP exposure compared to BP naïve patients.

Conflict of Interest: None declared

P381-S

CHARACTERIZATION OF BONE QUALITY IN HIP ARTROPLASTY: COMPARISON BETWEEN FRAGILITY HIP FRACTURE AND HIP OSTEOARTHRITIS

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Osteoporosis (OP) and osteoarthritis are two common diseases in elderly people and seldom coexist. OP is a chronic disorder characterized by reduced bone strength and increased susceptibility to fracture. Bone strength depends on both density and quality. Bone density and quality alterations worsen the ability of OP bone to support prosthetic implants, likely reducing primary (immediate support to implant by close bone) and secondary stabilization (due to bone ingrowth). Aim of the study was to evaluate potential skeletal differences in hip

artroplasty patients for fragility fracture or for osteoarthritis. We evaluated 18 subjects (82 ± 8.0 yrs) with fragility hip fractures (HFG), 35 subjects (70 ± 8.0 yrs) with arthritis of the hip (OAG) and 19 subjects (70 ± 5.5 yrs) with normal femoral bone mineral density (CTL). Serum and urinary bone metabolism markers were evaluated within 48 hrs after hip fracture and preoperatively cement hip artroplasty in the HFG, preoperatively non-cement total hip artroplasty in OAG and CTL. Histomorphometric analysis was performed on bone samples obtained during arthroplasty surgery to characterize differences in bone microarchitecture. A significant difference was found in Ca-PTH axis with secondary hyperparathyroidism in HFG vs OAG and CTL. Further, two markers of bone resorption, CTX and TRAP, were significantly increased in the HFG vs both OAG and CTL ($p < 0.0007$ and $p < 0.0039$ respectively for CTX and $p < 0.002$ and $p < 0.0007$ TRAP). No differences were found in 25-OH-vitamin-D3 levels among groups, but all subjects were vitaminD3 deficient. Histomorphometric data showed a better connectivity in OAG as compared to HFG. In conclusion, our data show significant differences in bone structure between patients who underwent hip artroplasty for a fragility hip fracture or for hip osteoarthritis. Finally, biochemical bone turnover markers might play a role in hip fragility fracture prediction. Therefore, it would be important to make an accurate preoperative evaluation of bone health status and also careful surgical planning in order to optimize hip artroplasty surgery.

Conflict of Interest: None declared

P382-M

USES OF TOPICAL BISPHOSPHONATES IN DENTAL PRACTICE. A PRELIMINARY REPORT ON COAGULATION

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Odontological surgical intervention as extractions, implants, etc., always affects the surrounding bone structure. Also, in subjects with bone metabolic diseases as osteoporosis; receiving bone catabolic agents as corticosteroids; and/or being affected by intra-oral immune or infectious conditions, such as peridental disease or ostheomielitis, the bone remodeling may result over-normal. In consequence a useful portion of local bone mass may be lost at that moment. For these cases, the use of topical bisphosphonates formulations is suggested in order to prevent the excessive resorption of bone, and/or to maintain the local supportive efficiency of calcified tissue. Bisphosphonates are potent adsorbers of minerals, including calcium and others, and may impair coagulation at the surgical site, this side event is favorable as it may allow the protracted interaction between the bisphosphonate and the exposed bone surface, avoiding a quick closure of the area. Under these circumstances, before testing the local antiresorptive efficacy of topical bisphosphonate formulations, we studied the effects of 3 different compounds on ex-vivo blood coagulation. Blood samples (3cm3) from veno-puncture, were obtained from a group of 7 healthy volunteers, young adults, 3 women and 4 men. Each sample-tube was mixed with two doses of 0,2 and 0,5mL of the following gels: placebo; disodium pamidronate 2.5%; monosodium alendronate and monosodium olpadronate crystal form A (IG9402) 1.0%. Tubes were incubated in 37 degrees. Coagulation was normally achieved (between 7 and 9 minutes) in all the placebo samples, but in none of the tubes having bisphosphonates (up to 4 hours of observation), including the lower and the higher dose. There was no need to add sodium chloride for the lapse without coagulation was considered long enough for the therapeutic purposes. It is concluded that all the tested bisphosphonates doses and compounds in gel formulations were effective to maintain a protracted bleeding time.

Conflict of Interest: Montangero, Univ. Maimónides, Grant Support

P383-T

DIFFERENT STRATEGIES FOR MONITORING THE BIOCHEMICAL RESPONSE TO RALOXIFENE THERAPY: RELATIONSHIP TO CHANGES IN BONE MINERAL DENSITY

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The compliance with osteoporosis therapy may change over time; this would result in changes in the level of bone turnover markers. A measurement made at the end of a period of therapy may not capture this well. The aim of our study was to examine the biochemical response to raloxifene in relation to change in bone mineral density (BMD, comparing use of frequent measurements of bone resorption markers, to use of a measurement made at the end of two years.

Fifty postmenopausal, osteopenic women (BMD T score between –1 and –2.5 at lumbar spine or hip), age 50 to 80 years were recruited to take part in a two-year randomised, controlled study. Subjects were randomized to raloxifene 60 mg/day plus elemental Ca 500 mg/day ($n = 25$) or no treatment ($n = 25$). Samples were collected at baseline and at 1, 2, 4, 8, 12, 24, 36, 48, 72, and 96 weeks. Second morning void urine was collected on 4 consecutive days prior to each visit. Urinary NTX was measured by automated analyzer Vitros ECi, ©Ortho Clinical Diagnostics (Rochester, NY, USA) and results corrected for creatinine. BMD of the lumbar spine (LS) and total hip (TH) was measured during 2 visits a week apart at baseline and 96 weeks (QDR 1000W Hologic Inc, Bedford, MA, USA). Change in NTX was calculated using the mean of 4 consecutive samples at each visit. Both the percentage change from baseline to 96 weeks (NTX % at 96weeks) and the mean percentage change across all study visits (NTX mean % over 96 weeks) were calculated. BMD change was calculated from the mean of the two baseline scans compared to the mean of the two 96 week scans.

The relationships between change in NTX and change in BMD over 96 weeks in the raloxifene treatment group are shown in the table below. The correlation between NTX and bone density changes was stronger when the mean change in NTX during weeks 1 to 96 was used. This measure integrates the NTX response and may be a truer reflection of the bone marker response than a single NTX measurement at 96 weeks compared to baseline. Using multiple time-points may also reduce noise which would improve the relationship. In conclusion there is a stronger relationship between NTX and bone density changes at 96 weeks when an average change in NTX of all time-points is used rather than a single time-point measurement.

Table:

	NTX % at 96 weeks	NTX mean % over 96 weeks
LS BMD %	$r = -0.51$, $P = 0.043$	$r = -0.65$, $P = 0.006$
TH BMD %	$r = -0.39$, $P = 0.14$	$r = -0.53$, $P = 0.036$

Conflict of Interest: KE Naylor, Unipath, Grant Research Support JA Clowes, Eli Lilly, Grant Research Support NFA Peel, Eli Lilly, Speakers Bureau R Eastell, Eli Lilly, Grant Research Support, Consultant R Eastell, Unipath, Grant Research Support, Consultant

P384-S

NO ASSOCIATION BETWEEN KNOWLEDGE ON OSTEOPOROSIS AND ADHERENCE WITH TREATMENT – A CROSS-SECTIONAL QUESTIONNAIRE STUDY

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Background: It is well-known that medical treatment of osteoporosis is combined with low adherence. Several studies show that after two years of treatment, adherence is lower than 50%. Identification of predictors of adherence is of interest in order to optimize patient care.

Aim: This study was undertaken to assess adherence with bisphosphonates and raloxifene and determinants of adherence (knowledge of osteoporosis, age, gender, education etc.) in two clinical cohorts.

Design: This cross-sectional study comprised 1074 patients in a University Clinic and 287 patients in a private practice who were started on specific anti-osteoporosis therapy between January 1999 and December 2003. Data on adherence and a number of possible confounders were collected using a questionnaire. In addition, knowledge on osteoporosis was assessed in the hospital cohort using a questionnaire. Questionnaires were issued in 2006. Reminders were issued after 1–2 months. All non-responders and patients who had died were coded as non-compliant.

Results: In the two cohorts, 145 and 35 patients had died, respectively. Similarly, 125 and 43 patients declined participation. A total of 223 men (age 33–93) and 915 women (age 36–93) were included in the study (response rate 84 %). The mortality in men was significantly ($p < 0.001$) higher compared with women. At 5 years, the adherence with therapy was 51% and 69% in the hospital and private clinic cohorts, respectively ($p < 0.010$). Adherence was not significantly associated with sex, age, knowledge on osteoporosis, or education. The study showed a trend towards higher adherence ($P = 0.08$) in patients with family members with osteoporosis. We found no significant difference between adherence to once daily and once weekly alendronate. Our study has some limitations. First, the study was cross-sectional. Second, our data reflects ''worst case'' since all non-responders were coded as ''non-adherent''. Third, patients were not randomly allocated to the different treatments.

Conclusion: Adherence with specific pharmacological therapy after 5 years was higher in patients treated in a private clinic compared with a hospital cohort. Knowledge of osteoporosis, level of education, age and sex were not significantly associated with adherence rate.

Conflict of Interest: B. Langdal, Eli Lilly, Consultant K. Brixen, Eli Lilly, Consultant, Speakers Bureau K. Brixen, Merch Sharp & Dohme, Grant/Research/Support, B. Langdal, Novartis, Consultant K. Brixen, Novartis, Consultant, Speakers Bureau D. Nielsen, Nycomed, Advisory board B. Langdal,

Nycomed, Consultant K. Brixen, Servier, Consultant, Speakers Bureau D. Nielsen, et al. Roche and Procter and Gamble, Research support

P385-M

PRIMARY- AND SECONDARY PROPHYLAXIS TO THE USE OF INHALED GLUCOCORTICOID IN PRIMARY HEALTH CARE

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Inhaled glucocorticoid (iGC) is widely used in asthma and COLD.

Aim: To investigate the extent of iGC treatment in general, and to what extent general practitioners (GP) manage the risk of GIO.

Method: Questionnaire was sent to all 3,617 GP's in Denmark.

Results: 904 of 3,617 GP's did respond. 535 questionnaires were valuable regarding iGC, representing 1,265,797 citizens corresponding to 23.4 % of the Danish population. Patients in short time iGC treatment: 25 (4.6 %) of the responding GP's recommended calcium + vitamin D (Ca + Vit D) to patients in iGC treatment without evaluation of DXA or X-ray. Their recommendations were dosage related. 24 % started at a budesonid dose of 200 μm/day, 16 % at 400 μg/day, 40 % at 800 μg/day and 20 % at 1, 600 μg/day. The remaining 510 (95.3 %) performed DXA or X-ray before prophylaxis. The 510 began primary prophylaxis after DXA. 61.4 % began at a T-score < -1, the percent increased to a total of 72.4 % at T-score < -2.5. 77.5% of the GP's recommended Ca + Vit D when X-ray verified osteoporotic fracture. Patients in long time iGC treatment; 6 (1.1 %) of the 535 GP's forwarded their patients for DXA and/or X-ray of the spine prior to or during treatment with iGC whereas the remaining 98.9% did nothing. Treatment with bisphosphonate and Ca + Vit D in iGC treated patients: 4 (0.7 %) of the GP's recommended bisphosphonate and Ca + Vit D without performing DXA or X-ray. The remaining 527 GP did not recommend bisphosphonate and Ca + Vit D without DXA or X-ray. The GP's were once again asked which demands they made for starting the primary- and secondary prophylaxis. The weight of demands were evidently. 86.7 % recommended prophylaxis if a DXA scanning showed a T-score < -2.5.

Conclusion: Most GP's in Denmark follow the same recommendations of primary- and secondary osteoporosis prophylaxis in iGC patients and in postmenopausal osteoporosis.

Conflict of Interest: P. Schwarz, Lilly, Consultant

P386-T

BIOCHEMICAL MARKERS OF BONE TURNOVER PREDICT BMD CHANGES AT CORTICAL AND CANCELLOUS SITES IN A 16-MONTH STUDY OF DENOSUMAB (A FULLY HUMAN RANKL ANTIBODY) IN AGED OVARIECTOMIZED CYNOMOLGUS MONKEYS

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Denosumab, a fully human monoclonal antibody against RANKL, causes marked suppression of bone turnover markers and increases BMD at cortical and cancellous sites in postmenopausal women and in non-human primates. Therapeutic suppression of bone turnover markers typically correlates with increments in BMD; we tested whether this relationship was maintained during long-term (16 month) denosumab therapy in aged OVX cynomolgus monkeys.

One month after OVX or sham surgery, OVX animals were treated with either vehicle (OVX-Veh) or denosumab at 25 or 50 mg/kg (SC, monthly) for 16 months ($n = 14–20$/ group). Shams received vehicle. Serum CTx, a resorption marker, and BSAP, a formation marker, were assessed at regular intervals. BMD was measured at several sites by DXA or pQCT, and terminal BMD was expressed as % change from baseline for statistical comparisons.

OVX-Veh was associated with significant increases in serum CTx and BSAP throughout the study ($p < 0.05$ vs Sham), and denosumab treatment of OVX animals caused marked reductions in CTx and BSAP ($p < 0.05$ vs OVX-Veh and Sham). OVX-Veh led to significant decreases in areal BMD (aBMD) of the lumbar spine, total hip, and central tibia ($p < 0.05$ vs Sham), and denosumab treatment led to significantly greater aBMD at each of these sites ($p < 0.05$ vs OVX-Veh and Sham). OVX-Veh was associated with significant reductions in cortical volumetric BMD (vBMD) at the radius and tibial diaphyses ($p < 0.05$ vs Sham), and denosumab treatment led to significant increases in vBMD at both sites ($p < 0.05$ vs OVX-Veh).

Linear regression analyses were performed across all groups to evaluate relationships between absolute Month 6 values for BSAP or CTx vs terminal BMD (% change from baseline). CTx and BSAP values were lower in each denosumab-treated animal compared to levels found in any untreated (OVX-Veh or Sham) animal. Denosumab-related turnover suppression was associated with increased aBMD at the lumbar spine (a predominantly cancellous site), and with increased vBMD of the radius diaphysis (a purely cortical site). Regression analyses suggested that CTx and BSAP values predicted 55–63% of the Month 16 changes in BMD at the lumbar spine and radius diaphysis.

In summary, denosumab suppressed bone turnover markers in aged OVX cynomolgus monkeys to levels that were significantly lower than in ovary-intact controls. This level of suppression was associated with significant increases in BMD at cortical and cancellous sites.

Conflict of Interest: MS Ominsky, Amgen Inc, Employee SY Smith, Amgen Inc, Paid contract DJ Farrell, Amgen Inc, Paid contract J Schroeder, Amgen Inc, Employee JE Atkinson, Amgen Inc, Employee PJ Kostenuik, Amgen Inc, Employee

P387-S

COMPARISON OF RISEDRONATE, RALOXIFENE, CALCITONIN AND ACTIVE VITAMIN D TREATMENTS IN POSTMENOPAUSAL OSTEOPOROSIS WITH LOW BACK PAIN

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The present study assessed the efficacy of risedronate, raloxifene, calcitonin and active vitamin D treatments in osteoporosis patients on chronic low back pain, bone mineral density (BMD) and bone resorption.

METHOD: A total of 140 postmenopausal women with lumbar spine BMD 2 SD or more below the young adult mean and without any fresh vertebral fractures in the lumbar spine were randomly assigned to one of four groups. All patients reported chronic low back pain persisting for more than 2 weeks. Thirty-five patients received oral risedronate 5 mg daily (risedronate group: RSD), Thirty-five patients received oral raloxifene 60 mg daily (raloxifene group: RLX), Thirty-five patients received eel calcitonin 20 IU intramuscularly once a week (calcitonin group: CLC), and Thirty-five patients received oral active vitamin D (1α-OH-D3) 1 microgram daily (vitamin D group: Vit.D) for four months. Back pain was serially evaluated by a visual analogue scale (VAS) at the first examination, and after 1 month and 4 months of treatment. BMD of the distal end of radial bone was measured by double X-ray absorptiometry (DXA) at the first examination and after 4 months of treatment. As a bone resorption marker, the level of urinary cross-linked N-terminal telopeptides of type I collagen (NTx) was measured by enzyme-linked immunosorbent assay at the first examination and after 4 months of treatment. There were no significant differences in baseline characteristics including age, VAS, BMD and NTx among the four groups.

RESULT: Back pain was improved in all groups after 4 months of treatment. The RSD group showed significantly greater improvement than the Vit.D group after 1 month and 4 months of treatment ($p = 0.0035$). The RSD and CLC group also showed significantly greater improvement than the Vit.D group after 4 months of treatment (RSD: $p = 0.0091$, CLC: $p = 0.0434$). The urinary NTx levels were reduced in the RSD, RLX and VitD groups, but not in the CLC group. BMD was increased in the RSD groups and reduced in the Vit.D group. There were no serious side effects in any of the patients during follow-up.

CONCLUSION: These findings suggest that risedronate and calcitonin have the same level of efficacy in treating chronic pain associated with osteoporosis unrelated to vertebral fractures. And risedronate show earlier analgesic efficacy than calcitonin.

Conflict of Interest: None declared

P388-M

PRIMARY CARE PHYSICIANS' PERCEIVED CERTAINTY OF THEIR PATIENTS RISK FACTOR STATUS INCREASES FOLLOWING A MULTIFACETED OSTEOPOROSIS EDUCATIONAL INTERVENTION: CANADIAN QUALITY CIRCLE (CQC) NATIONAL PROJECT

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Background: Quality Circles (QCs) methodology was utilized to improve primary care physicians' (PCPs) management of osteoporosis in accordance with the Osteoporosis Canada 2002 Guidelines.

Methods: The project consists of five phases: wave I data collection, 1st educational intervention, wave II data collection, 2nd educational intervention, and wave III data collection. During the educational intervention QC's met to discuss profiles of the physician's management of osteoporosis and to participate in an osteoporosis workshop. This interim analysis (wave I & II) examined the change in risk factor awareness. All risk factors were derived from the guidelines

and included age ≥ 65 years; prior hip, wrist or spine fracture; family history of a fracture; menopause before age 45 years; any other major risk factor, two or more minor risk factors. A total of 340 (wave I) and 301 (wave II) PCPs formed 34 QC. For each wave, PCPs collected data from different patients via chart reviews and a standardized collection form. A total of 8376 (wave I) and 7354 (wave II) patient records were selected at random and analyzed. All patients were women 55 years and older. The generalized estimating equations approach was used to evaluate differences in appropriate utilization of BMD testing pre and post educational intervention. An exchangeable correlation matrix was used for the analysis. Odds ratios and 95% confidence intervals (CI) were calculated.

Results: At baseline, physician perceived uncertainty of their patients risk factor status ranged from 0.0% for age to 50.7% for family history of fracture. Univariate results indicated that PCPs perceived certainty increased for prior hip fracture 6.6 (95% CI: 2.5, 17.5), prior wrist fracture (OR 2.5; 95% CI: 1.4, 4.3), prior vertebral fracture (OR 1.5; 95% CI: 1.1, 1.9), family history of fracture (OR 2.9; 95% CI: 2.5, 3.5), menopause before the age of 45 years (OR 2.0; 95% CI: 1.7, 2.4), any other major risk factor (OR 2.6; 95% CI: 1.5, 4.5), and two or more minor risk factors (OR 3.8; 95% CI: 2.6, 5.6).

In conclusion, the use of QCs was an effective knowledge translation approach that improves family physicians' awareness of their patients risk factor status. This improvement will hopefully have a beneficial effect on clinical outcomes.

Conflict of Interest: B Kvern, Procter & Gamble/Sonofi-Aventis, consultant G Ioannidis, None A Hodsman, Eli Lilly, Merck Frosst, NPS, Zelos Therapeutics, Servier, Procter & Gamble/Sonofi-Aventis, Medical advisory boards, speaker fees A Papaioannou, Eli Lilly, Merck Frosst, Amgen, Procter & Gamble/Sonofi-Aventis, Novartis, grants, consultant L Thabane, none A Gafni, none D Johnstone, Employee of Procter & Gamble C Crowley, Employee of Procter & Gamble JD Adachi, Eli Lilly, Merck Frosst, Amgen, Procter & Gamble/Sonofi-Aventis, Novartis, grants, consultant

P389-T

THE MICRODAMAGE ACCUMULATION IS MORE FREQUENT IN PATIENTS WITH IMPAIRED TRABECULAR BONE MICROSTRUCTURE

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Microdamage accumulation and impairment of trabecular (tb) structure may indicate deterioration in bone quality. Little is known about the relationship between these in human. Fifty postmenopausal women with osteoporosis (mean age of 68.2 ± 6.7 years, BMD T-score of −1.6 ± 0.9 at total hip and −2.8 ± 0.9 at lumbar spine; 56% with prevalent fractures) were investigated cross-sectionally. Twenty-five had been treated previously with alendronate 10 mg/day to 70 mg/week (mean duration 56.5 months) while twenty-five were treatment-naïve. Nine trabecular histomorphometric indices, crack density (Cr.Dn) and crack surface density (Cr.S.Dn) were determined and correlated in exploratory analyses without multiplicity adjustment. In the pooled patient group, 7 and 4 out of 9 correlation analyses between trabecular structure and Cr.Dn (Tb.Ar, Tb.Ard, Tb.Th-2D, Tb.N-2D, Tb.N-3D, Tb.Sp-2D, Tb.Sp-3D) and Cr.S.Dn (Tb.Ar, Tb.Ard, Tb.Th-2D, Tb.Sp-2D), respectively could be determined as significant. These correlation results were largely driven by findings from patients in the alendronate group. In alendronate-treated patients, the analyses of the same relationships resulted in the detection of 8 and 7 significant correlations, respectively (Table). In contrast, in treatment-naïve patients these were only 3 (Tb.Ard, Tb.Th-2D, Tb.Th-3D) and 0. In alendronate-treated patients, up to 20% to 30% of the variability of the microcrack parameters could be explained by the trabecular microstructure. In conclusion, the results indicate that impaired trabecular microarchitecture is associated with accumulation of microdamage. The more significant correlations seen in the alendronate-treated patients might also be due to selection bias.

Table: Correlations in patients treated with alendronate

	Cr.Dn r, p-value	Cr.S.Dn r, p-value
Tb area (mm^2)	−0.54, 0.005	−0.46, 0.020
Tb area-derived (%)	−0.54, 0.005	−0.48, 0.015
Tb perimeter (mm)	−0.47, 0.018	−0.40, 0.048
Tb thickness 2-D (μm)	−0.47, 0.019	−0.38, 0.058
Tb number 2-D (/mm)	−0.46, 0.021	−0.41, 0.043
Tb separation 2-D (μm)	0.54, 0.005	0.42, 0.035
Tb number 3-D (/mm)	−0.48, 0.014	−0.46, 0.021
Tb separation 3-D (μm)	0.49, 0.012	0.44, 0.029

Conflict of Interest: I. Pavo, Eli Lilly and Company, Employee and Shareholder A. Sipos, Eli Lilly and Company, Employee and Shareholder H. Petto, Eli Lilly and Company, Employee J. J. Stepan, Eli Lilly and Company, Grant/Research Support J. Li, Eli Lilly and Company, Grant/Research Support D. Michalská, Eli Lilly and Company, Grant/Research Support D. B. Burr, Eli Lilly and Company, Grant/Research Support H. Dobnig, Eli Lilly and Company, Grant/Research Support

P390-S

ROLE OF THE ULTRASOUNDS DEVICES IN THE MONITORING OF BONE STRENGTH IN PEDIATRIC PATIENTS AT RISK OF OSTEOPOROSIS

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BACKGROUND Osteoporosis is characterized by loss of both bone mass and microarchitectural integrity, resulting in an increased risk of fractures with associated morbility and mortality.

Bone mass in the elderly is related to the rate of involutional bone loss and mainly to the peak bone mass reached in childhood and adolescence. The peak bone mass appears as being the key issue problem in prophylactic strategy of osteoporotic therapy. It is determined around for 70–80% by genetic factors. Among the acquired factors some illnesses or the medical therapies for their control can already cause a reduction of the bone mass in childhood, preventing from reaching an ideal value of peak bone mass.

METHODS We present the results of bone strength analysis in children and adolescent affected by pathologies that can determine osteoporosis.

Between neurological pathologies we had analysed 23 patients affected by myelomeningocele; 17 patients affected by Duchenne (DMD), 5 by Becker muscular dystrophy and 10 by Spinal Muscular Atrophy.

We have also examined 21 HIV infected patients, 23 patients in treatment with local steroids for bronchial asthma, 41 patient affected by scoliosis.

The age range was from 1 ys to 18 ys.

The bone strength was evaluated by Sunlight Omnisense, a device designed to measure SOS (Speed of Sound) at multiple skeletal sites, by axial transmission method.

In order to critically interpret the sonographic results of the children included in our study, we have drawn a diagram of reference with the data obtained by the same device in the control group. Speed of sound values, indicatory of bone density, obtained in children affected by various pathologies were compared with those obtained by means of the same device in a control group of 700 Italian healthy children and adolescents aged 6–19 years.

RESULTS In the submitted patients both to QUS that to DXA results obtained with the two different methods and equipment are quite comparable: of course it should be considered the different anatomical region where the exam is performed.

CONCLUSION In therapeutic protocol of patients affected by pathologies at risk for osteoporosis we suggest to use QUS as a screening tool in order to decide which should have DXA.

Conflict of Interest: None declared

P391-M

ATORVASTATIN AND BONE MASS

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Background. Statins are drugs that are useful for the reduction of cardiovascular morbimortality. They are used in the treatment of vascular risk factors and in the secondary prevention of atherosclerosis. Experimental studies have demonstrated their ability to increase bone mass. The objective of this study is to evaluate the effect of atorvastatin on phospho-calcium metabolism parameters, bone remodeling markers and bone mineral density in patients with acute ischemic heart disease.

Methods. Eighty-three patients (52 male and 31 female) with acute coronary syndrome were studied. They received treatment with atorvastatin using low doses (20 mg) and high doses (40 mg–80 mg) according to their basal cholesterol levels and their risk indexes. The patients were monitored during one year. Initial and final cholesterol, triglyceride, calcium, phosphorus, magnesium, PTHi, 25-hydroxyvitamin D and urinary deoxypyridinoline levels were obtained from every patient. Spine and hip densitometries were performed at the beginning and one year later.

Results. In addition to cholesterol and triglyceride levels reduction, treatment with atorvastatin led to a decrease in serum magnesium (8%, $p = 0.0001$) and osteocalcin (25%, $p = 0.0001$), and an increase in PTHi (89%, $p = 0.012$) and vitamin D (33%, $p = 0.007$), as well as a statistically significant reduction in the number of individuals with vitamin D insufficiency. Bone mineral density in-

creased in the spine (1.31%, p = 0.02), but it was statistically significant only in male patients.

Conclusion. Atorvastatin has a beneficial effect on bone metabolism in patients with acute ischemic heart disease (mainly males) by incrementing bone mass through an anti-catabolic mechanism.

Conflict of Interest: Grant Pfizer, Spain

P392-T

A COMPUTER-BASED MEASURE OF IRREGULARITY IN VERTEBRAL ALIGNMENT IS A BMD-INDEPENDENT PREDICTOR OF FRACTURE RISK IN POSTMENOPAUSAL WOMEN

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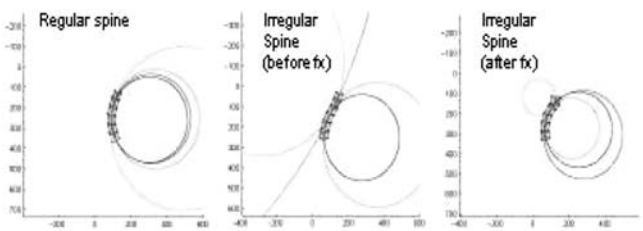
To investigate whether the degree of lordosis or irregularities in vertebral alignment are independent contributors to fracture risk in elderly women.

Participants were 72 healthy elderly women (36 maintained skeletal integrity, whereas 36 sustained at least one fracture over a 7.5-year study period). The two groups were matched for age, BMI, spine BMD L1–L4, smoking habits, and physical activity at baseline. On digitized lateral radiographs, a radiologist annotated the corner points of each lumbar vertebra (L1–L4). Using a computer program, local curvature for each lumbar vertebra was calculated as 1/radius of the curve connecting the midpoint of the respective vertebra with the midpoints of its two neighbors. Lordosis was quantified as the mean of individual curvatures. Irregularity was defined as the average of absolute differences between the individual curvatures and the mean curvature.

At baseline, the measure of irregularity, but not the measure of lordosis, was significantly higher in those who later sustained a fracture compared with those who did not ($1.5x10^{-3}$ vs. $2.0x10^{-3}$, p = 0.002). A vertebral fracture also increased the measure of irregularity ($2.8x10^{-3}$, p < 0.001), whereas no significant changes were detectable in those maintaining vertebral integrity ($1.4x10^{-3}$, p = 0.58).

These findings suggest that measuring irregularities of vertebral alignment might provide a useful supplement to current diagnostic tools (e.g. DEXA) used for fracture risk estimation.



Conflict of Interest: None declared

P393-S

RISK FACTORS ASSOCIATED WITH LOW BONE MINERAL DENSITY AND OSTEOPOROTIC FRACTURES IN BRAZILIAN WOMEN – THE SAO PAULO OSTEOPOROSIS STUDY (SAPOS)

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Background/ Aims: Several cross-sectional studies have confirmed significant association between bone mineral density (BMD) and clinical risk factors (CRF) in Brazilian women. However, the relationship is not very consistent due to size of sample and heterogeneous of the subjects. SAPOS is the first large cross-sectional observational registry of pre and postmenopausal Brazilian women initiated in 2004 with purpose to evaluate the ability of CRF and BMD to predict osteoporotic fracture. Subjects and Methods: A total of 4332 pre and postmenopausal Brazilian women were enrolled in this study who were recruited from primary physicians offices, without secondary osteoporosis. At enrollment, each participant completed a risk assessment questionnaire that included details concerning lifestyle, diet, hormonal factors and drug use and had performed spine and femur BMD (DPX MD +, GE-Lunar). The association between BMD and fracture was assessed using logistic regression, adjusted for important covariates. The risk prediction was similar whether calculated from the manufacturers' young normal values (T scores) or using SDs from the mean age of the NHANES III. Results: mean age, weight, height and BMI was 60.1 ± 10.1 years, 66.1 ± 13.1 kg, 1.54 ± 0.06 m and 27.8 ± 5.2 kg/ m2, respectively. A majority of this population was white (75.2%) and postmenopausal (90.9%). Low-impact fracture was reported by 734 subjects (16.9%) with mean age 52 ± 17.2 years. After adjustments for age and clinical variables, we have found that age (OR 1.072, 95% CI 1.063; 1.081), menopause (OR 2.166, 95% CI 1.494; 3.141), current smoking (OR 1.446, 95% CI 1.127; 1.855), and calcium supplementation (OR 1.64, 95% CI 1.342; 2.003) were significantly related to low BMD, while body mass index, milk consumption, current physical activity and current hormone replacement therapy had significant protective effect on bone mass. On the other hand, age (OR 1.047, 95% CI 1.036; 1.058), menopause (OR 4.16, 95% CI 1.809; 9.563), familial history of hip fracture (OR 3.588, 95% CI 2.883; 4.467) and calcium supplementation (OR 1.451, 95% CI 1.125; 1.872) were significantly associated with osteoporotic fracture. Conclusions: Our results have demonstrated that CRF could be used to predict low bone mass and osteoporotic fractures in pre and postmenopausal Brazilian women.

Conflict of Interest: None declared

P394-M

SHORT-TERM EFFICACY OF ALENDRONATE IN WHEELCHAIR BOUND PATIENTS

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The aim of the study was to establish efficacy of one-year alendronate treatment in 10 wheel-chair bound subjects (6 males, 4 females); age 23.1 +/−2.8 y., immobilized 19.3 +/−7.8 y. Bone status was assessed at heel and wrist (BMD, g/cm^2) and hand phalanges (Ad-SoS, m/s). Laboratory variables included ICTP and bone alkaline phosphatase (bALP). Mean values of bone measurements are shown in Table 1 and did not differ between baseline and follow-up, and mean value of ICTP non-significantly increased and bALP dropped (p < 0.01).

In Figure 1 are shown individual changes. Bold – significant change in bone measurement.

Concluding, in wheel-chair bound subjects short-term alendronate therapy does not reduce bone resorption and causes only small non-significant improvement in skeletal status.

Table: Table 1

	Baseline	Follow-up	Baseline Z-score	Follow-up Z-score
Ad-SoS	2085 +/−72	2100 +/−69	−0.66 +/−1.58	−0.27 +/−1.58
Heel BMD	0.306 +/−0.11	0.290 +/−0.08	−2.73 +/−0.93	−3.09 +/−1.01
Wrist BMD	0.363 +/−0.11	0.402 +/−0.06	−2.42 +/−1.75	−2.25 +/−0.99

Mean values of bone measurements

Nr, Sex	Ad-SoS	Heel BMD	Wrist BMD	ICTP	Bone Alkaline Phosphatase
1 M	-15	**-0.01**	**+0.15**	**0.219**	**-9.1**
2 M	**+35**	**-0.087**	**+0.181**	**1.931**	**0.9**
3 M	**+33**	not available	**+0.045**	**-1.879**	**-14.7**
4 M	-19	0	**-0.043**	**0.529**	**-5.7**
5 M	0	**-0.247**	**+0.149**	**-2.167**	**-3.8**
6 M	**+30**	**+0.22**	**-0.03**	**4.478**	**-7.3**
7 M	**+47**	**+0.009**	-0.008	**-4.327**	**-11.1**
8 F	**-36**	**-0.107**	-0.002	**0.272**	**-13.4**
9 F	+11	**+0.008**	-0.007	**1.712**	**0.2**
10 F	**+66**	**+0.064**	**-0.044**	**2.437**	**-9.8**

Conflict of Interest: None declared

P395-T

REGIONAL THINNING OF CORTICAL BONE IN THE FEMORAL NECK IN HEMIPLEGIA: A PILOT STUDY

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Sideways falls (which typically lead to hip fracture) severely compress the superior–posterior (SP) cortex of the femoral neck and may result in catastrophic local failure of thin cortices. Cortical thinning of the femoral neck SP cortex with age has been reported ex-vivo, possibly reflecting reduced loads on the ageing hip and may predispose to hip fracture. In this study, we used stroke-induced hemiplegia as a model of disuse in humans to measure differences in femoral neck cortical thickness (C.Th) between the hemiplegic and unaffected hips with clinical QCT. 9 patients were selected from the placebo arm of an RCT

evaluating the effects of zoledronate on hip BMD following acute stroke. The subjects (2F, 7M mean age 71.9 ± 11.3) had a >3% decrease in BMD at the hemiplegic hip over a year. They further consented to a single CT scan of both hips (1mm slice thickness, 0.59 mm pixel size) at 22 ± 10.9 months after stroke. Mindways CTXA software (Austin, Texas) was used to reconstruct images and measure bone architecture using a contour tracking algorithm after automatic calibration of greyscale CT images to mineral density equivalents (using a K2HPO4 phantom). A single cross-section of the femoral neck was automatically extracted at a reproducible location (ratio of 1.4: 1 'in plane' to 'out of plane' diameter) for analysis of regional C.Th. To allow for anatomical variation and variable sector placement in between-subject and within-subject comparisons, a nested ANOVA model with random effects was used. This tested differences in C.Th between the unaffected and hemiplegic hips in the SP, superoanterior, infero-posterior and infero-anterior regions. When this showed a significant effect further tests were used to locate the principal regional differences. Longitudinal drift was <1% and the CV for C.Th was 1.9%. The overall p-value for the effect of region (relative to variation between sectors within region) was $p = 0.035$. C.Th in the SP region was significantly lower (mean regional difference −0.37mm 95%CI; −0.08, −0.66) in the hemiplegic hip than in the unaffected hip. There were no significant differences in the other regions. In conclusion, lower limb hemiplegia thinned the SP cortex of the femoral neck in this small sample, suggesting the need for a larger prospective study. This work also indicates that femoral neck cortical thickness can be reliably measured in vivo by CT raising the possibility of better detection of those at risk of hip fracture.

Conflict of Interest: J. K. Brown, President of Mindways Software, Inc.

P396-S

EXPERIMENTAL AND CLINICAL RESEARCH INTO EFFECTIVENESS OF CALCEMIN FOR PROPHYLAXIS AND TREATMENT OF OSTEOPOROSIS

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The aim of this study was to estimate the effectiveness of calcemin drug for prophylaxis and treatment of osteoporosis in otherwise healthy elderly people, and in patients with proximal hip fractures in anamnesis.

Materials and methods. During the experimental research 18 adult rats were examined and divided into 3 groups (I group – intact animals, II group – rats after surgical castration, group III – operated animals treated with calcemin). 12 somatically healthy postmenopausal women with structural–functional disorders of bone (osteoporosis or osteopenia) and 10 patients aged 58–78 years with hip fractures (duration of postmenopausal period up to 6 months) were clinically examined and prescribed 1 tablet of calcemin twice a day. Besides, we've examined 20 patients who were not treated with calcium preparations during the period of follow-up (9 of them with hip fractures in anamnesis). The bone state was evaluated by its solidity, chemical composition, mineral component's ultrastructure (experimental research), questionnaire, ultrasound densitometry (clinical research).

Results. Experimental research showed that application of calcimine for adult rats after two-sided ovariectomy brings about considerable improvement of bone solidity, changes in chemical bone composition.

Analysis of calcemin's effectiveness in treatment of postmenopausal patients revealed that application of the drug leads to the reliable decrease of vertebral pain syndrome over 6 month of treatment (index dynamics by visual analogy scale, $-2{,}4 \pm 0$, 4 sm, $p < 0{,}05$) compared to lack of reliable index dynamics of ultrasound densitometry, testifying effectiveness of the drug for prophylaxis of postmenopausal osteoporosis.

Application of calcemin to treat patients with proximal hip fractures led to the reduction of pain syndrome in the damaged extremity (index dynamics according to the data of visual analogy scale: $-2{,}3 \pm 0$, 4 sm, $p < 0{,}05$), improvement of functional status of the extremity (index dynamics by Neverov's scale: $-0{,}8 \pm 0{,}3$ points, $p < 0{,}05$).

Calcemin is an effective means of prophylaxis and treatment of osteoporosis. It certainly decreases intensity of pain syndrome, brings about increase of indexes of structural–functional bone state, and improves functional abilities of patients.

Conflict of Interest: None declared

P397-M

LOW USAGE OF CALCIUM AND VITAMIN D WITH BISPHOSPHONATE THERAPY IN POST-MENOPAUSAL OSTEOPOROTIC WOMEN IN FRANCE AND IN SPAIN

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Objective: Calcium and Vitamin D (Vit-D) supplementation are recommended as co-medication to antiresorptive therapy by European guidelines for osteoporosis treatment. However, co-prescription of calcium and Vit-D with bisphosphonate across Europe does not seem to be very high in clinical practice. The aim of this study was to determine in patients with osteoporosis taking once a week (OAW) bisphosphonates the actual co-intake of calcium and Vit-D and to evaluate how they use their supplements.

Material and Methods: A total of 400 women (200 France, 200 Spain) taking OAW bisphosphonate for at least 3 months, were selected for telephone interviews (15 minutes) by an independent market research organization. Participants were asked about the type of medications taken for the treatment of osteoporosis, including information on supplementation. The participants interviewed in Spain had been 3 months to 5 years or > taking bisphosphonates for an average of 2 years (range more) and participants in France had been taking bisphosphonates for an average 3 months to 5 years or more). > of 1.6 years (range

Results: The study shows that a large proportion of patients, 46% and 40% in Spain and in France respectively, do not take any calcium and/or Vit-D supplementation with their bisphosphonates treatment. Those women that take calcium and Vit-D get it by prescription 91% and 88% in Spain and France, respectively. Few patients buy supplementation themselves. 14% of women in France and 7% in Spain incorrectly take their calcium supplementation with their bisphosphonate (at the same time or within 30 minutes of their BP intake).

Conclusions: Despite clear recommendations for supplementation of calcium and Vit-D for osteoporotic patients taking OAW bisphosphonates, study results show that co-prescription and adherence to supplementation are suboptimal. The low level of co-prescription by physicians is not compensated by self intake. There are patients who take their calcium supplements at the same time as their bisphosphonates, potentially reducing the effectiveness of the therapy.

There is a clear need to improve the usage and the adherence to calcium and Vit-D supplementation in osteoporotic patients taking OAW bisphosphonate therapy.

Conflict of Interest: JM Quesada: Procter & Gamble, consultant B Mann: Procter & Gamble, employee

P398-T

INSUFFICIENCIES IN TREATED AND NON-TREATED POSTMENOPAUSAL OSTEOPOROTIC SPANISH WOMEN: CALCIUM INTAKE AND SERUM VITAMIN D. THE PREVICAD STUDY

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OBJECTIVE: To assess calcium intake and serum vitamin D status in postmenopausal women treated and non-treated for osteoporosis, and to evaluate possible influences on prevalence of different sunlight exposures.

MATERIAL AND METHODS: Cross-sectional, observational epidemiologic study in different hospitals and primary care centres from Spain. Centres were classified according to sunlight exposure (more or less than 2500 sunlight hours/year). Demographic data, medical background, fracture history, pharmacological treatments, and dietary calcium intake survey were collected among postmenopausal women with and without osteoporosis treatment by the participating investigators. Patients gave signed informed consent and a blood sample to measure serum 25OHD in a central laboratory using a validated HPLC fully automated method.

RESULTS: 336 postmenopausal women aged 71.2 (±4.9) were enrolled into the study: 190 treated and 146 non-treated for osteoporosis. Among treated patients, 69.5% took bisphosphonates [BP], 11.1% selective estrogenic receptor modulator [SERM], 1.6% received both treatments (BP + SERM), and 17.8% received other osteoporosis treatments. Dietary calcium intake was insufficient (<1500 mg/day) in 91.0% of all patients, with an average value of 1018.6 mg/day in the whole sample. Mean 25OHD levels were 25.5 ng/ml., and 68.9% presented vitamin D insufficiency (<30 ng/ml). Treated women showed significantly higher 25OHD serum levels than non-treated patients ($p = 0.01$). Vitamin D levels were not different between patients living in areas with more than 2500 sunlight hours per year and those with less sunlight ($p > 0.05$). Bone turnover markers, PTH and 25OHD levels were not significantly influenced by calcium intake ($p > 0.05$).

CONCLUSION(S): Insufficient calcium intake and vitamin D serum levels have an important prevalence both in treated and untreated postmenopausal osteoporotic Spanish women. The importance of an adequate calcium and vitamin D intake should be emphasized among Spanish postmenopausal women in order to adjust the calcium intake and vitamin D status, and in consequence to optimize the osteoporosis therapeutic response.

Conflict of Interest: JM Quesada, Procter & Gamble Pharmaceutical Iberia, Research Support J Delgadillo, Procter & Gamble Pharmaceutical Iberia, Staff Member E Ramírez, Infociencia, Research Support

P399-S

ORAL IBANDRONATE PRESERVES TRABECULAR MICROARCHITECTURE: MICRO COMPUTED TOMOGRAPHY FINDINGS FROM THE BONE STUDY

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In the BONE study, daily and intermittent oral ibandronate increased lumbar spine and proximal femur BMD, reduced bone turnover (urinary CTX) and reduced vertebral fracture incidence (3-year fracture risk reduction: 62%).[1] However, bone strength also depends on the quality of bone microarchitecture. Micro computed tomography (micro CT) provides a quantitative three-dimensional (3D) method to assess trabecular architecture. In BONE, postmenopausal women with osteoporosis were randomised to daily oral ibandronate (2.5mg), intermittent oral ibandronate (20mg every other day for 12 days every 3 months) or placebo. All participants received daily calcium and vitamin D supplements. Of the 110 single transiliac bone biopsies obtained after 22 or 34 months of treatment,[2] 84 evaluable samples were analysed using micro CT (28 from the placebo arm and 56 from the pooled ibandronate arms). Analysis was performed in one laboratory (Creighton University, Omaha, Nebraska) using a Scanco μCT 40 scanner (Scanco Medical, Bassersdorf, Switzerland). Deterioration in trabecular structure due to osteoporosis is characterised by a change from plate to rod elements. The structural model index (SMI) using the 3D view quantifies the bone structure in terms of rods and plates using a differential analysis of the triangulated bone surface. Micro CT analysis demonstrated that SMI was significantly lower with ibandronate vs placebo (median: 1.001 vs 1.365, respectively; 90% CI: −0.626, −0.033), indicating that trabecular bone in the ibandronate-treated group contained more plate elements. In addition, connectivity density was significantly greater with ibandronate vs placebo (median [/mm^3]: 3.904 vs 3.112, respectively; 90% CI: 0.159, 1.517). Micro CT analysis of structural elements associated with bone strength showed greater preservation of trabecular bone after ibandronate treatment compared with placebo. This is consistent with the quantitative histomorphometric analysis conclusions.[2] Further, it supports the results obtained for BMD, bone turnover[1] and hip structural analysis[3] in the BONE study. Thus the reduction in fracture incidence following ibandronate therapy[1] is accompanied with an overall increase in bone strength.

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2. Recker RR, et al. Osteoporos Int 2004;15: 231–7.
3. Fuerst T, et al. J Bone Miner Res 2006;21(Suppl. 1): S287 (Abstract SU323).

Conflict of Interest: RR Recker, Merck, Lilly, Wyeth, P&G, Amgen, Roche, GSK, Novartis and NPS Alleli, Consultant D Masanauskaite, F. Hoffmann-La Roche Ltd employee D Ethgen, GlaxoSmithKline employee

P400-M

THE MOBILE STUDY LONG-TERM EXTENSION: PROGRESSIVE IMPROVEMENTS IN EFFICACY WITH ORAL IBANDRONATE (150MG) WHEN ADMINISTERED MONTHLY

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The material and structure of bone contribute to its strength along with bone mineral density (BMD) and bone turnover, which can be assessed in clinical studies. In the 2-year MOBILE study, monthly oral ibandronate (50 + 50mg, 100mg, 150mg) was at least as effective as 2.5mg daily in gaining BMD and reducing serum CTX.[1, 2] In fact, 150mg was superior with gains in lumbar spine BMD of 6.4% (vs 4.8% with 2.5mg; $p < 0.05$; per-protocol population) and gains in total hip BMD of 3.8% (vs 2.3% with 2.5mg; $p < 0.05$).[2] A total of 1,291 patients completed the MOBILE study, of whom 719 were enrolled into a long-term extension (LTE) study. Patients from the 100mg and 150mg arm continued to receive the same medication, patients in the 50 + 50mg or 2.5mg daily arms were re-randomised to either 100mg or 150mg. Adverse events are being continually monitored. A total of 693 patients (347 100mg, 346 150mg) were eligible for inclusion in the intent-to-treat analysis of BMD. Patients in both arms had further increased lumbar spine BMD after the first year of LTE on top of the original gains at 2 years (1.1% additional increase with 100mg, 1.5% additional increase with 150mg). Of the 346 patients in the 150mg arm, 168 had received 150mg ibandronate during the initial 2 years and thus completed 3 years of treatment with 150mg. In this population, a substantial increase of 7.6% in lumbar spine BMD was achieved after 3 years ($p < 0.0001$ vs baseline). For patients receiving 100mg monthly for 3 years, the increase in lumbar spine BMD was 6.4% ($p < 0.0001$ vs baseline). Total hip BMD also significantly increased (4.1%, 150mg and 3.4%, 100mg; $p < 0.0001$). Reductions in the marker of bone turnover, serum CTX, seen in the 2-year MOBILE study[1,2] continued to be maintained in the first year of MOBILE LTE. Monthly oral ibandronate continued to be well tolerated, with no clinically significant differences in adverse events between the two arms. There were 8.4% (100mg) and 7.8% (150mg) treatment-related adverse events. The incidence of clinical osteoporotic fractures was low (3.6% 100mg, 2.2% 150mg) and only 1.7% (100mg) and 1.9% (150mg) of patients withdrew due to adverse events. These data confirm that monthly ibandronate 150mg provides continuous improvements in efficacy over 3 years of treatment and confirm the good tolerability of monthly ibandronate seen in the original 2-year MOBILE study.

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2. Reginster J-Y, et al. Ann Rheum Dis 2006;65: 654–61.

Conflict of Interest: J-Y Reginster, Servier, Novartis, Negma, Lilly, Wyeth, Amgen, GlaxoSmithKline, Roche, Merckle, Nycomed, NPS, Theramex); Lecture fees when speaking at the invitation of a commercial sponsor (Merck Sharp and Dohme, Lilly, Rottapharm, IBSA, Genevrier, Novartis, Servier, Roche, Merckle, Teijin, Analis, Theramex, Nycomed, Novo-Nordisk, Consultancy Fees J-Y Reginster, Bristol Myers Squibb, Fondation Leon Frédéricq (Liège), Standard de Liège, Merck Sharp & Dohme, Novartis, Roche, GlaxoSmithKline, Research/Grant support F Sedarati, F. Hoffmann-La roche Ltd employee C Neate, F. Hoffmann-La roche Ltd employee JA Stakkestad, F. Hoffmann-La roche Ltd, GlaxoSmithKlineGrant/Research Support

P401-T

FAVOURABLE TOLERABILITY WITH CONTINUED ADMINISTRATION OF QUARTERLY INTRAVENOUS IBANDRONATE INJECTIONS: THE DIVA STUDY LONG-TERM EXTENSION

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For some patients with postmenopausal osteoporosis, intravenous (i.v.) bisphosphonate therapy may be preferable to oral treatment for reasons including gastrointestinal intolerance or difficulty in complying with oral dosing requirements. Ibandronate is a potent nitrogen-containing bisphosphonate that is available for both i.v. and oral treatment. A randomised, double-blind phase III trial, DIVA, showed that 2-monthly or 3-monthly i.v. ibandronate injections were at least as effective as daily oral treatment (2.5mg) in increasing bone mineral density (BMD) and reducing markers of bone resorption during 2 years of treatment.[1] I.v. ibandronate was generally well tolerated and in particular there was no evidence of renal toxicity. A long term extension study (LTE) is currently being conducted to determine the efficacy and safety of i.v. ibandronate over a further 3 years. Eligible patients who completed 2 years of treatment in DIVA continue to receive the same dosing regimen of i.v. ibandronate in this open label study; patients receiving daily oral ibandronate in DIVA were re-randomised to i.v. treatment. After the first year of the LTE, safety data are available for 781 patients (381 receiving 2mg every 2 months; 400 receiving 3mg every 3 months). Overall, ibandronate continued to be well tolerated, with the pattern of adverse events similar to that seen in the 2 years of the randomised study and with no evidence for late or cumulative toxicity. The proportion of patients reporting any adverse event during the first year of the LTE (third year of continuous treatment) was 83% in the 2-monthly group and 81% in the 3-monthly group, while treatment-related adverse events occurred in 20% and 16% of patients, respectively. Serious adverse events occurred in 12% or fewer patients in each group (only 2 events deemed treatment-related) and only 9 patients (all in the 2-monthly group; 2.4%) withdrew because of adverse events. These results indicate that quarterly ibandronate i.v. injections continue to be well tolerated after 3 years of continuous treatment.

1. Delmas PD, et al. Arthritis Rheum 2006;54: 1838–46.

Conflict of Interest: DM Reid, Roche Pharmaceuticals, Grant/Research support, Consultant L Hyldstrup, F. Hoffmann-La Roche Ltd, GlaxoSmithKline, Eli-Lilly Denmark, MSD, Novartis, Novo-Nordisk and Nycomed, Consultant R Grant, F. Hoffmann-La Roche Ltd employee C Neate, F. Hoffmann-La Roche Ltd employee C Leigh, F. Hoffmann-La Roche Ltd employee S Adami, F. Hoffmann-La Roche Ltd, GlaxoSmithKline, Grant/Research support, Consultant

P402-S

ERYTHROMYCIN INHIBITS IMPLANT WEAR- INDUCED OSTEOLYSIS THROUGH MODULATION OF VEGF SIGNALING IN A MOUSE MODEL

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BACKGROUNDS: Two VEGF receptors have been identified, VEGF receptor-1 (Flt-1) and VEGFR-2 (Flk-1). Activation of VEGF/Flt-1 signaling might be critical in the development of aseptic loosening (AL). The aim of this study was to investigate the therapeutic effects of erythromycin (EM) on ultra high molecular weight polyethylene (UHMWPE) particles-induced osteolysis and VEGF signaling activation in a mouse model.

METHODS: UHMWPE particles were introduced into established air pouches on BALB/c mice, followed by implantation of calvaria bone from syngeneic littermates. EM treatment started two weeks after bone implantation (2 mg/kg/day, ip). Both EM untreated mice and mice injected with saline alone were included as controls (10 mice per group). Pouch tissues were harvested two weeks after EM treatment for analysis. Osteoclast formation was determined by tartrate-resistant acid phosphatase (TRAP) staining, and expression of VEGF and Flt-1 was measured by immunostaining. Implanted bone degradation was analyzed by micro-CT. Statistical analysis was performed using ANOVA method.

RESULTS: Immunostaining showed that both VEGF and Flt-1 was significantly increased by UHMWPE particle stimulation. EM treatment resulted in a significant reduction of VEGF and Flt-1 staining ($p < 0.05$). Intense TRAP staining was observed in pouches stimulated with UHMWPE. EM treatment significantly reduced TRAP+ cells ($p < 0.05$). In consistent with these findings, micro-CT analysis showed a significant alteration of plateau surface contour of implanted calvaria in UHMWPE containing pouches, as compared to saline control pouches. EM treatment significantly improves UHMWPE particles-induced bone degradation. The bone protective effects of EM treatment were further confirmed by bone mineral density (BMD) measurement.

CONCLUSION: The interactive network of VEGF/Flt-1 pathway and RANKL/RANK pathway may play important roles in the initiation, progression, or resolution of AL. Data shown here indicate that EM treatment inhibits UHMWPE particle-induced tissue inflammation, gene expression of VEGF, Flt-1 and TRAP+ cell formation, and hence improves established UHMWPE particle-induced inflammatory osteolysis in a mouse model. These data suggest that EM down regulates VEGF and Flt-1 gene expression. The molecular mechanism of EM action on VEGF/Flt-1 signaling warrants further investigation.

Conflict of Interest: None declared

P403-M

CHANGES IN SERUM CATHEPSIN K LEVELS, CTX AND BONE MINERAL DENSITY IN POSTMENOPAUSAL WOMEN TREATED WITH ALENDRONATE

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Introduction: Cathepsin k is a member of the cysteine protease family that cleaves both helical ad telopeptide regions of collagen I, the major type of collagen in bone. The enzyme is restricted to osteoclast at sites of active cartilage and bone remodelling. It appears at the ruffled border of actively resorbing osteoclast, so it could be a specific marker of bone resorption. There are few data about the changes of cathepsin K in patient on treatment with bisphosphonates. Aims: To evaluate the performance of serum cathepsin K as a biochemical marker of bone resorption in women with postmenopausal osteoporosis before and after 3, 6 and 12 months of treatment with alendronate. To compare serum cathepsin K levels between premenopausal healthy women, postmenopausal women without osteoporosis and osteoporotic women. Patients and methods: We selected 46 patients (64 ± 7 years) with densitometric criteria of osteoporosis (T-score < 2.5 SD) that started alendronate treatment (70 mg/weekly). Serum samples were obtained at baseline and after 3, 6 and 12 months for measurements of biochemical markers of bone remodelling. DXA was performed after 12 months of treatment. Serum cathepsin K levels were measured by ELISA (Biomedica Medizinprodukte GhbH & Co KG Wien, Austria). Serum CTX was measured by ELISA (Elecsys β CrossLaps, Roche Diagnostics SL, Barcelona, Spain). We also measured bone turnover markers and serum cathepsin K in 20 postmenopausal women without osteoporosis (58 ± 6 years) and 20 premenopausal healthy women (29 ± 5 years). Results: Serum levels of cathepsin K were higher in postmenopausal women with osteoporosis (9.4 ± 11 pmol/L) compared with postmenopausal women without osteoporosis (6.8 ± 8.1 pmol/L) and premenopausal women (6.3 ± 5 pmol/L). We observed a significant reduction in cathepsin K levels after alendronate treatment (17 % at 3 months, 22 % at 6 months and 41 % at 12 months, $p < 0.05$). Serum CTX levels decreased 63 % at 3 months, 64 % at 6 months and 59 % at 12 months ($p < 0.05$). There was no correlation between changes in serum cathepsin K and CTX levels. Changes in CTX were negatively correlated with BMD changes in lumbar spine at 12 months. Conclusions: Serum cathepsin K seems to be a good resorption marker in postmenopausal women on treatment with alendronate and may identify women with osteoporosis. Serum CTX can be useful in the prediction of response to treatment.

Conflict of Interest: None declared

P404-T

THE IMPACT OF STEOPOROSIS ON PERIPROSTHETIC BONE MINERAL DENSITY

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Background. The poor condition of periprosthetic bone stock is a risk factor for the implants loosening. BMD around implants may be influenced by some factors.

Objectives. To evaluate the influence of osteoporosis on periprosthetic BMD loss and recovery after uncemented hip arthroplasty within 1–15 months after surgery.

Methods. Total uncemented hip replacement with Zweymueller endoprosthesis was made in 118 coxarthrotic women (46–79 years). All patients were divided into two groups: those with and without osteoporosis. The groups did not differ significantly in the mean age, axial and initial periprosthetic BMD. Bone density around prosthesis stem was measured in Gruen zones by DXA (Lunar Prodigy) 3, 6, 9, 12, 15 months following surgery.

Results. In 6 months, BMD loss was detected in all Gruen zones. The highest BMD decrease was evident in both groups (without and with osteoporotic): R6 (−14.5 % and −20,3% , r = −0,98), R7 (−16,2% and −25,4%, r = −0,95) zones. Subsequent periprosthetic bone deficit in groups was different: in 12 months R1 (−5,2% and −13,3), R2 (−2% and −5,2%), R6 (−5% and −9,7%), R7 (−2% and −7%; $p > 0,05$). By month 15, BMD tended to increase in all zones except for R1, R2 and R6 for the group with osteoporosis (−2% , −2,3% and −3,2% as compared with initial values). The difference between groups with osteoporosis and without in R 6 was found significant in 15 months ($p < 0,05$).

Conclusions. Osteoporosis causes an unfavourable effect on periprosthetic bone recovery after hip uncemented arthroplasty, and may therefore be regarded as a risk factor of implant loosening. Antiresorptive agents may considerably increase BMD.

Conflict of Interest: None declared

P405-S

THE IMPACT OF CALCIUM INTAKE ON PERIPROSTHETIC BONE MINERAL DENSITY

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Background. The poor condition of periprosthetic bone stock is a risk factor for the implants loosening. BMD around implants may be influenced by some factors.

Objectives. To evaluate the influence of calcium consumption on periprosthetic BMD loss and recovery following uncemented hip arthroplasty.

Methods. Total hip uncemented replacement with Zweymueller endoprosthesis was made in 50 coxarthrotic women (46–79 years). All patients were divided into two groups (25 pts) with usual calcium intake more than 900 mg and less than 600 mg per day. The groups did not differ significantly in the mean age, axial and initial periprosthetic BMD. Bone density around prosthesis stem was measured in Gruen zones by DXA (Lunar Prodigy) 3, 6, 9, 12, 15 months following surgery.

Results. BMD loss was the greatest in 6 months in all Gruen zones (varying from 15,9% to 23,0%). Differences between the groups were not significant (total decrease 17,3% and 19,1%, respectively). Subsequent periprosthetic bone recovery in calcium-replete group was almost complete within 12 months (from 7,7% BMD loss in 7 zone to 3,8% increase in 4 zone; total decrease 2,2%; $p > 0,05$). In 15 months, BMD tended to increase in all zones except for zones 1 and 7 (total increase 5,9% as compared with initial value). In calcium-deficient group, BMD around prosthetic stem considerably decreased within 12 months (from 10,6% in 6 zone to 7,2% in 3 zone; 8,8% BMD loss in total). Even within 15 months BMD did not recover, except for zone 5 (4,2% total periprosthetic BMD loss). The difference between calcium-replete and calcium-deficient groups was significant in 15 months ($p < 0,05$).

Conclusion. Calcium deficiency causes an unfavourable effect on periprosthetic bone recovery following hip uncemented arthroplasty, and may be considered a risk factor of implant loosening.

Conflict of Interest: None declared

P406-M

SHORT TERM ORAL PAMIDRONATE IMPROVES OSTEOPENIC SITES AT THE HUMAN MANDIBLE. A PQCT DENSITOMETRIC ANALYSIS

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The human mandible owns a particular bone metabolic rate, which is 4 to 10 fold higher than in most other parts of the skeleton, and it suffer from different types of osteoporosis, mainly postmenopausal, disuse, and overuse (high remodeling rate). Consequently, the bone supporting teeth and prosthesis may get lost affecting their functionality during mastication. High doses (intermittent schedules) of the very potent bisphosphonates may impact adversely on mandible (osteonecrosis), but daily doses of moderate potent compounds has been long standing used, and never related to such type of adverse events. Hence, daily, low dose oral pamidronate, can be a good option to prevent or treat osteopenic conditions located at the jaw. We tested 24 adult patients, in an open clinical observation, having mandible osteopenia, and also lumbar or hip osteopenia. Therefore they received gastric resistant soft capsules 200mg/d oral pamidronate (Gador SA, Buenos Aires), during a 6 month period. Bone mineral content was assessed by pQCT (XCT 3000; Stratec, Porfzheim), using threshold analysis in order to detect the internal areas within the peridental mandible. Areas above 430mg/cm3 are considered having abundant bone structure (type I), and those below 190mg/cm3 are considered osteoporotic (type IV), being type II and III areas of intermediate quality separated by the 310mg/cm3 threshold. Areas up to 700mg are considered as sub-cortical, according to previous established cut-off criteria. All the patients were studied before and after treatment (± 2weeks). After 6 months, whole mandible section did not change outside the range of variation of the method, but particularly the sub-areas type III improved (+4.7% ± 2.2) at the expense of type IV (−1,9% ±1,6; P < 0,05). Sub-cortical proportion of the medullar space was found reduced in 83% of the cases (p < 0,05), and remain steady in the rest, suggesting a trend to type III/II tissues predominance under pamidronate treatment. No serious side events were reported. We conclude that oral, daily pamidronate can be an effective treatment to impact at the osteopenic sites of the jaw, during up to 6 months.
Conflict of Interest: Gador SA, Advisor

P407-T

SUSTAINED EFFECT OF PTH (1–84) ON RISK OF VERTEBRAL FRACTURES 12 MONTHS AFTER CESSATION OF THERAPY

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Introduction: PTH (1–84) has proven to be efficacious and safe in clinical trials for 18 months of treatment. The effect of additional 6 months of treatment with PTH (1–84) and the effect of PTH (1–84) 12 months after cessation of therapy have not been reported.

Methods: The long term efficacy of PTH (1–84) therapy was studied in the Open-label Extension Study (OLES), which included 781 women treated with PTH (1–84) in the TOP Study. The Treatment of Osteoporosis with PTH (TOP) study, an 18-month, multinational, randomized, double-blind, placebo-controlled trial, assessed the effect of recombinant human PTH(1–84) on vertebral fracture incidence in postmenopausal women with osteoporosis. The women in OLES received a total of 24 months PTH (1–84) therapy (18 months in TOP and 6 months in OLES) and were followed up for an additional 12 months. Subjects in the TOP study included postmenopausal women with low bone mass without (n = 2056) and with (n = 471) prevalent vertebral fracture at baseline, 55 years of age or more with spine, femoral neck, or total hip T-score below or = −2.5 (or below or = −2.0 of the women had a vertebral fracture + prevalent vertebral fracture), or 45–54 years of age with a T-score below or = −3.0 (or below or = −2.5 + prevalent vertebral fracture). All received 700 mg calcium + 400 IU vitamin D. The demographics of the OLES subpopulation did not differ from the TOP population.

Results: At the completion of TOP 1.5% (95% CI: 0.67–2.40) vs 3.9% (95% CI: 2.63–5.15) of the women had a new vertebral fracture respectively in the PTH (1–84) group and in the placebo group at 18 months. After additional 6 months of open labelled treatment with PTH(1–84), 1 new vertebral fracture in the former PTH(1–84) group corresponding to an incidence of 1.7% (95% CI: 0.77–2.56) was seen. In the 12 months of OLES placebo treatment only 2 new vertebral fracture was seen corresponding to an incidence of 1.9% (95% CI: 0.96–2.88). From the OLES baseline to Month 18, there were 4 worsened vertebral fractures in the former PTH(1–84) treated group.

Conclusion: In conclusion, the consistency of efficacy of PTH throughout 24 months of therapy is supported by the low number of new fractures from 18 months to 24 months of treatment. After cessation of PTH (1–84) treatment the number of new vertebral fractures remains low in a one year follow-up period.
Conflict of Interest: Prof. C Roux, Nycomed company, consultant Dr. Jesper Clausen, International Medical Affairs, Nycomed Denmark

P408-S

GROWTH HORMONE-DERIVED PEPTIDE (AOD9604) PREVENTS BONE LOSS AND FRAGILITY IN THE OVARIECTOMIZED RAT MODEL OF OSTEOPOROSIS

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A synthetic human Growth Hormone (hGH) 16AA C-terminus peptide, AOD9604 (AOD, Tyr-hGH177-191), has been shown to modulate fat metabolism and is currently being developed as a novel weight-loss drug. However, hGH is known to effect both adipocytes and osteoblasts, which suggests that AOD may also affect bone. Therefore, this current study targets the skeletal effects of two different dosages (0.25mg/kg/day, AOD-0.25) and 0.5mg/kg/day, AOD-0.5) of orally administered drug over a 12-wk period in the ovariectomized (OVX) rat. Each group contained 15 animals. We have assessed bone mass using the traditional DXA and bone fragility using mechanical testing techniques (three-point bending, torsion, femoral neck fracture and vertebral compression). Recently, we have also assessed bone quality through analysis of structure (diaphysis cross-section, strut analysis), mineralization (back scattered electron imaging (BSE), microhardness) and remodeling (histomorphometry). We have previously reported a slight increase in femoral BMD and a 10–20% increase in femoral strength with both AOD doses. In the vertebrae, with AOD-0.25, we reported a 10–15% increase in strength and a 30% increase in toughness while with AOD-0.5 we reported a 30% increase in stiffness. Structurally, we have now associated the decreased femoral fragility with a 15–20% increase in cortical shell thickness although we see no significant differences in cortical mineralization with BSE. In the trabecular bone of the proximal tibia, we observed an increase in connectivity with AOD-0.25, which may influence the increase in toughness and strength. Mineral profiles of the trabecular bone show that both doses cause a 7% increase in mineralization, which may explain some of the increased strength in AOD-0.25 and increased stiffness in AOD-0.5 dosed groups. In conclusion, we have shown a relationship between fat and bone and provided evidence of a skeletal effect of AOD. Specifically, AOD appears to prevent the bone loss and the increase in bone fragility that results from OVX. Furthermore, the dose response of AOD differs in cortical and trabecular bone.
Conflict of Interest: Metabolic Pharmaceuticals Ltd., Grant Research Support

P409-M

REDUCTION IN BONE TURNOVER RESULTS IN A TRANSIENT HIGHLY UNIFORM MINERALIZATION STATE

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On the material level, trabecular bone is a patchwork of bone packets with different mineral contents. This heterogeneity of mineralization results from continuous remodeling, where a small bone volume is resorbed and replaced by an unmineralized bone packet. After deposition, mineralization leads to an increase in mineral content in the bone packet described by the mineralization law. The heterogeneous mineralization of trabecular bone is characterized by a frequency distribution, the bone mineralization density distribution (BMDD). While experimental information about the mineralization law is limited to the first weeks after new bone deposition, the BMDD is experimentally easily accessible, e.g., in the electron microscope looking at the backscattered electrons (qBEI). For healthy individuals it was found that the bell-shaped BMDD is independent of skeletal site, sex, ethnicity and age. Using a mathematical model which considers both processes, mineralization and remodeling, we obtained an expression connecting the BMDD and mineralization law. It allows us to deduce from the experimentally obtained reference BMDD, a mineralization law which defines the ongoing increase in the mineral content in a bone packet over timescales of years rather than weeks. The knowledge of the mineralization kinetics can then be used to predict the full evolution of the BMDD as a function of a change in turnover and also the rate at which this change is applied. When reducing the turnover (to mimic antiresorptive therapy) the BMDD displays transiently a shape with a sharp peak corresponding to bone with an unusual uniformity in its mineral content. The peak in the transient BMDD becomes more pronounced with decreasing turnover. In the context of high turnover osteoporosis the evolution of the BMDD was also assessed as a function of the starting point of an antiresorptive treatment. Our results allow the manipulation of the shape of the BMDD once drugs with a well-defined effect on the turnover are available. The long-term aim is to design patient-specific therapies which bring an abnormal BMDD back to its original healthy state.
Conflict of Interest: None declared

P410-T

LOW LEVELS OF FREE TESTOSTERONE INDEX ARE SIGNIFICANTLY ASSOCIATED WITH OSTEOPOROSIS IN MEN WITH HIP FRACTURES. RESULTS FROM A 1 YEAR POPULATION BASED COHORT

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It is well known that hypogonadism (HG) is a risk factor for male osteoporosis and that osteoporosis is highly prevalent in patients with hip fractures (HFx). However, only few studies have examined the testosterone levels and the prevalence of HG in these patients. Aim: To elucidate the prevalence of HG and the relationship between free testosterone index (FTI) and osteoporosis in male HFx patients. Methods: During 2005 a total of 144 males were admitted to our hospital with a low energy HFx. Excluding patients with dementia, severe comorbidities, and pathological fractures, 106 patients (74 %) were referred for evaluation by DXA, Instant Vertebral Assessment (IVA), biochemical screening, and clinical assessment. Osteoporosis was defined as prevalent vertebral fracture (VFx) and/or BMD T-score < -2.5, defining VFx as a reduction of vertebral height by IVA of at least 25 %. A population based cohort of 783 males, aged 20–39 years, participating in the Odense Androgen Study (OAS), served as controls. FTI was calculated as serum total testosterone (TT)/Sex Hormone-Binding Globulin (SHBG). Low FTI and TT were defined as values below the 2.5 percentiles of these measures (0.471 and 13.2 nmol/l, respectively) in healthy, non-obese men of the young cohort. Results: A total off 78 (54 %) patients aged 77 [46–94 years] showed up for evaluation. Of these patients 52 (67 %) had T-score < -2.5 in either total hip or spine BMD and 49 (63 %) patients had at least one VFx making the overall prevalence of osteoporosis 85 %. Similarly, 55 % of the patients had HG with TT (median, range: 12.7, [0.4; 40.5]) below the 2.5 percentile of young. SHBG rose significantly ($R = 0.28$, $p < 0.05$), FTI declined significantly ($R = -0.39$, $p < 0.001$) while no significant decline of TT was seen with age. SHBG, TT or FTI did not correlate significantly with BMD, BMI, or VFx. Patients (93 %) with FTI below the 2.5 percentile of young had significantly more often osteoporosis compared to patients with normal FTI ($OR = 2.2$, $p < 0.05$). No significant difference was seen between the 2 groups regarding current smoking, alcohol intake, BMI, previous fractures, or prevalent VFx. Conclusion: Our study shows that low FTI and osteoporosis are prevalent in men with HFx and that FTI is significantly associated with osteoporosis. In order to evaluate the importance of this finding we suggest the initiation of intervention studies.

Conflict of Interest: J. Ryg, Danish Centre for Evaluation and Health Technology Assessment, Grant Research Support K. Brixen, Eli Lilly, Consultant K. Brixen, Eli Lilly, Speakers Bureau J. Ryg, Eli Lilly, Other (traveling expenses) M. Andersen, Ipsen, Grant Research Support M. Andersen, Ipsen, Speakers Bureau K. Brixen, Merck Sharp & Dohme, Grant Research Support J. Ryg, Merck Sharp & Dohme, Grant Research Support J. Ryg, Merck Sharp & Dohme, Other (speakers honoraria, traveling expenses) K. Brixen, Novartis, Consultant K. Brixen, Novartis, Speakers Bureau M. Andersen, Novo Nordic, Speakers Bureau J. Ryg, Nycomed, Other (speakers honoraria) M. Andersen, Pfizer, Speakers Bureau J. Ryg, Roche, Other (speakers honoraria) K. Brixen, Servier, Consultant K. Brixen, Servier, Speakers Bureau J. Ryg, Servier, Other (speakers honoraria) J. Ryg, University of Southern Denmark, Grant Research Support

P411-S

ADJACENT FRACTURES AFTER BALLOON KYPHOPLASTY OF OSTEOPOROTIC VERTEBRAL FRACTURES – A PROSPECTIVE TWO YEAR FOLLOW UP

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Introduction: Balloon-kyphoplasty is a minimally invasive procedure for stabilization of osteoporotic vertebral fractures. Aim of the prospective follow up was to evaluate the long-term results of balloon kyphoplasty regarding pain reduction and restoration of vertebral body height.

Materials and methods: In this prospektive study, 63 patients (43 female, 20 male) with 96 osteoporotic vertebral compression fractures (VCF) could be followed up for 24 months.

Preoperatively, ap and lat x-ray-examination as well as CT and/or MRI-scans were performed. Pre- and postoperatively, a visual analog scale concerning pain and the oswestry score were recorded. Vertebral body height and angulation were measured and recorded on conventional x-ray examination pre- and postoperatively and after 3, 6, 12 and 24 months.

Results: After treatment, a long term pain reduction ($p < 0{,}001$) was evident. The oswestry score showed significant better results ($p < 0{,}001$) in comparison to preoperatively. A significant height restoration and reduction of kyphosis angle was achieved using the balloon technique ($p < 0{,}05$). Between 2 weeks and 22 months, in 9 Patients (15.8%) an adjacent VCF occured. The VCF was asymptomatic in 4 patients, 5 patients had to be treated with balloon kyphoplasty again. Asymptomatic cement leakage was observed in 12 out of 96 VCF (12.5%).

During 24 months follow up, the vertebral body height which was achieved postoperatively, could be maintained.

Conclusion: The treatment of osteoporotic VCF, balloon kyphoplasty led to a immediate and prolonged pain reduction. A long term restoration of vertebral body height and reduction of kyphosis angle was achieved.

Conflict of Interest: Dr. Robert Pflugmacher, Paid instructor

P412-M

ACCURATE DETECTION OF ABDOMINAL AORTIC CALCIFICATION ON LATERAL SPINE DXA (VFA) IMAGES

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Background: Radiographic abdominal aortic calcification (AAC) is a significant predictor of incident fatal and non-fatal cardiovascular disease (CVD), independent of other clinical CVD risk factors. Post hoc analysis of a previous small pilot study showed that AAC can be accurately assessed with lateral spine DXA images intended for vertebral fracture assessment (VFA), compared to undigitized lateral spine radiographic films.

Objective: To evaluate the accuracy of AAC assessment on VFA images compared to digital lateral abdominal radiographs.

Methods: One reader (JTS) assessed VFA images for AAC in 153 women, mean age 69.2 (SD 7.8) years, first with a previously validated 24 point scale (AAC-24) and subsequently with a simpler 8 point scale (AAC-8), blinded to his AAC-24 readings. One month later, electronic digital lateral abdominal radiographic images were assessed for AAC-24 and AAC-8, blinded to the prior VFA readings.

Results: Considering radiographic AAC-24 score as the gold standard, the areas under receiver operator characteristics (ROC) curves were 0.953 (95% C.I. 0.911 – 0.996) for radiographic AAC-8, 0.872 (95% C.I. 0.797 – 0.948) for VFA AAC-24, and 0.855 (0.779 – 0.930) for VFA AAC-8.

The bootstrapped non-parametric intra-class correlation between VFA and radiography for AAC-24 was 0.80 (95% C.I. 0.69–0.88) and for AAC-8 was 0.76 (95% C.I. 0.65–0.84).

Conclusion: Using a simplified 8-point scale, spine images obtained with bone densitometry to detect vertebral fracture can also be used to accurately detect radiographic AAC, a significant risk factor for incident CVD.

Conflict of Interest: John Schousboe, Grant support, Hologic Inc. Thomas Hangartner, Grant support, Hologic Inc.

P413-T

THREE YEARS EXPERIENCE WITH THE CLINICAL USE OF BALLOON KYPHOPLASTY

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The most frequently occurring vertebral fractures are osteoporotic of origin and mainly occurring in older patients. Conventional management of vertebral fractures, consisting of pain medication and immobilization represents a serious socio-economic burden.

Balloon kyphoplasty is a minimally invasive percutaneous technique for the management of vertebral body fractures, allowing stabilization 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 for use of viscous cement and low pressure deposition 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. Balloon kyphoplasty can be performed under short general anesthesia, which makes it also suitable for older patients.

In our clinic we selected balloon kyphoplasty for the management of vertebral body fractures and treated since May 2004 over 110 patients. The majority suffered osteoporotic fractures between T6 and L5, though also younger patients suffering traumatic fractures A1 or A3.1 and patients with benign and malignant infiltration were treated. When treating vertebral body fractures in patients younger than 50 years we used the bioresorbable calciumtriphosphate cement.

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 when compared to pre-fracture height.

In the patient group already evaluated at 3 year follow-up, a maintained 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 correction at follow-up. In only one case subsequent vertebral fractures were detected on X-ray.

During the presentation we will provide information on the pathophysiology of osteoporotic vertebral fractures, demonstrate the balloon kyphoplasty 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: None declared

P414-S

RELATION BETWEEN ADIPONECTIN AND BONE MINERAL DENSITY IN PATIENTS WITH POSTMENOPAUSAL OSTEOPOROSIS

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INTRODUCTION: Adiponectin is a novel adipocyte-derived hormone that regulates energy homeostasis and has anti-infammatory and anti-atherogenic effects. Growing evidence suggests that positive associations between fat mass and bone mineral density (BMD). In vitro studies have reported that adiponectin is implicated in OPG-RANK-RANKL system. Regulation of osteoclastic activity is critical for understanding bone loss associated with the postmenopausal period. However, the role of adiponectin in postmenopausal osteoporosis is controversial.

AIMS: Evaluate adiponectin serum levels in patients with osteoporosis postmenopausal and its relation with bone mineral density and osteoclastogenesis markers.

MATERIAL AND METHODS: We selected 54 untreated women with postmenopausal osteoporis (T-score < −2.5 DS) attending in Bone Metabolic Unit. We determined antropometric data, adiponectin (HDK1-61K-A01, RAFER), OPG (OSTEOPROTEGERIN OPG ELISA BI-20402, BIOMEDICA-GRUPPE) y ultrasensible estradiol (E2) (DSL-39100 3rd Generation Estradiol RIA, Diagnostic System Laboratories, Inc) and Bone Mineral Density (BMD) in lumbar spine, femoral neck and total hip.

RESULTS: Adiponectin serum levels of our patients (medium age 63 ± 7 years) was 36.9 ± 25.9 μg/ml. Circulating adiponectin was significantly related to age ($r = 0.38$; $p = 0.012$) and OPG serum levels ($r = 0.3$; $p = 0.04$), but not significantly associated with weight, height, BMI, E2 and BMD in lumbar spine, femoral neck and total hip. In multivariate analysis waist circumference was independently associtated ($p < 0.05$) with adiponectin levels after adjusting for antropometric data (age, BMI, waist circunference and hip). OPG was independently associated ($p < 0.01$) with adiponectin levels after adjusting for hormonal status, BMD and OPG.

CONCLUSIONS: Adiponectin levels could be implicated in the regulation of OPG-RANKL system as suggested by invitro studies. This mechanism should be explored in future clinical studies.

Conflict of Interest: None declared

P415-M

THE EFFECTS OF ESTROGEN RECEPTORS AND AROMATASE GENE POLYMORPHISMS ON BONE MINERAL DENSITY, LIPID PROFILE AND THE RESPONSE TO HORMONE REPLACEMENT THERAPY IN POSTMENOPAUSAL WOMEN

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Estrogen receptor alfa (ER α) genes' polymorphisms have been widely studied in the evaluation of several chronic disorders, such as breast cancer, Alzheimer's dementia, cardiovascular disease (CVD), and osteoporosis. However, results are often conflicting. Controversely limited information is available on the relation of ER b genotype with osteoporosis, with the body mass index and with ovarian disfunction. The effect of ER β polymorphism on lipid profile as well as on the response to hormone replacement therapy (HRT) has never been investigated before The aromatase is an enzyme that catalyze the formation of estrogens from C19 steroids both in men and in women and its polymorphisms, together with the ones of the ER alfa, have been associated with low BMD in postmenopausal women.

The purpose of our study was to evaluate the influence of ER a, ER b and aromatase genes' polymorphisms on both lipid profile and BMD in a population of healthy postmenopausal women. Addictionally in a subpopulation who completed one year treatment with HRT, the potential influence of ERs and aromatase genotypes on the response to HRT was evaluated.

Segregation analysis of two polymorphisms in the ER a gene (PvuII and XbaI) and one polymorphism in the ER b gene (AluI) as well as the TTTA repeats of the aromatase gene with bone mineral density at the lumbar spine and forearm and with lipid profile was performed in 1098 women. In a subpopulation of 280 women who completed 1 year of treatment with HRT, the response of both bone phenotype and lipid profile to treatment was compared with genotypes.

Results: Baseline BMD, incidence of spinal fractures and response to HRT did not significants relate to ER a gene polymorphisms as well as to aromatase gene polymorphism, while a borderline difference in baseline vertebral BMD with the ER b gene polymorphism was found ($p = 0.07$).

Additionally, the ER b gene polymorphism was significantly associated with the response in total cholesterol during treatment with HRT after 1 year ($p = 0.02$).

Conclusions: In a Caucasian population of postmenopausal women no association between ER a and aromatase gene polymorphisms was found with bone mineral density and lipid profile at baseline as well as with their response to one year hormone replacement therapy. Conversely, the ER b genotype appeared to segregate with bone loss and lipid profile in response to hormone replacement treatment.

Conflict of Interest: None declared

P416-T

MEASUREMENT OF QUALITY OF LIFE (QOL) IN PATIENTS ENROLLED IN THE ICARO STUDY (INCIDENCE AND CHARACTERIZATION OF "INADEQUATE TREATMENT RESPONDER PATIENTS" IN OSTEOPOROSIS): RESULTS AT FOLLOW-UP AFTER 12 MONTHS

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ICARO is a multicentre observational project aimed at assessing the outcome at the 12-month follow-up (longitudinal phase) in patients with severe osteoporosis and "inadequate response to antiresorptive treatment", defined as patients prescribed with antiresorptive drugs (alendronate, risedronate and raloxifene) for at least 1 year and presenting a new fragility fracture (vertebral or non-vertebral). Health-related quality of life (HRQOL) was measured by using the QUALEFFO-41 questionnaire, which includes the following domains: pain, activities of daily living, housework, mobility, leisure/social activities, general health perception, and mood.A score = 1 indicates the worst impact and a score = 5 is the best impact on HRQOL. The scores were linearly transformed to obtain a 0 to 100 score, corresponding to the best and the worst HRQOL, respectively. The mean (± SD) changes from baseline in patients with and without new incidental fractures in the follow-up period who answered at least 70% of items at both baseline and endpoint are shown in the following table.P-values refer to the comparisons between subgroups (n = number of evaluable patients). The occurrence of new fractures in the follow-up period caused a worsening of HRQOL (except changes of mood) and the difference between the two subgroups was significant ($p < 0.05$) for mobility and general health perception. The comparison in patients with traumatic and non-traumatic fractures showed that the former group had higher worsening in any domain (not significantly between groups), while the analysis in patients with symptomatic and symptoms-free fractures showed that symptoms caused a more marked worsening of HRQOL (significantly between groups for pain, $p = 0.040$, and mood, $p = 0.003$). These data show that the occurrence of new fractures is associated with a deterioration of HRQOL parameters, being impairment of mobility and of ability in daily activities the most important factor that caused a poor general health perception.

Table: QUALEFFO-41 domains score

	No new fractures	New fractures	P value
Global score	0.18 ± 12.9(n = 740)	3.20 ± 15.2(n = 87)	0.078
Pain	−1.79 ± 22.4(n = 706)	1.76 ± 22.0(n = 84)	0.171
Activities of daily living	0.67 ± 17.1(n = 738)	3.62 ± 20.1(n = 88)	0.189
Housework	0.80 ± 19.0(n = 744)	4.90 ± 23.6(n = 87)	0.122
Mobility	0.20 ± 15.9(n = 743)	4.83 ± 17.9(n = 86)	0.012
Leisure/social activities	1.06 ± 24.5(n = 680)	1.37 ± 28.2(n = 81)	0.918
General health perception	−0.91 ± 18.6(n = 736)	4.69 ± 19.8(n = 87)	0.009
Mood	1.02 ± 15.6(n = 727)	0.13 ± 16.3(n = 85)	0.621

Conflict of Interest: Sandra Silvestri and Raffaella Gentilella are employed at Eli Lilly Italia

P417-S

ICARO STUDY (INCIDENCE AND CHARACTERIZATION OF "INADEQUATE TREATMENT RESPONDER PATIENTS" IN OSTEOPOROSIS): RESULTS AT ONE-YEAR FOLLOW-UP

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ICARO is a multicentre observational project aimed at assessing the incidence of fractures (frs) at the one-year (1Y) f-up (longitudinal phase) in patients (pts) with severe osteoporosis and "inadequate response to antiresorptive treatment", defined as pts prescribed with antiresorptive drugs (alendronate, risedronate and raloxifene) for at least 1Y and presenting a new fragility fracture (vertebral or non-vertebral). The occurrence of frs in the f-up phase was diagnosed by lumbar or sacral X-ray. Of the 1421 pts evaluable at baseline, 862 completed the follow-up at 1Y.90 pts (10.4%), developed a new fracture. Traumatic frs occurred in 24 pts (26.7% of those with frs), non-traumatic and symptomatic in 39(43.3%), non-traumatic and non-symptomatic in 27(30.0%).A number of 796 pts had a prescribed therapy: frs occurred in 85 of them and in 5 with no prescription. The following table shows the incidence of frs in pts prescribed with the 3 anti-resorptive drugs given with or without Ca + VitD supplements: rates of frs were 8.0% and 14.7%, respectively in the two subsets. Rates of frs (irrespective of the use of Ca + VitD) were lower in pts receiving raloxifene (7.5%) than with alendronate (9.3%) and risedronate (14.6%). There was no evidence of differences in rates of pts with traumatic and non-traumatic (symptomatic and non-symptomatic)frs in pts receiving anti-resorptive therapy alone or supplemented with Ca + VitD. Among the pts with a prior therapy, frs occurred in 7.4%, 9.7% and 13.8%of pts treated with the 3 drugs, respectively. The concomitant use of Ca + VitD supplements was associated with a slightly lower rate of pts with frs compared to those prescribed with anti-resorptive therapy alone [45/459(9.8%)vs.45/397(11.3%)]. These results show that approximately 10% pts developed a new fracture during 1Y of f-up. The supplementation of the prescribed anti-resorptive therapy with Ca + VitD was associated with a lower rate of pts with new frs.

Table: Incidence of fractures by prescribed drug

	With Ca + VitD (n = 476)		Without Ca + VitD (n = 320)	
	No new fractu	New fractures	No new fractu	New fractures
Alendronate	279 (63.7%)	20 (52.6%)	167 (61.2%)	26 (55.3%)
Risedronate	98 (22.4%)	12 (31.6%)	71 (26.0%)	17 (36.2%)
Raloxifene	46 (10.5%)	4 (10.5%)	28 (10.3%)	2 (4.3%)
Mixed therapy	2 (0.5%)	0 (0.0%)	4 (1.5%)	0 (0.0%)
Other	8 (1.8%)	2 (5.3%)	3 (1.1%)	2 (4.3%)
None of the 3 drugs	5 (1.1%)	0 (0.0%)	–	–
Total number of patients	438 (100.0%)	38 (100.0%)	273 (100.0%)	47 (100.0%)

Conflict of Interest: Sandra Silvestri and Raffaella Gentilella are employed at Eli Lilly Italia

P418-M

COMPARISON OF EFFECTS OF GENISTEIN, RALOXIFENE AND ESTRADIOL ON DEVELOPMENT OF OVARIECTOMY-INDUCED OSTEOPENIA IN RATS IN VIVO AND ON MURINE OSTEOBLAST ACTIVITY IN VITRO

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There is growing interest in the discovery or development of compounds that provide the benefits of estrogen replacement therapy but do not cause estrogen-dependent side effects. Genistein, a major phytoestrogen of soy, is considered a potential drug for prevention and treatment of postmenopausal osteoporosis. Raloxifene is a selective estrogen receptor modulator, used in the treatment of osteoporosis.

The aim of the present study was to compare the effects of genistein, administered at a dose of 5 mg/kg p.o. daily for 4 weeks to mature ovariectomized Wistar rats, with those of raloxifene hydrochloride (5 mg/kg p.o. daily) and estradiol (0.1 mg/kg p.o. daily). Bone mass, mineral and calcium content, macrometric and histomorphometric parameters were examined.

Also the effects of genistein, raloxifene and estradiol on murine osteoblast activity were compared. Osteoblasts were isolated from neonate mouse calvariae by sequential digestions with 0.1% collagenase and 0.05% trypsin. The investigated compounds were added to the culture media at concentrations of 10^{-9}–10^{-7} M for 48 h. The expression of mRNA specific for alpha2 procollagen type I chain (COL) and alkaline phosphatase (ALP), as well as for factors involved in regulation of generation and activity of osteoclasts: receptor activator of nuclear factor kappaB ligand (RANKL), osteoprotegerin (OPG), macrophage colony-stimulating factor (M-CSF) and interleukin 6 (IL-6) was examined. Reverse transcriptase-polymerase chain reaction (RT-PCR) and densitometry were employed for semiquantitative mRNA expression measurements.

In vivo, estrogen deficiency resulted in increased bone resorption and impaired mineralization. Genistein, raloxifene and estradiol inhibited bone resorption. Raloxifene and estradiol, but not genistein, improved bone mineralization.

In the *in vitro* studies, genistein, raloxifene and estradiol decreased expression of mRNA of IL-6 and RANKL, although only raloxifene decreased the RANKL mRNA/OPG mRNA ratio. All compounds significantly increased expression of ALP mRNA and tended to decrease expression of COL mRNA.

Concluding, genistein may have some potential as an antiosteoporotic drug, however weaker than raloxifene.

Acknowledgement: This study was supported by grant No 2 P05D 092 30 from the Ministry of Science and Higher Education, Poland.

Conflict of Interest: None declared

P419-T

BONE MINERAL DENSITY, MOTOR FUNCTION AND MUSCLE STRENGTH IN DUCHENNE MUSCULAR DYSTROPHY

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Duchenne muscular dystrophy (DMD), the most common muscular dystrophy in childhood, implies an increased risk of osteoporosis. Few studies have been conducted on the bone health in DMD boys. This cross-sectional study examined bone mineral density (BMD), motor function and muscle strength in 24 boys with DMD (2.3 – 19.7 years), most of whom were being treated with low-dose prednisolone, and BMD in 24 age-matched healthy boys (2.7 – 19.6 years). BMD was measured by DXA (GE Lunar Prodigy) and DXL (Demetech). Motor function was classified according to the Vignos scale. Isometric muscle strength was measured using an electronic handheld myometer. Measurements of the knee extensor strength were used for further analysis. In order to determine muscle strength in the knee extensors regardless of the age of the patients, quotients were calculated between values obtained by the patients and reference values.

BMD for total body (TB), TB head excluded (HE), spine, heel, and hip, were lower in the DMD group compared with the control group ($P < 0.001$), as were Z-scores for TB and spine BMD ($P < 0.001$). BMD, at all sites, increased with increasing age in the control group. BMD values obtained from the hip and the heel decreased within the patient group with increasing age while values obtained from the spine remained at the same level during childhood and adolescence. The differences in BMD values between patients and controls increased significantly with increasing age ($P < 0.001$).

Correlations were found between heel BMD and the Vignos grade ($r = -0.80$, $P < 0.001$) and heel BMD and isometric muscle strength ($r = 0.76$, $P < 0.001$). Quotients calculated to show the impairment in knee extensor muscle strength in relation to reference values correlated with the hip BMD values ($r = 0.54$, $P = 0.016$) and even stronger with heel BMD ($r = 0.82$, $P < 0.001$). No correlations were found between results of the motor function tests or muscle strength tests and the BMD values in TB, TBHE or spine. Six out of the 24 DMD boys (25%) had sustained 11 fractures. Seven of these were located in the lower extremities, of which five occurred after loss of ambulation. Nine controls (37.5%) had sustained 13 fractures of which four were located in the lower extremities.

We conclude that BMD is generally decreased in DMD patients compared with healthy matched controls and that these differences in BMD values increase

with age. Moreover that muscle strength seems important to BMD in the lower extremities.
Conflict of Interest: None declared

P420-S

WATER-BASED EXERCISE PROGRAM IMPROVES BALANCE AND QUALITY OF LIFE IN POSTMENOPAUSAL WOMEN WITH OSTEOPOROSIS

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Aquatic environment may be beneficial to individuals who are frail, suffer from pain, are severely kyphotic or have poor balance, such as the osteoporotic population. In our study, we aimed to compare the effects of water-based and land exercise programs on balance, anxiety and quality of life on postmenopausal women with osteoporosis.

60 postmenopausal women with osteoporosis were randomized either to water-based or land exercise groups. Group I (n = 30) received a 3-week water-based exercise program conducted by a physiotherapist at an aquatic centre, five times a week for one hour. Group II (n = 30) received a 3-week land exercise program at home five times a week for an hour. There were strengthening exercises for back and lower limbs muscles and balance exercises in both programs. Beck Depression Inventory (BDI), Short Form 36 (SF-36) questionnaire and step test were performed before and one month after the exercise programs in each group. Paired t-tests are used for analysis.

The mean ages were 55.8 ± 4.6 and 56 ± 4.1, respectively in group I and group II. In group I, mean BDI scores before and after intervention were 11.8 ± 4.7, 7.3 ± 3.5 and 15.7 ± 5.2, 14.7 ± 4.5 in group I and II respectively. Mean BDI scores significantly decreased in group I ($p < 0.001$), but the decrease in BDI scores was not significant in group II ($p > 0.05$). In group I, the mean scores in SF-36 questionnaire before and after intervention were 23.1 ± 3.1, 25.1 ± 2.7 ($p < 0.001$) for physical function, 6.1 ± 1.3, 7.0 ± 1.1 ($p < 0.001$) for role-physical, 7.5 ± 1.9, 8.7 ± 1.4 ($p = 0.003$) for bodily pain, 17.4 ± 3.7, 19.8 ± 3.6 ($p = 0.001$) for general health, 16.8 ± 3.4, 18.7 ± 3.6 ($p = 0.001$) for vitality, 8.2 ± 1.7, 8.6 ± 1.3 ($p = 0.009$) for social function, 4.8 ± 1.1, 5.3 ± 0.8 ($p = 0.007$) for role-emotional and 19.9 ± 3.8, 21.8 ± 3.7 ($p = 0.001$) for mental health, respectively. There was significant improvement in all scales of SF-36 in group I. But in group II, only physical function scale changed significantly ($p < 0.05$). There was a significant improvement in step test, used to measure balance, on the right and left sides after intervention (for right side $p = 0.004$, for left side $p = 0.001$) in group I, but there was no significant improvement in group II (for right side $p = 0.5$, for left side $p = 0.4$).

It is concluded that water-based exercise program rather than land exercise program produced significant improvements in quality of life, anxiety and balance in postmenopausal women with osteoporosis.
Conflict of Interest: None declared

P421-M

SELF-REFERRAL FOR OSTEOPOROSIS ASSESSMENT WITH DXA – WHAT IS THE DIFFERENCE TO PATIENTS SENT BY THEIR PHYSICIAN?

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Objectives: The aim of the study is to assess incidence of diagnosis of osteoporosis (OP) in two groups with different motivation. The first group (Group A) were women referring themselves to an OP evaluation as a reaction on an advertisement in a popular Swiss weekly journal, aimed of informing the general public about OP. The second group (Group B + C) are female patients, referred by their general practitioner.

Materials and Methods: All measurements where performed with dual-energy X-ray absorptiometry (DXA) (Hologic QDR 4500 Elite). The T-scores of three arias were used for comparison; the total hip (T-hip), hip neck (T-neck) and the lumbar spine (T-sp). In a retrospective analysis of 770 consecutive patients undergoing DXA analysed.

The analysis was restricted to those patients with an age of ≥ 40 yrs and young patients with a variety of medical problems such as anorexia nervosa, cystic fibrosis or Crohn's disease were excluded from the analysis. Also patients known with OP and patient treated with high dose of steroids (high OP risk) were excluded.

The self-referred Group A n = 104 aged (mean ± SD) 63.1 ± 8.6 yrs, group B n = 269 aged (mean ± SD) (59.7 ± 9.4 yrs) send by their physician based on risk factors without an underlying clinically evident disease and group C n = 104 patients, aged (mean ± SD) 68.9 ± 10.3 yrs send by their physician based on a fracture.

Results: The T-scores of the groups are: T-hip (mean ± SD) A = (-0.6 ± 1.1), B = (-0.7 ± 1.0), B2 = (-1.7 ± 0.9); T-neck A = (-1.1 ± 1.1), B = (-1.2 ± 1.1), C = (-2.0 ± 0.9); T-sp A = (-1.1 ± 1.5), B = (-1.2 ± 1.4), C = (-2.2 ± 1.3).

Group A was not significantly different group B, but it was significantly different to Group C (independent samples T-test; $p < 0.00$). There was a significantly difference of age between A and B ($p < 0.00$) but not between A and C.

Conclusion: Wide spread public information, which inform about the risk factors for osteoporosis and promote the use of DXA, recruit similar women as physicians sending female patients with risk factors. However, with advertisements a relatively older population is addressed. Nevertheless the professional assignment of a general practitioner does locate patients at higher osteoporotic risk such as patients with a fracture.
Conflict of Interest: None declared

P422-T

PERIPHERAL LEPTIN ADMINISTRATION TO NORMAL FEMALE RATS MAINTAINS BONE MINERAL DENSITY, BONE ARCHITECTURE AND MECHANICAL STRENGTH IN SPITE OF SIGNIFICANT WEIGHT LOSS

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We have previously demonstrated the presence of leptin and leptin receptor in human osteoblasts, and shown that leptin stimulates bone formation. Leptin administered subcutaneously has been shown to reduce ovarectomized-induced bone loss in rats, in contrast to studies showing that leptin administered centrally results in a decrease in bone formation. In the present study we wanted to investigate the skeletal effects of leptin administered subcutaneously to normal female rats.

Thirty-six Fischer rats 15 weeks of age were divided into groups and assigned to the following treatments for 9 weeks: 1. Vehicle (saline) 2. leptin 100 ug/daily, 3. leptin 200 ug/daily, administered continuously by osmotic minipumps (Alzet). Body weight was registered before and during study, and bone mineral density (BMD) was measured by dual X-ray absorptiometry. Biomechanical properties were tested by three-point bending experiments, and bone architecture parameters examined by μCT. Plasma levels of insulin, monocyte chemotactic protein-1, (MCP-1), osteocalcin and bone resorption marker (Collagen 1 fragments) were measured at the end of the study.

At the end of the study, the body weight was significantly lower in the rats receiveing leptin compared to controls. The leptin concentrations in the groups 2 and 3 were14 fold and 33 fold increased, respectively. There were no significant differences in femoral BMD. Whole body BMD was significantly lower in the low dose leptin group, while there was no difference between the high dose leptin group and the control, indicating that the negative effect of weight reduction was overcome by the higher leptin concentration. Both femoral and whole body BMD were significantly higher in both the leptin groups compared to controls, when corrected for body weight or fat mass. Mechanical tests and bone architecture parameters showed that despite the weight loss, the high dose group had similar bone strength and architecture as controls. The low dose group, however, had decreased bone strength and altered Moment of Inertia (MOI). This suggests that the low dose leptin group had altered bone geometry. Plasma levels of MCP-1 and osteocalcin were equal for all groups at the end of the study.

In conclusion, our data show that leptin given at high doses maintains BMD, bone architecture and mechanical strength in spite of the significant decrease in body weight.
Conflict of Interest: None declared

P423-S

3-HYDROXY-3-METHYLBUTYRATE ADMINISTRATION DIMINISHES FUNDECTOMY-INDUCED OSTEOPENIA OF THE LUMBAR SPINE IN PIGS

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Experimental and clinical studies have shown that total or partial gastrectomy leads to severe bone metabolism disturbance and strong osteopenic changes in skeletal system. However, no data are available in humans or animals concerning the effects of 3-hydroxy-3-methylbutyrate (HMB) administration on skeletal system properties under conditions of long-term fundectomy. The aim of the study was to test the hypothesis whether oral administration with HMB diminishes osteopenia of axial skeleton induced by fundectomy. Considering physiology and anatomy of the gastrointestinal tract

and skeletal system, we intended to use the pig model as optimal model for humans. Eighteen male pigs were divided into 3 weight-matched groups at the age of 40 days of life. Animals from the first (n = 6) and the second (n = 6) group were subjected to experimental fundectomy while the third group (n = 6) was sham operated. Starting the day after fundectomy, the first and the second group received placebo (CaCO3 0.05 g/kg of body weight/day) and calcium 3-hydroxy-3-methylbutyrate monohydrate (CaHMB 0.05 g/kg of body weight/day), respectively. Animals were sacrificed at the age of 8 months to obtain L5 and L6 vertebrae for analysis. 3-hydroxy-3-methylbutyrate treatment increased the weight of vertebrae, bone mineral density, bone mineral content, total bone volume, trabecular bone mineral density, mean volumetric bone mineral density and calcium hydroxyapatite density in the trabecular and cortical bone in the fundectomized pigs, when compared to the control animals ($P < 0.05$). Furthermore, mechanical endurance of the spine, expressed by the values of ultimate force, Young's modulus, ultimate stress, stiffness and work to the ultimate force point was increased in HMB-treated pigs, when compared to the control group ($P < 0.05$). In conclusion, oral administration with 3-hydroxy-3-methylbutyrate to pigs effectively diminished fundectomy-induced osteopenia.

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Conflict of Interest: None declared

P424-M

PRECISION EVALUATION OF LUNAR IDXA BODY COMPOSITION MEASUREMENTS

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Dual-energy x-ray absorptiometry (DXA), the gold standard for osteoporosis diagnosis, is increasingly used for body composition assessment. Precision error is a measure of the ability of a DXA system to detect small changes in a patient's body composition and bone mineral density (BMD). Lower precision error reduces the least significant change (LSC), allowing a smaller change in BMD or fat and lean mass to be identified as biological rather than related to instrument variability. Recently, GE Healthcare introduced a new imaging densitometer, the Lunar iDXA. We studied the precision error of Lunar iDXA total body measurements: BMD, bone mineral content (BMC, g), %fat, fat mass (g), and lean mass (g).

We scanned 24 women and 7 men for a total of 31 subjects (mean age 56.9 yrs, SD 13.1; mean BMI 28.4, range 17.3 – 39.6). 13 subjects had BMI values in the obese category (above 30), with a mean total %fat of 38% for the entire group. Each subject's total body was scanned two times, with repositioning between scans. Precision (%CV) was calculated as the root-mean-square standard deviation.

Precision values for Lunar iDXA total body BMD and BMC were 0.56% and 0.57% respectively. Body composition precision values were 0.63% for %fat, 0.59% for fat mass, and 0.45% for lean mass. We conclude that the Lunar iDXA provided excellent precision for total body measurements, including BMD, BMC and body composition.

Table:

iDXA (n = 31) Total Body	Mean	CV
BMD (g/cm^2)	1.065	0.56%
BMC (g)	2318	057%
%Fat	38%	0.63%
Fat (g)	28779	0.59%
Lean (g)	42783	0.45%

Conflict of Interest: None declared

P425-T

EFFECTIVE TRACING OF OSTEOPOROSIS AT A FRACTURE AND OSTEOPOROSIS SCREENING PROGRAM IN GP PRACTICE, EXPERIENCE WITH A MOBILE DEXA FACILITY

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Background / aims: To analyse the results from a fracture and osteoporosis screening program, mobile dual-energy X-ray absorptiometry (DEXA) facility, at a general practitioner clinic in order to achieve efficient case-finding for osteoporosis in patients of 60 years and older with a fracture due to low-energy trauma. Methods: Almost following the publication of new Dutch professional guidelines (NHG-guidelines) for case-finding and treatment of osteoporosis, a fracture case finding program was performed at a general practitioner practice. Patients of 60 years and older with a fracture due to low-energy trauma could be referred for further diagnosis and treatment after initial trauma. Bone-mineral density of the lumbar spine, hip and instant lateral assessment was performed with DEXA. Patients with manifest osteoporosis, defined as having a fracture and a T-score < or = −2 SD (1) at one of the measured sites, were diagnosed as osteoporosis. The results from 36 patients (scandate June 1st & 2nd 2006) were analysed. Results: 36 of the patients were scanned in the mobile DEXA-facility. June 19th 2006 the patients completed the diagnostic process / analysis. A total of 18 (50%) patients had manifest osteoporosis, 6 osteopenia and 12 had normal bone density. Unrecognised vertebral fractures were found in 2 patients. Conclusion: a fracture and osteoporosis screening program in GP practice, with a mobile DEXA facility, proved to be effective and useful for identifying and treating a population at risk of osteoporosis without the intervention of a second or third line hospital facility.

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Conflict of Interest: None declared

P426-S

EFFECTS OF TREATMENT WITH FLUORIDE ON BONE MINERAL DENSITY AND FRACTURE RISK – A META-ANALYSIS

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Background: Fluoride stimulates osteoblastic bone formation. However, the biomechanical competence of the newly formed bone may be reduced compromising antifracture efficacy.

Material and methods: We performed a systematic search of PubMed (1951 and onwards), Embase (1974 and onwards), Web of science (1945 and onwards) using the terms ''fluoride'' AND (''bone mineral'' or ''fracture''). Eligible studies were those reporting original data on changes in BMD or fracture risk with fluoride treatment compared to no treatment, placebo or another treatment regime in a randomised setting. The search yielded 2,028 references.

Results: A total of 25 eligible studies were identified. Spine BMD increased by 7.9%, 95% CI: 5.4–10.5%, and hip BMD by 2.1%, 95% CI: 0.9–3.4% ($p < 0.01$). The estimates were heterogeneous ($p < 0.01$). A meta-regression showed an increase in spine BMD with increasing treatment duration (5.04 ± 2.16%/year of treatment), while there was no effect of fluoride formulation (sodium fluoride, monofluorophosphate or combinations of fluoride with other drugs, $p = 0.83$) or fluoride dose (0.20 ± 0.35 %/mg F equivalents, $p = 0.57$). Overall, there was no statistically significant effect of fluoride treatment on the risk of vertebral (OR = 0.8, 95% CI: 0.5–1.5, test for heterogeneity: $p < 0.01$) or non-vertebral fracture risk (OR = 0.8, 95% CI: 0.5–1.4, test for heterogeneity: $p < 0.01$) in a meta-analysis. Results on fracture risk were rather heterogeneous, and a meta-regression on fracture risk showed a decreasing risk reduction with increasing daily fluoride dose. This was confirmed in a subgroup analysis. With a daily dose of < = 20 mg of fluoride equivalents (152 mg monofluorophosphate/44 mg sodium fluoride) there was a statistically significant reduction in both vertebral (OR = 0.3, 95% CI 0.1–0.9, 6 studies) and non-vertebral (OR = 0.5, 95% CI: 0.3–0.8, 6 studies) fracture risk. However, with a daily dose >20 mg of fluoride equivalents there was no significant reduction, but rather a trend towards an increase in both vertebral (OR = 1.3, 95% CI: 0.8–2.0, 11 studies) and non-vertebral (OR = 1.5, 95% CI: 0.8–2.8, 7 studies) fracture risk.

Conclusions: Fluoride treatment increases BMD in the spine and the hip depending on treatment duration. Overall there was no effect on hip or spine fracture risk. However, in subgroup analyses a low dose of fluoride (< 20 mg/day fluoride equivalents) was associated with a reduction in fracture risk.

Conflict of Interest: None declared

P427-M

EFFECTS OF STRONTIUM RANELATE ON BONE MINERAL DENSITY AND FRACTURE RISK – A META-ANALYSIS

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Aim: To study the effects of strontium ranelate (SR) on changes in bone mineral density (BMD) and fracture risk.

Methods: Randomised placebo controlled trials on SR in men or women retrieved from PubMed (1951 to present), Web of Science (1945 to present), or

Embase (1974 to present). Search date was November 1, 2005. A total of six studies on SR were identified in the initial search, and of these four were included.

Results: SR reduced vertebral (OR = 0.55, 95% CI: 0.48–0.64) and non-vertebral (OR = 0.84, 95% CI: 0.72–0.99) fracture risk and increased spine (6.9%, 95% CI: 2.5–11.3%) and hip BMD (3.9%, 95% CI: 0.8–6.9%) during 24–36 month of follow-up. The increase in spine BMD with SR was larger with 2g per day than with other doses. There was a linear relationship between daily dose of strontium and increase in spine Z-score.

Conclusion: SR is effective in preventing vertebral and non-vertebral fractures and in increasing BMD in patients with osteoporosis.

Conflict of Interest: None declared

P428-T

SEVELAMER RESTORES BONE QUALITY IN OVARIECTOMIZED RATS

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Sevelamer hydrochloride (Renagel®), a non-calcium phosphate binder, is capable of reducing progressive coronary artery and aortic calcification.

We have recently reported that sevelamer could also prevent and restore bone loss that follows ovariectomy in rats (Vukicevic et al., J Bone Miner Res 2005, S289). In the present study our aim was to determine the biomechanical properties of bones from rats treated with sevelamer. Sprague Dawley rats were ovariectomized (OVX) at 6 months and the sevelamer therapy started immediately in following groups: (1) Sham; (2) Sham + sevelamer 3%; (3) OVX; (4) OVX + sevelamer 1%; (5) OVX + sevelamer 3% and continued for 25 weeks in the prevention mode. In the restoration mode sevelamer treatment started 3 months after ovariectomy in these groups: (1) Sham; (2) OVX; (3) OVX + sevelamer HCl 3%; (4) OVX + sevelamer calcium carbonate 1%, (5) OVX + sevelamer calcium carbonate 3%, (6) OVX + calcium carbonate 3%. Therapy continued for 12 weeks.

In order to evaluate the biomechanical properties of rat bones we used the three-point bending and indentation tests. The intrinsic properties, stress, elastic modulus and toughness were calculated from the maximum load, stiffness, energy absorbed, anterior–posterior diameter, and the moment of inertia. In the restoration mode, rats treated with sevelamer HCl 3% had increased maximum load for 14.75% and stiffness for 12, 85% as compared to OVX animals. The cancellous bone was tested by using the indentation test in the marrow cavity of the distal femoral metaphysis. Sham animals treated with sevelamer 3% increased the maximum load for 26.5%, energy absorbed for 25, 3% and the ultimate strength for 25.9% as compared to sham rats. Sevelamer 3% in OVX rats also increased the maximum load for 69, 2%, stiffness for 70, 1% , energy absorbed for 57% and the ultimate strength for 75% as compared to OVX control animals. In the restoration mode sevelamer hydrochloride 3% had better effect on the biomechanical parameters than other treatment groups. It increased maximum load for 91, 5%, stiffness for 74, 2%, energy absorbed for 91, 4% and the ultimate strength for 89, 5% as compared to OVX animals.

These results suggest that sevelamer strongly effects bone strength with a particular reference to the trabecular bone in OVX rats, and could have a positive effect on bone parameters, not only in patients on dialysis, but also in patients with osteoporosis.

Conflict of Interest: Kuber T. Sampath, Genzyme Drug Discovery and Development, employee

P429-S

LOW SERUM TSH CORRELATES WITH THE NUMBER OF VERTEBRAL FRACTURES IN POSTMENOPAUSAL WOMEN WITH OSTEOPOROSIS

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We recently demonstrated that systemically administered TSH at low doses prevents bone loss and restores bone mass in aged OVX rats through anabolic and anti-resorptive effects on bone remodeling (Sampath et al, Calcif Tiss Int, 2006). In the present study, we examined whether circulating TSH levels correlate with reduced BMD and vertebral fractures in post menopausal women with osteoporosis. We selected 939 postmenopausal women with low BMD as determined by ultrasound during population screening tests in our local hospitals. We measured BMD at the lumbar spine and femoral neck using DEX-a, serum TSH concentrations using immunoluminometry, and the number of vertebral fractures determined by two independent radiologists using standardized methods (Genant et al, J Bone Miner Res, 1994). Out of 939 women, 727 had a clinical sign of osteoporosis and a BMD equal or lower than −2.5 T score, while remaining women were osteopenic. After adjustments for age, years since menopause and the body mass index, we found 85% of osteoporotic women had lower TSH serum levels (0.3–1.5 mU/L) without having any disturbances in serum T4 and T3 concentrations. A total of 581 vertebral fractures were recorded on vertebral X-rays in 516 patients (74%). The majority of fractures were in osteoporotic patients who had either one (n = 167; 24.5%) or two (n = 225; 33%) or 3 (n = 124; 18%) fractures. Two hundred and eighty four patients with 1 to 5 vertebral fractures had low TSH levels (0.3–1.0 mU/L), while 142 patients with (132) fractures had TSH levels between (1.01–1.5 mU/L). In patients with TSH above 1.51 mU/L only 8% had 1 or 2 fractures. Ninety nine osteoporotic patients did not have a fracture whose TSH levels were in the range from 0.3 to 5.5 mU/L. These results suggest that the majority of osteoporotic patients with low lumbar BMD and with 1–3 vertebral fractures have TSH serum levels in the lower normal range of 0.3–1.1 mU/L, suggesting circulating TSH levels correlates with decreased BMD and the number of vertebral fractures in postmenopausal women with osteoporosis.

Conflict of Interest: None declared

P430-M

VITAMINN D RECEPTOR (VDR) GENE FOKI POLYMORPHISM AS INDICATOR OF ALENDRONATE THERAPY EFFECTIVENESS

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Background: Osteoporosis is a systemic metabolic bone disease, characterized by changes in microarchitecture and loss of bone mass. Decrease of bone strength and increase of vulnerability for bone fractures are further consequences. Osteoporotic bone fracture often leads to physical disability.

Each decision concerning pharmacotherapy is undertaken on the basis of individual case analysis. The most precise way of estimation of bone fracture risk and optimal treatment is still unknown. The genetic background of the disease suggests that identification of gene polymorphisms can bring answers to both questions. Vitamin D3 receptor gene (VDR) is one of the best surveyed genes in search for correlation between bone mass and the presence of fractures.

The aim of the study was to examine the association of the bisphosphonates treatment effectiveness with VDR gene FokI polymorphism. The association of FokI VDR genotypes with bone mass and low-energy fractures was searched.

Material and methods: We observed 112 female patients from Endocrinology Outpatient Clinic of The Clinical Hospital No2 in Poznan, admitted with diagnosis of osteoporosis in age 49–88 (mean 65,9) treated with alendronate. The study group was divided into 3 subgroups: FF, Ff, ff depending on VDR gene FokI polymorphism. Effects of annual therapy were compared in subgroups with different polymorphic variants FokI (FF, Ff, ff) of VDR gene. In this study we also examined the frequency of certain polymorphisms in the whole group. We performed not only clinical assessment but also densitometry of the hip and lumbar spine with DXA method and genotyping of VDR with method PCR-RFLP.

Results: The prevalence of VDR gene FokI polymorphism in this group was 24 for FF, 62 for Ff and 26 for ff. The largest increase ($\Delta = 0{,}044 g/cm^2$) of BMD in hip was observed in FF group after annual antyresorptive therapy with alendronate. An increase $\Delta = 0{,}033 g/cm^2$ was observed in heterozygotes Ff. The smallest change $\Delta = 0{,}002 g/cm^2$ was in ff group.

Conclusions: 1. The largest increase in BMD after annual antyresorptive therapy with alendronate given daily was observed in female patients with FF genotype for FokI VDR polymorphism. 2. VDR gene FokI polymorphism can be used as an indicator of bisphosphonates therapy effectiveness.

Conflict of Interest: None declared

P431-T

EFFECT OF CATHEPSIN K INHIBITION ON BONE RESORPTION MARKERS IN HEALTHY POSTMENOPAUSAL WOMEN

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Postmenopausal osteoporosis is a common disorder characterized by an increase in bone resorption relative to bone formation, generally resulting in an increase in bone turnover. The bone loss is brought about by an imbalance between bone resorption and bone formation. Osteoclastic bone resorption requires 2 processes: demineralization of the inorganic bone components and degradation of the organic bone matrix. Cathepsin K is the most abundant cysteine protease expressed in the osteoclast and is necessary for bone matrix

degradation required for bone resorption. It was hypothesized that MK-0822, a potent cathepsin K inhibitor, would inhibit bone resorption.

A randomized, double-blind, placebo-controlled, sequential group, multiple oral dose study was conducted in 30 postmenopausal women to assess safety, tolerability, pharmacokinetics, and the pharmacodynamic profile of MK-0822 during multiple oral dosing.

Three panels of 10 postmenopausal female subjects (8 active, 2 placebo) were administered 0.5, 2.5, and 10 mg MK-0822 or placebo once-daily for 21 days. Subjects participated in only one panel and received only one dose level. Blood and urine samples were obtained at selected time points pre- and postdose for measurement of serum CTx, urinary NTx/Cr, bone specific alkaline phosphatase (BSAP), and osteocalcin.

Robust reductions in urine NTx (~80%) were seen following daily administration of 2.5 and 10 mg for 21 days. More modest reductions in urine NTx (~15%) were seen with 0.5 mg daily for 21 days. The effects of MK-0822 on serum CTx were similar to those seen on urine NTx. Serum CTx was suppressed ~15%, ~70%, and ~80% following 21 days of dosing MK-0822 at 0.5, 2.5, and 10 mg, respectively. No significant effect was seen on bone formation markers (BSAP and osteocalcin). MK-0822 was generally well tolerated, with no subjects discontinuing due to adverse experiences.

In conclusion, the cathepsin K inhibitor, MK-0822, results in robust suppression of bone resorption markers (urine NTx/Cr, serum CTx) when administered once-daily to postmenopausal women for 21 days. These data suggest that the cathepsin K inhibitor MK-0822 may be developed as an effective therapeutic agent for osteoporosis.

Conflict of Interest: All authors are employees of Merck Research Laboratories.

P432-S

BONE EFFECTS OF A CATHEPSIN K INHIBITOR IN THE ADULT ESTROGEN DEFICIENT RABBIT

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Cathepsin K (Cat K), an enzyme that degrades Type I collagen, is prominent in osteoclasts, and may play an important role in bone resorption. Compound J (J) reversibly inhibits human Cat K with an IC50 of 0.2nM, with greater than 4000-fold selectivity for Cat K vs. cathepsins B, L, and S; its potency on rabbit Cat K is 1.5nM. The goal is to contrast the effect of J in an estrogen deficient animal model with cancellous and cortical bone remodeling, to that of alendronate (ALN).

Adult (7 months old, 3.9kg) rabbits were OVX'd (N = 48) or Sham-OVX'd (N = 12) during early June. OVX rabbits were given J (0, 2, or 10mg/kg, orally once daily [N = 12 each]) or ALN (0.125mg/kg; subcutaneous, 3X/wk [N = 12]) for 27 weeks. After in vivo dual calcein labeling, all were necropsied. Lumbar vertebrae (LV) 2–4 and whole femurs were excised and fixed in 70% ethanol. LV3 was DXA-scanned. LV4 was sectioned parasagitally at 6 μm and analyzed for cancellous (c) bone formation surface. 100 μm sections of the mid-femur were analyzed in cross-section for endocortical (e), and periosteal (p) mineralizing surface (MS/BS; double + half-single label), mineral apposition rate, and number of double labeled Haversian systems (HS)/mm2.

Significant OVX-induced bone loss occurred in LV3. This loss was partially prevented by 2mg/kg J and fully prevented by 10mg/kg J and ALN. This bone loss was accompanied by increased formation rate at all surfaces and in Haversian bone. 2 mg/kg J partially blocked ($P < 0.1$), and both 10 mg/kg J and ALN completely blocked OVX-induced bone loss ($P < .01$) in LV3. While ALN reduced MS/BS at cancellous and endocortical surfaces and reduced Haversian labeling, neither dose of J affected MS/BS at these surfaces. Mineral apposition rate at any surface was unaffected by treatment with J. These results suggest that estrogen deficiency bone loss is prevented to a similar extent by Cat K inhibition and ALN. In contrast to the suppression of bone formation observed with ALN, inhibition of resorption by a Cat K inhibitor does not appear to be accompanied by suppression of bone formation.

Conflict of Interest: All authors are employees of Merck Research Laboratories.

P433-M

BONE EFFECTS OF CATHEPSIN K INHIBITORS IN THE GROWING RABBIT

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Cathepsin K (Cat K) is prominent in osteoclasts, degrades Type I collagen, and may play an important role in bone resorption. However, it has significant interspecies sequence variation, rodent Cat K being only 88% homologous to human Cat K. While Cat K of higher species (rabbit and non-human primate) is 94–98% homologous to human Cat K, its low homogeneity with rodent Cat K presents challenges for efficient in vivo testing of human Cat K inhibitors in the usual rodent bone test models.

Female rabbits (7 wks old, 1.4–1.5kg) were used as the tertiary screen for reversible human Cat K inhibitors previously proven efficacious in enzyme inhibition and in vitro bone resorption (pit) assays. Rabbits (N = 11/grp) were treated orally for 10d with multiple doses up to 30 mg/kg/d of the Cat K inhibitor, always with comparison to vehicle and 0.1mg/kg/d alendronate (ALN) subcutaneously. The whole right femur was taken at necropsy. The distal 6cm of the femur was DXA-scanned (Hologic 4500A). Bone mineral density analysis (BMD; Table) focused on the region located 0–3cm from the distal end.

This "rabbit Schenk" assay, patterned after the growing rat model used for testing anti-resorptive efficacy of bisphosphonates (Schenk et al., Calc Tiss Int 38: 342–349 (1986)), relies on inhibiting normally ongoing resorption in rapidly-growing animals at both the periosteum and the distal aspect of trabeculae in the marrow cavity of the metaphysis of long bones. Histologic examination of specimens from selected experiments showed that both ALN and Cat K inibitors were active at these sites. ALN, the positive control, consistently increased BMD by 11–22%. This assay identified human Cat K inhibitors that increased BMD in a dose–effect fashion, and were generally as efficacious as ALN. On the basis of these results, compounds J and K were tested in estrogen deficient non-human primates, where they reduced bone resorption markers.

We conclude that the rabbit Schenk assay is a valid and consistent in vivo screen for anti-resorptive activity. When the rat is inappropriate, the rabbit can serve as a rapid, cost-effective, and reliable intermediate model before non-human primate testing.

Conflict of Interest: All authors are employees of Merck Research Laboratories.

P434-T

EFFECTS OF CATHEPSIN K INHIBITORS ON BONE RESORPTION MARKERS IN ESTROGEN-DEFICIENT NON-HUMAN PRIMATES

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Cathepsin K (CatK), an enzyme in osteoclasts, degrades Type I collagen, and may play an important role in bone resorption. Detecting the action of CatK inhibitors (CatKi) non-invasively in a large animal species is essential to demonstrating their action. In this study, we use human biomarkers of bone resorption in estrogen deficient monkeys to detect the action of human CatKis that had previously been validated in enzyme inhibition and in vitro bone resorption assays, and a primary in vivo bone screen in rabbits.

Ovariectomized (OVX) female rhesus monkeys (15–19 years old, 5.5–9.5kg) were studied at 4–8 years post-OVX. They were randomized by body weight and untreated level of urinary N-telopeptides (uNTx). After three days of pretreatment with vehicle, monkeys (N = ~11/each) received either vehicle or active drug orally once daily for six days (Days 1–6). 24hr urine specimens were collected from pans in the monkeys' home cages on Days 0, 2, 4, 5, 6, 8, 11, and 14. uNTx was measured with manufacturers' kits (Osteomark; Seattle, WA USA)

Compound H at 20mg/kg/d reduced uNTx by ~80%; the suppression resolved within one week of cessation of dosing. Compound J at 3 or 15mg/kg/d reduced uNTx by ~65–80%; the suppression resolved within one week. A no effect level of Compound J was identified at 0.6mg/kg/d (uNTx reduced by ~25–30%). Compound K at 0.6mg/kg/d reduced uNTx by ~65%, while smaller reductions were seen with 0.3mg/kg/d. We conclude that urinary NTx assay in rhesus monkeys is a rapid, valid, and consistent in vivo screen for anti-resorptive activity that can differentiate compounds according to in vitro activities, and provide data similar to that eventually to be collected in humans.

Table: uNTx (% Difference between treated and vehicle animals)

Day (mg/kg)	Cpd H (20)	Cpd J (15)	Cpd J (3)	Cpd J (0.6)	Cpd K (0.6)	Cpd K (0.3)
0	+23	−1	+22	−7	+11	−10
2	−87&	−81&	−73&	−29*	−59&	−51&
4	–	−76&	−65&	−29	−68&	−46*
5	−78&	–	−71&	−24	−61&	−63&
6	−75&	−79&	−65&	−40*	−73&	−38*
8	−58&	−82&	−47&	−32*	−59&	−13
11	–	−65&	−14	+2	+22	−6
14	−28	−12	–	–	–	–

vs. Vehicle ($P < 0.005$& ; $P < 0.05$*)

Conflict of Interest: All authors are employees of Merck Research Laboratories.

P435-S

DIFFERENCE IN CENTRAL INTRAVERTEBRAL PRESSURES DURING VERTEBROPLASTY AND BALLOON KYPHOPLASTY?

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Aim: High intravertebral pressures during PMMA injection have frequently been proposed as the reason for a higher cement leakage rate of vertebroplasty (VP) in contrast to balloon kyphoplasty (BKP). It has been shown that the intravertebral pressure measured in the shell of the vertebral body is much lower than the pressure applied to the injection syringe. However, the pressure relations in the center of the vertebral body are not known. This in vitro study investigates the different intravertebral pressures measured in close proximity to the injection cannula during VP and BKP.

Methods: Vertebroplasty and balloon kyphoplasty were performed in 8 lumbar cadaver spines. A pressure sensor (Peekel Instr., Rotterdam, NL) was placed close to the tip of the injection cannula. In the VP subgroup a total volume of 6 cc of PMMA cement was delivered in 1.5 cc increments. In the BKP subgroup balloon dilation up to a volume of 4 cc was made prior to cement injection of 6 cc (1.5 cc increments). Room temperature, cement mixing time and constant volume flow during cement injection were recorded.

Results: During the administration of the first 1, 5cc of PMMA the average intravertebral pressure for the VP was 18,9 kPa versus 1,3 kPa for BKP. For the 2nd filling an average pressure of 36,4 kPa (VP) and 3,9 kPa (BKP) was recorded. The average intravertebral pressure during the 3rd injection was 56,1 kPa (VP) and 13,8 kPa (BKP). The pressure of the last 1,5 cc averaged in 59,0 kPa (VP) and 24,5 kPa (BKP). The differences of pressures were statistically significant ($p < 0{,}05$).

Conclusion: The intravertebral pressure measured during the PMMA filling of the vertebrae of cadaver spines was lower for balloon kyphoplasty than for vertebroplasty. In the balloon kyphoplasty group an increase of the intravertebral pressure was registered at the end state of cement augmentation this is at complete cavity fill. The differences in pressures were statistically significant at all time points measured.

Conflict of Interest: None declared

P436-M

THE EFFECT OF HEPATIC OR RENAL IMPAIRMENT ON THE PHARMACOKINETICS OF PTH (1–84)

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The purpose of this study was to compare the pharmacokinetics of ALX1-11 (full length recombinant human parathyroid hormone, PTH [1–84]) given as a 100-μg subcutaneous injection in subjects with moderate hepatic or mild-to-moderate renal impairment to gender- and BMI-matched subjects with normal function. This was an open-label, single-dose study in 12 hepatically-impaired subjects (6 women and 6 men), 16 renally impaired subjects (8 women and 8 men) and 28 matched normal subjects. Following injection of PTH (1–84), blood samples were collected over 24 hours for plasma PTH and serum total calcium determination. In addition, the following safety parameters were collected: 12-lead electrocardiograms, physical examinations, vital signs, clinical laboratory evaluations (hematology, serum chemistry, urinalysis), and adverse events (AEs). Plasma PTH data were corrected for baseline levels of PTH and subjected to non-compartmental analysis. Cmax and AUC values were log-transformed and fit by an analysis of covariance model with impairment group as a factor, and age and BMI as covariates. The mean and individual plasma PTH concentration–time profiles were comparable between the impaired and normal subjects. Regardless of the type of impairment, the mean ratios of baseline-corrected PTH exposures between the impaired and normal groups were in the range of 1.0 to 1.4. The 90% confidence intervals for the mean ratios were wide, exceeding 200% in most cases. There were no significant differences in the serum total calcium concentration–time profiles between the impaired and normal groups. The AEs were generally mild in intensity and were consistent with other studies using a similar dosing regimen. In conclusion, moderate hepatic and renal impairment lead to small mean increases in mean PTH (1–84) exposure of less than 40% and do not warrant dose-adjustment.

Conflict of Interest: D. S. Wells, employed at NPS Pharmaceuticals Inc

P437-T

EFFECTS OF TERIPARATIDE ON SERUM CALCIUM

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Introduction: Teriparatide 20 mcg/day [rhPTH (1 34), TPTD] increases bone turnover and bone mineral density and reduces the risk of vertebral and nonvertebral fractures in postmenopausal women with osteoporosis. The present study examined the effects of TPTD on serum calcium (Ca) in patients previously treated with alendronate (ALN) or raloxifene (RLX).

Methods: Postmenopausal women with osteoporosis previously treated for at least 18 months with ALN (70 mg/wk or 10 mg/d) or RLX (60 mg/d) continued their usual ALN or RLX during a 2-month antiresorptive phase. Patients previously treated with ALN were randomized to add TPTD ($n = 52$) or switch to TPTD ($n = 50$). Patients previously treated with RLX were randomized to add TPTD ($n = 47$) or switch to TPTD ($n = 49$). Patients were supplemented with at least 500 mg/day elemental Ca and 400–800 IU/day vitamin D. Serum samples from hydrated patients were obtained at 0, 1, and 2 months during the antiresorptive phase, and prior to TPTD dosing at 1, 3, and 6 months during the TPTD phase. The primary analysis was change in mean predose serum Ca between the antiresorptive and TPTD phases.

Results: In the patients previously treated with ALN, mean serum Ca did not significantly change in the group randomized to add TPTD but significantly increased by 0.04 mmol/L in the group randomized to switch to TPTD. In the patients previously treated with RLX, mean serum Ca did not significantly change in the group randomized to add TPTD but significantly increased by 0.05 mmol/L in the group randomized to switch to TPTD. One prior ALN patient had serum Ca > 2.76 mmol/L during the antiresorptive phase. One prior ALN patient who added TPTD and one prior RLX patient who switched to TPTD had serum Ca > 2.76 mmol/L at a single visit during the TPTD phase; one prior ALN patient with 2 of 3 serum calciums above the normal range during the antiresorptive phase discontinued after serum Ca was > 2.76 mmol/L at 2 visits during the TPTD phase.

Conclusions: Patient groups previously treated with ALN or RLX who added TPTD had no significant change in mean predose serum Ca. Patient groups switched from prior ALN or RLX to TPTD had small but not clinically meaningful increases in mean predose serum Ca.

Conflict of Interest: L Xie; EV Glass; and JH Krege are employees of ELi Lilly and Company

P438-S

PRECISION IMPROVEMENTS WITH DXA

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At the 2005 Position Development Conference, the International Society for Clinical Densitometry published a position paper with a meta-analysis of 58 recent peer reviewed short-term precision studies[1]. This review found statistically significant differences in the precision between manufacturers for the AP Spine and Femoral Neck, but not for the total hip. The paper did not report the precision results by manufacturer.

Using the same 58 peer reviewed studies from that manuscript; the studies were analyzed to determine the precision by manufacturer. This analysis shows that in both cases where a statistical significant difference in precision was found, Hologic scanners had the best precision (see table below).

However, with the introduction of the new QDR Apex software, there have been further improvements in the precision of BMD on Hologic scanners, particularly at the total hip where the previous peer reviewed studies found no statistically significant difference between manufacturers.

This analysis looked at the precision of the total hip BMD of six previously acquired precision studies. These six studies had a total of 414 replicate measurements. Three of the studies used the 60s scan mode, one study used the 30s scan mode, and two studies used the 10s scan mode.

In the reanalysis with the QDR Apex software, precision of the total hip improved for all studies. The minimum precision improvement was 13% and the maximum improvement was 37%. The % coefficient of variation (%CV) of BMD of the six studies after reanalysis with the new software varied from a high of 0.95% to a low of 0.54%, with the average %CV of the six studies of 0.78%.

The QDR Apex software provides more precise BMD analysis than previous QDR software versions at the total hip for all scan speeds.

[1]Journal of Clinical Densitometry, Vol. 9, no. 1, 31–36, 2006.

Table:

Region	All Manufacture	Hologic	GE/Lunar	Norland	p value
AP Spine	1.17%	1.08%	1.22%	1.58%	0.02
Femoral Neck	1.85%	1.50%	1.97%	2.30%	0.03

Average precision from the 58 peer reviewed studies.

Conflict of Interest: K. Wilson, Hologic Inc., Employee C. Ruth, Hologic Inc., Employee T. Kelly, Hologic, Inc., Employee

P439-M

PREDICTING THE CLINICAL POTENCIES OF BISPHOSPHONATES: DIVERGENCE OF HYDROXYAPATITE AND FARNESYL DIPHOSPHATE SYNTHASE BINDING AFFINITIES

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Potencies of bisphosphonates (BPs) are dependent on both binding affinities for bone mineral and inhibitory actions on osteoclasts. In the case of nitrogen-BPs osteoclast inhibition is mediated by selective targeting of Farnesyl Diphosphate Synthase (FDPS). The extent to which each factor contributes to the properties of individual BPs can now be better distinguished by using quantitative assays for mineral binding and for FDPS activity. Here we have used a newly developed highly reproducible mineral binding assay based on column chromatography on ceramic hydroxyapatite (HAP). Kinetic analyses were used to measure the inhibitory potencies of BPs on FDPS. The results indicate that the rank order for mineral binding of a series of N-BPs to HAP is independent of their inhibitory potencies on FDPS (Table 1). The major determinants of mineral binding are the P-C-P group, an OH on R1, and the orientation of the N group on the R2 side chain. For FDPS inhibition the PCP group is also essential (via $Mg2+$ binding), combined with the specific orientation of the N in the R2 in relation to the enzyme binding pocket. Modelling studies show how different 3-D configurations of the R2 side chain in relation to the overall BP structure can account independently for HAP binding and FDPS inhibition.

In conclusion, distinctive chemical structures of N-BPs separately determine mineral binding and FDPS inhibition and contribute independently to overall in vivo potency of N-BPs. It is therefore now possible to modulate the HAP binding affinity to produce BPs with weak or strong mineral binding affinities and this activity is separable from the underlying potency on FDPS.

Table 1. HA column retention times and inhibitory potencies on FDPS of N-BPs

N-BPs	Retention time (min, mean±SE)	Ki (nM)
NR58029	ND	11.6
NR58052	ND	92,900
NR10790 Bis -phosphonocarboxylate	4.30±0.06	40,000
NR58086 Dihydropyridine BP	4.83±0.09	428
NR58043 3-pyridylethane BP	5.83±0.17	5.7
NR1810 3-Me 2-pyridylaminoethane BP	5.93±0.07	134.5
NR1818 Pyridyl aminoethane BP	6.57±0.12	2.2
NR97220 (3-Me 2-pyridyl aminomethane BP)	6.53±0.15	1.1
NR58022 (Phenyl analogue of Ris)	7.77±0.15	302.8
Minodronate (NR0848)	8.70±0.10	0.09
Risedronate (NR58051)	9.67±0.09	79.9
Risedronate	9.97±0.09	0.34
Zoledronate	12.53±0.06	0.07
NR1807 (a 2-pyridyl aminomethane BP)	12.83±0.17	2.3

Conflict of Interest: None declared

P440-T

OSTEOPOROSIS SCREENING IN PATIENTS WITH PREVIOUS FRACTURES

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Background and Aim: Previous fractures may be indicator of osteoporosis. However, patients are not usually asked about fractures, osteoporosis is not screened and patients are often left untreated.

Method: 1027 consequtive patients admitted to our outpatient clinic for comprehensive geriatric assessment were examined. History of previous fractures were taken. Patients were asked if they had known osteoporosis diagnosis, and if they were on medication for osteoporosis.

Results: 1027 patients (649 (%63.2) female, mean age = 72.2 ± 6.0) were asked for previous fractures and screened for osteoporosis. 124 (12.1%) of them reported previous fracture.29 of the fractures (24.2%) was hip fracture. Mean age of the patients with history of fracture was 73.8 ± 7.3, and 89 (71.8%) were female. Within these patients with fracture, only 25 (28.7%) were diagnosed as osteoporosis and treatment was prescribed in the primary care. Within these patients receiving therapy, 3 was on calcitonine, 22 was on bisphosphonate treatment. After taking this history from the patients admitted to our outpatient clinic, bone mineral densitometry was performed to every patient. Results revealed that 87 (71.9%) of the patients with reported fracture had osteoporosis, and 4 (3.3%) of them had osteopenia. After making the diagnosis, appropriate treatment was initiated.

Conclusion: Although osteoporosis is a well known disease, it is commonly underdiagnosed especially in elderly patients. Previous fracture should be asked to elderly patients and patients who report fractures should be carefully examined in order to prevent future fractures.

Conflict of Interest: None declared

P441-S

BONE MINERAL DENSITY IN RATS WITH IRON ENRICHED DIET AND REPEATED BLOOD LOSSES

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The aim of our study was to assess the influence of dietary iron (over-supply) and repeated blood withdrawals (insufficiency) in rats on bone mineral density (BMD) in different regions of interest (R1 – spine, R2 – tail, R3 – femur).

Methods: After institutional approval 24 Wistar male rats were divided into 4 groups. The halves of the rats were fed with the standard laboratory diet (SD, 27 mg Fe/kg). The second half of rats were fed with iron enriched diet (FE, 400 mg Fe/kg). We used repeated blood withdrawals (-w) from retroobital plexus for simulation of blood donors clinical situation. Group 1 (M-SD) was fed by SD diet, and group 2 (M-FE) was fed by FE diet, in these groups was performed one blood withdrawal in 9th week. Group 3 (M-SD-w) was fed by SD, and group 4 (M-FE-w) was fed by FE, there was performed the blood withdrawal once a week, 9 times totally. The rats were sacrificed by exsanquination from abdominal aorta and then BMD (g/cm^2) was measured by DEXA densitometer using Small animal software (Hologic Delphi A, Hologic, MA, USA) in ROI: distal spine (R1), proximal tail (R2), femoral region (R3). Statistical analyses were performed by t-test using SigmaStat software (Jandel Scientific, CA, USA). Results are presented as mean ± SEM.

Results: BMD in spine increased in rats with blood withdrawals, significantly in M-FE-w (0.230 ± 0.005) versus M-SD (0.217 ± 0.004). BMD in tail increased in rats with blood withdrawals, significantly in M-FE-w (0.234 ± 0.004) versus M-SD (0.210 ± 0.008) and versus M-FE (0.217 ± 0.007). BMD in femur not significantly decreased in rats M-FE-w (0.176 ± 0.007) versus M-SD (0.181 ± 0.009) and versus M-FE (0.185 ± 0.015).

Conclusions: We assume that increased iron turnover (iron over-supply with repeated bood withdrawals) is associated with increased BMD in vertebrae but not in femur region (extremities). The explanation of these facts could be different ratio of erythropoesis in spine in comparison with femur.

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Conflict of Interest: None declared

P442-M

BONE MINERAL DENSITY IN MALE RATS WITH DIET INDUCED LIVER IMPAIRMENT

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Liver diseases are being diagnosed more frequently in male than in female and often accompanied by skeletal alteration. The aim of study was to assess the influence of dietary induced liver steatosis in adult male rats on bone mineral density (BMD).

Methods: After institutional approval 32 male Wistar rats were divided into four groups and fed by different diets ad libitum for 30 days as follows: group 1(control): standard laboratory diet (SLD), group 2: diet with addition of 4 % of cholesterol (CHOL), group 3: methionin cholin deficient diet (MET), group 4: diet with 1 % of orotic acid (ORO). Rats were sacrificed by exsanguination in anesthesia and then BMD (g/cm^2) was measured by DEXA densitometer using Small animal software (Hologic Delphi A, MA, USA) in: distal spine (R1), proximal tail vertebrae (R2), femoral region (R3). Histological examination of liver and serum transaminase activity were performed. Statistics: t-test (mean ± SEM) was performed using SigmaStat software (Jandel Scientific, CA, USA).

Results: Liver steatosis was histologically proved in groups 2 and 4. Serum transaminases were significantly higher in group ORO and MET vs. SLD group. Lumbar spine BMD in ORO (0.213 ± 0.004) and MET (0.214 ± 0.004) groups were not significantly higher than in CHOL (0.192 ± 0.005) and SLD (0.193 ± 0.003) groups. The similar situation was in R2: ORO (0.206 ± 0.005), MET (0.209 ± 0.003), CHOL (0.199 ± 0.003) and SLD (0.195 ± 0.009). There were no differences in femoral region BMD: ORO (0.172 ± 0.006), MET (0.163 ± 0.006), CHOL (0.169 ± 0.009) and SLD (0.175 ± 0.008).

Conclusions: Male rats develop steatosis after short time diet treatment. Surprisingly it is sufficient time to develop differences in BMD (though not statistically significant) among groups. Despite of histologically proved liver steatosis in ORO and CHOL groups they had different BMD in R1 and R2 region. We suppose that during this short time BMD is influenced more likely by diet itself than by liver impairment. R1 and R2 regions seem to be more susceptible for above mentioned influences than R3 region.

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Conflict of Interest: None declared

P443-S

INSULIN LEVELS CONTRIBUTE TO BONE LOSS RATES AND BMD IN HEALTHY POSTMENOPAUSAL WOMEN OVER AND ABOVE THE ASSOCIATION EXPLAINED BY FAT MASS

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BACKGROUND: Previous studies have yielded conflicting results regarding the importance of lean versus fat mass on BMD. In addition, it is unclear if the influence of fat mass reflects insulin levels, gonadal hormones or other factors. We assessed the contribution of fat mass, lean mass, and plasma insulin to BMD in 55-year old women.

STUDY POPULATION AND METHODS: 435 healthy postmenopausal women (mean age 55.3y), who had 5y previously been randomized to HRT ($n = 224$) or no treatment ($n = 211$) and remained compliant. BMD and body composition was measured using Hologic QDR-2000 devices.

RESULTS: Median insulin levels were 17% lower with HRT ($p < 0.01$). A positive correlation was found between spine/hip BMD and LBM, FM and insulin levels in both treatment groups. Multiple regression showed that both HRT ($p < 0.01$) and lean body mass ($p < 0.01$) contributed to BMD. Plasma insulin added to the prediction of both spine ($p < 0.05$) and hip ($p < 0.01$) BMD, whereas FM did not ($p = 0.12$ to 0.16). There was a significant correlation between FM and log(insulin), $r2 = 0.19$, $p < 0.01$, but no significant interaction between insulin and FM in the prediction of BMD. Over 10y, insulin explained 10% of the variation in bone loss at the spine (table 1) and 5% at the hip in untreated participants ($p < 0.01$).

CONCLUSIONS: Plasma insulin levels are correlated with spine and hip BMD and with bone loss rates in healthy postmenopausal women; this adds to the prediction offered by fat mass, lean body mass and HRT status. Fat mass did not affect BMD when controlled for insulin levels.

Table 1: Multiple regression analysis for bone loss rate as a function of HRT, body composition and insulin levels

Spine ΔBMD $r^2=0.37$	p	$r_{partial}$	B	95% CI for B
HRT	< 0.001	0.59	11.90	(9.82; 13.97)
Lean body mass	0.69	0.03	0.04	(-0.16; 0.25)
Fat mass	<0.05	0.16	0.17	(0.03; 0.30)
Log(insulin)	<0.01	0.18	5.53	(1.62; 9.43)

Conflict of Interest: B. Abrahamsen, advisory board, Nycomed B. Abrahamsen, grant support, Roche

P444-M

PREVALANCE OF BONE MINERALIZATION DEFECTS IN GERMANY – A HISTOMORPHOMETRIC ANALYSIS AFTER THE INCLUSION OF 440 PATIENTS

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A potential vitamin D and calcium deficit in the population of middle and northern europe gains increasing attention. So far it remains however to be determined to what extend this potential deficit in vitamin D indeed leads to pathologic changes of skeletal mineralization.

To address this question we have performed transiliac crest biopsies in 440 patients (270 males, age 6 to 95 years (mean age: 57,2 years), and 170 females, age 11 to 97 years, (mean age: 70,29 years)) who had no secondary osteopathies during autopsy. All biopsies were undecalcified embedded in methylmetacrylate and histologically processed. Static structural analysis was performed using the Osteomeasure histomorphometry system according to ASBMR standard. Beside age, sex, and body mass index analysis included the histomorphometic parameter bone volume (BV/TV), osteoid volume (OV/BV), osteoid surface (OS/BS), trabecular thickness (Tb.Th), trabecular number (Tb.N), and trabecular separation (Tb.Sp). Statistical analysis consisted of testing for the lack of correlation and the similarity of the correlation coefficient in males and females.

The histologic results demonstrate that in the present study cohort not only patients with osteoporosis but also another group of patients with pathologic bone mineralisation, i.e. with volume and/or surface osteoidosis, was identifyable. The histomorphometric quantification documents an age-related, sex-unrelated decrease in BV/TV, Tb.N and Tb.Th as well as an unexpected high number of patients with a pathologic increase in osteoid. Indeed 25% of the analyzed patients presented with an osteoid surface of more than 20%. Furthermore a pathologic increase in osteoid volume per bone volume of more than 2% was detected in 20 % of the patients. Most interestingly these mineralization defects were found throughout all ages and affected both females and males respectively. Based on the definition described by Delling in 1975 that an OV/BV of $> 1.2\%$ is pathologic, it becomes clear that the prevalence of manifest skeletal mineralization defects is by far higher and more dramatic than expected until now.

These results clearly demonstrate the paramount importance of a sufficient calcium and vitamin D supplementation in general and even more in patients with osteoporosis, furthermore they provide strong arguments for the need of further subgroup analysis after study extension.

Conflict of Interest: M. Amling, MSD, Speakers Bureau M. Amling, Procter & Gamble, Speakers Bureau and Grant Research Support M. Amling, Roche, Speakers Bureau

P445-T

HIGH CA INTAKE PREVENTS DELETERIOUS EFFECTS OF REDUCED PROTEIN INTAKE ON SKELETAL GROWTH

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During skeletal development, both bone mass and size are critical for an optimal bone quality. Low protein intake is associated with suppressed somatotrop axis and reduced intestinal Ca absorption and secondary hyperparathyroidism. Both hormonal disorders could be in turn influenced by Ca intake. To better understand the selective effect of protein and Ca intake on skeleton development, 1-month old rats were fed an isocaloric diet containing 10, 7.5 or 5% casein. Each type of diet was available with normal (1.1%) and low (0.2%) Ca contents; the Ca/Pi ratio was maintained constant. After 8 weeks under these six different regimens, rats were sacrificed. Bone apparent area (AREA) and bone mineral content (BMC) were measured using DXA, bone dimensions using digital calliper. Anthropometric measurements of the tail tibia were also evaluated. Values are means ± SEM, * $p < 0.05$ vs casein 10 and normal Ca and ° $p < 0.05$ vs. casein 10 and low Ca as evaluated by an ANOVA. Reduction of protein intake was associated with lower BMC, AREA and of outer-diameter in rats fed 5% casein when Ca intake was normal and in rats fed 7.5 and 5% casein when Ca intake was low. In rats fed a normal Ca diet tibia length were 37.2 ± 0.2, 36.5 ± 0.2 and 34.7 ± 0.3*°, in rats fed a 10, 7.5 or 5% casein diet, and in rat fed a low Ca diet 36.5 ± 0.4, 36.5 ± 0.2 and 34.7 ± 0.3*°. Tibia and tail length were significantly lower in animals fed a 5% casein independently of the Ca intake. These results indicate that isocaloric reduced protein intake resulted in poor acquisition of bone mass and size. The deleterious effects of moderate low protein intake on bone diameter and mass (determinant of bone strength) are prevented when the Ca intake is high. These results indicate that a high Ca intake can prevent deleterious effects of suboptimal protein intake on skeletal growth and open new clinical questions regarding optimal growth and nutrition.

Table:

Casein	BMC Normal Ca	BMC Low Ca	AREA Normal Ca	AREA Low Ca	Diameter Normal Ca	Diameter Low Ca
10 %	131 ± 7	125 ± 3	605 ± 16	602 ± 11	254 ± 5	254 ± 4
7.5 %	127 ± 2	112 ± 3*°	594 ± 8	558 ± 17*°	252 ± 3	240 ± 4*°
10 %	104 ± 2*°	108 ± 3*°	520 ± 8*°	537 ± 11*°	235 ± 3*°	235 ± 3*°

Conflict of Interest: None declared

P446-S

RISK FACTORS OF WRIST FRACTURES IN GREEK POPULATION

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Aims. Wrist fractures are common osteoporotic fractures especially in perimenopausal women and contribute to significant morbidity. The aim of this study was to evaluate the relative contribution of risk factors to the occurrence of wrist fractures in Greek community.

Method. The study subjects comprise 489 women that attended the outpatient clinics of a University Hospital between October 2005 and August 2006. None of the 489 women, aged 22–84 years, were known to suffer from any disease or receive medications affecting bone metabolism. Bone mineral density (BMD) was measured by DXA at the lumbar spine, total hip and femoral neck. Forearm BMD was measured in a subgroup of 110 women. Detailed questionnaires on medical history and life style variables were completed by qualified nurses.

The effect of BMD and other risk factors on wrist fracture occurrence was examined using Poisson regression analysis and was expressed as incidence rate ratios (with 95% confidence intervals).

Results. The mean (SD) age of the women was 56.99 (12.13) years. Forty low trauma carefully ascertained wrist fractures were reported. Among the different sites measured, femoral neck BMD was the strongest predictor of wrist fractures and the only one remaining when data were adjusted for age. The adjusted rate ratio for wrist fractures decreased by 0.56 (95% CI = 0.35–0.88) for every unit increase in standardised BMD of the femoral neck. The crude rate ratio for wrist fractures decreased by 0.55 (95% CI = 0.39–0.79) and 0.64 (95% CI = 0.43–0.95) for every unit increase in standardised BMD of the total hip and lumbar spine respectively, but lost its significance when adjusted for age. Forearm BMD was not predictive for wrist fractures, possibly due to the smaller number of subjects (n = 110) with forearm BMD measurements.

Various risk factors including BMI, smoking, alcohol consumption, physical activity and menstruation history were examined. Delayed menarche over the age of 15 years was associated with an increased risk of wrist fractures (RR = 3.09 95% CI = 1.35–7.03), while none of the other risk factors examined showed any consistent association with the occurrence of wrist fractures.

Conclusions. Our findings considered representative of the Greek population are in accordance with large epidemiological studies. They support the importance of femoral neck BMD as major predictor of wrist fractures and the modest role of lifestyle and anthropometric factors in the occurrence of these fractures.
Conflict of Interest: None declared

P447-M

SCREENING FOR OSTEOPOROSIS – FRACTURE PATIENTS CARE! THREE YEAR FOLLOW-UP OF 239 FRACTURE PATIENTS FROM A PROSPECTIVE AND CONSECUTIVE OSTEOPOROSIS SCREENING PROGRAM

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Background: Future fractures can be prevented if patients with osteoporosis are identified and treated. Previously we have reported the results of a screening program, were 239 fracture patients at our orthopaedic department between 50 and 75 years of age with fragility fractures were measured regarding Bone Mineral Density (BMD) using a DEXA scan, diagnosed with osteoporosis, osteopenia or normal BMD and encouraged to see their primary care physician for further examination and treatment. We report a follow-up of the patients from this first year of our screening.

Patients and methods: A questionnaire was sent to patients included in the screening program after a fracture between November 2002 and November 2003. Questions included whether they had seen their doctor, if blood tests were taken, what type of treatment they received and questions about their opinion of osteoporosis.

Results: 90 % of the 236 patients answered the questionnaire. 90 % of those with osteoporosis, 70 % with osteopenia and 31 % of those with normal BMD had seen a doctor. Of these, blood tests were taken on 60 % of the osteoporosis patients, 50 % of the osteopenic and 27 % of the normal patients. 64 % of the osteoporotic, 17 % of the osteopenic and 0 % of the normal patients received bone resorption-reducing treatment, while 29 % of the osteoporotic, 54 % of the osteopenic and 45 % of the normal patients received calcium supplement monotherapy. 5 % of the osteoporotic, 28 % of the osteopenic and 55 % of the normal patients received no treatment. All patients regarded osteoporosis as important for them to know about and most felt they could influence their skeletal health.

Interpretation: Screening fracture patients for osteoporosis is an effective way of identifying patients with low bone mineral density. In our follow-up we had a high percentage of patients who went to see a doctor regarding osteoporosis and the treatment they received roughly reflects their post-bonescan diagnosis. Extrapolation of how many fractures this screening possibly prevents in the future is difficult, but cautious calculations suggest better results than what has previously been proposed.
Conflict of Interest: Åstrand, MSD, Grant/Research Support

P448-T

THE PATTERN OF BONE DENSITY IN MEN 50–65 YEARS WITH HIP OR DISTAL RADIAL FRACTURES

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Introduction; In general men peak in the incidence of distal radial fracture early in life compared to women who peak after the menopause. The value of a hip or radial fracture as a predictor of a second osteoporotic fracture is well recognized and the efforts to find, diagnose and treat are even more justified in men than in women.

Methods; Between October 2002– October 2004 all patients at the department of Ortopedics at Lund University with an ostoeoporotic fracture were invited to a dexa scan. If found osteoporotic or osteopenic they are sent to the family care physician for initiation of treatment. In the total cohort of 670 patients 98 were men between 50–65 years of age who were sent an invitation for a DEXA scan together with a short enquiry of risk factors. 77 patients came for the scan.

Results; 45 men had a distal radial fracture and 32 a hip fracture. In the distal radial group 6 had a normal DEXA scan, 12 showed osteopenia and 2 osteoporosis compared to the hip fracture group where 0 were normal 8 had osteopenia and 11 osteoporosis. The mean BMI in the first group was 27.8 compared to 22.8 in the second group. The hip fracture group also had more comorbidities.

Discussion In a cohort of osteoporotic fractures the predictive value of the fracture per se for the risk of a second fracture is well accepted. Within the group of younger men the ones with a hip fracture appear more sick and suffer fom an increased burden of comorbidities compared with the distal radial fracture group. In both groups large efforts are justified to bring these patients into a programme for preventing the second fracture

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Missed Opportunities in Patients with Osteoporosis and Distal Radius Fractures.
Clin Orthop Relat Res. 2006 Sep 21; [Epub ahead of print]
PMID: 17006362 [PubMed – as supplied by publisher]
Conflict of Interest: J. Åstrand, MSD, Speakers Bureau

P449-S

THE PREVALENCE OF OSTEOPOROSIS INCREASES QUICKLY THROUGH THE EARLY MENOPAUSE

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Introduction: It is well established that the prevalence of osteoporosis begins to increase in the menopause, but there is not known enough how it increases through the first years of menopause.

Subjects, material and methods: 183 women with natural menopause were randomly selected in the province of Albacete, Spain (mean age 53.7 ± 1.6 yr). They did not have any diseases and were not taking drugs to affect bone metabolism. Lumbar spine BMD (L2–L4) was measured at the start and two years later (Norland XR Mark 26).

Results: The prevalence of osteoporosis was 9.8% and the prevalence of osteopenia was 38.2% at the beginning of the study. Two years later, when the control DXA was performed, the prevalence of osteoporosis was 17.1% and the prevalence of osteopenia was 40.8%. The mean annual bone change was −1.83 ± 1.83%. In the osteoporotic women at the beginning of the study, the annual bone change was −1.36 ± 1.96%, and in the women who developed osteoporosis along the study it was −3.1 ± 1.74%, with difference in bone change between both groups (p = 0.024).

Conclusion: The prevalence of osteoporosis is about 10% at the start of the menopause but increases sharply through the early postmenopausal period due to the existence of a group of fast bone losers.

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Conflict of Interest: None declared

P450-M

RISEDRONATE ONCE A WEEK IS EFFECTIVE IN MEN WITH OSTEOPOROSIS IRRESPECTIVE OF BASELINE TESTOSTERONE LEVEL OR PREVALENT VERTEBRAL FRACTURE STATUS

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The efficacy and safety of 35 mg risedronate once a week in men with osteoporosis compared to placebo was evaluated in a 2-year, double-blind, randomized, placebo-controlled, parallel-group, multicenter study. Two hundred and eighty-four men (95% Caucasian), 36 to 84 years of age, inclusive, who had osteoporosis (T-score: lumbar spine ≤ −2.5 and femoral neck ≤ −1 SD or

lumbar spine ≤ −1 and femoral neck ≤ −2 SD) were randomized (2: 1) to 35 mg risedronate once a week (N = 191) or placebo (N = 93). The patients also took daily supplementation of calcium (1000 mg) and vitamin D (400–500 IU). The primary endpoint was percent change from baseline in lumbar spine (LS) BMD at Month 24 LOCF (last observation carrier forward). BMD was measured at Months 6, 12, and 24. Subgroup analyses evaluated the mean percent change in LS BMD at Month 24 for patients with low baseline testosterone (< 23 nmol/L; 72 placebo, 153 risedronate) and normal baseline testosterone (> 23 nmol/L; 21 placebo, 37 risedronate); no prevalent vertebral fracture (52 placebo, 114 risedronate) and at least 1 prevalent vertebral fracture (28 placebo, 59 risedronate).

The primary endpoint (Month 24 LOCF) showed a statistically significant difference between placebo and risedronate in mean percent change from baseline in LS BMD (4.53% [95% CI: 3.46, 5.60]). This difference was demonstrated regardless of whether men had baseline testosterone levels that were low (4.02% [95% CI: 2.83, 5.22]) or normal (6.69% [95% CI: 4.17, 9.20]). Similarly, a statistically significant mean percent difference between placebo and risedronate for LS BMD was observed regardless of prevalent vertebral fracture status; patients with no prevalent vertebral fracture had a 4.55% (95% CI: 3.16, 5.940) difference and patients with ≥ 1 prevalent vertebral fracture had a 3.96% (95% CI: 2.19, 5.74) difference.

Approximately 70% of patients in both the placebo and risedronate groups experienced an adverse event (73% placebo, 70% risedronate).

In this 2-year study, 35 mg risedronate once a week had a safety profile similar to placebo and was effective for the treatment of osteoporosis in men. Lumbar spine BMD increases were observed, irrespective of baseline testosterone levels or prevalent vertebral fracture status.

Conflict of Interest: S. Boonen: Procter & Gamble, consultant E. S. Orwoll: Procter & Gamble, consultant D. Wenderoth: Procter & Gamble, employee K. J. Stoner: Procter & Gamble, employee R. Eusebio: Procter & Gamble, employee P. D. Delmas: Procter & Gamble, consultant

P451-T

A BOUT OF RESISTANCE EXERCISE INCREASES SERUM OSTEOPROTEGERIN IN HEALTHY YOUNG MEN

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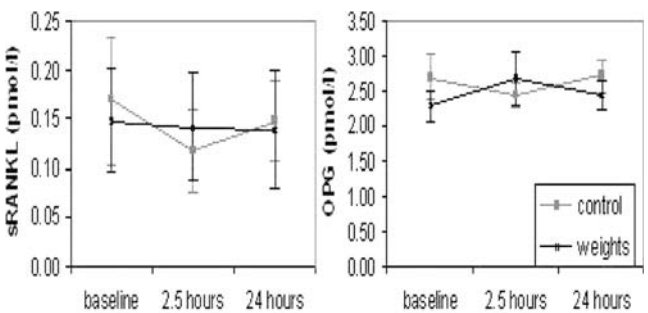
In vitro studies have reported increased production of osteoprotegerin (OPG), and reduced production of RANKL, in response to loading. There is little information as to the effects of loading on circulating OPG or soluble RANKL (sRANKL) in humans. This study thus aimed to determine whether a bout of resistance exercise influences serum OPG or sRANKL.

Participants were 7 healthy young men, who each completed a resistance exercise trial, and a control trial, in random order. The resistance exercise trial consisted of 3 sets of 12 repetitions of 10 resistance exercises, at 80% of 12-repetition maximum. During the control trial participants undertook sedentary activity in the laboratory. Food intake was matched for 48 hours before, and during the trials. Serum OPG and sRANKL were determined before exercise, after 2.5 hours (30 minutes after the exercise bout) and after 24 hours. sRANKL values for one subject were excluded as they were below assay detection limits. Repeated measures analysis of variance was used to determine whether values differed over time and/or between trials.

Plasma volume did not change significantly in either trial. Changes in sRANKL were not statistically significant, either within or between trials (Figure 1). For OPG, levels increased after the weights session, whilst a non-significant reduction occurred following the control session (Figure 1). The interaction term in analysis of variance was significant, indicating that changes differed significantly between trials ($p = 0.03$).

Resistance exercise increased serum OPG in healthy young men, but did not influence sRANKL. The OPG/RANKL/RANK system may be involved in bone response to mechanical loading.

Figure 1: Mean (standard error) serum concentrations of soluble RANKL and OPG over control and weights trials.



Conflict of Interest: None declared

P452-S

DETERMINANTS OF PEAK BONE MASS IN YOUNG FEMALE WOMEN

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Background: Many studies indicated that physical activity and calcium supplementation are beneficial for achiving and perserving adequate bone mass.

Aims: to analyze the influence of dietary intake and physical activity on bone mass in healthy female student population (N = 196), aged 18 to 25 years.

Subjects and Methods: Bone mineral density (BMD) was measured, using DXA technique, in spine, proximal femur, distal third of the radius and in total body. The Quantified Food Frequency Questionnaire (FFQ) was used for detailed dietary assessment. Physical activity was recorded by specialized designed questionnaire.

Results: significant negative correlation between age and bone mass was found in all skeletal regions except in cortical part of radius. Body weight and physical activity were the most significant positive predictors of bone density in all measured sites. Students who were permanently involved in sport activities (N = 55) had significantly higher bone mass in all skeletal regions. Calcium intake did not significantly correlated with bone mass.

Conclusion: In this study, physical activity, rather than calcium intake, plays an important role in maximizing bone mass in young people.

Table: Multiple regression with BMD as dependent variable

	Sign. predictors:		
	Age	Weight	Physical activity
BMD Spine	B = -0.009; $p < 0.05$	B = 0.005; $p < 0.0001$	B = 0.008; $p < 0.01$
BMD Femur	B = -0.017; $p < 0.05$	B = 0.004; $p < 0.01$	n.s.
BMD Radius	n.s.	B = 0.004; $p < 0.001$	B = 0.007;$p < 0.001$
BMD Tot. body	n.s.	B = 0.004; $p < 0.0001$	B = 0.001;$p < 0.05$

Conflict of Interest: None declared

P453-M

PHYSICAL ACTIVITY IN ELDERLY MEN AND WOMEN: ASSOCIATIONS WITH BONE LOSS, MUSCLE STRENGTH, FUNCTIONAL PERFORMANCE AND FRACTURES IN A 10-YEAR PROSPECTIVE POPULATION BASED STUDY

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Background: Exercise training can improve multiple musculoskeletal risk factors for fracture, but the long-term effects of habitual physical activity (PA) on muscle and bone, function and fractures remains uncertain. The aim of this 10 yr prospective population based study was to examine age-related changes in BMD relative to muscle strength, balance and gait in older men and women, and to assess the long-term PA effects on the musculoskeletal system and fracture risk. Methods: Men (n = 152) and women (n = 206) aged 50, 60, 70 and 80 yrs in 1988–89 had BMD (distal radius), grip strength, balance, gait velocity, PA and fractures (questionnaire) reassessed after 10 yrs. The effect of varying habitual PA histories was assessed by categorizing participants into a moderate (M) or low (L) activity group at baseline and follow-up to form four groups: LL, ML, LM and MM. Data for men and women were pooled as there were no gender by activity interactions. Results: For the age-related changes, there was a significant gender by age-change category interaction for BMD ($p < 0.05$); women 60–70 and 70+ yrs had greater losses compared to women aged 50–60 yrs (−1.5 vs −0.7%/yr, $p < 0.05$), whereas the rate of loss was similar (−0.9%/yr) for all men. There were no gender interactions for strength, balance or gait. The rate of loss in muscle strength was similar in all age-change categories for men and women (~2.6%/yr). The decline in balance was greatest in 70+ yr old men, whereas for women the deterioration was greatest in those 60–70 and 70+ yrs ($p < 0.001$). Gait velocity only declined in men and women aged 70+ yrs ($p < 0.001$). For PA, the rate of bone loss was 0.5–0.6%/yr less in the MM vs LL ($p < 0.01$) and ML ($p = 0.05$) groups. Annual changes in balance were also less in the MM and ML vs LL or LM groups ($p < 0.05$). These results were independent of age, gender, adolescence PA, changes in disability and medication or baseline measures. There was no

effect of PA on changes in muscle strength or gait. The proportion of new fractures (total, distal forearm or other fragility fractures) was lowest in the MM group (total: LL 15%; LM 13%; ML 12%; MM 7%), but the between group differences were not significant. Conclusion: Older adults who maintain an active lifestyle have reduced bone loss and better balance, but not muscle strength or gait. While absolute fracture incidence was halved in those who maintained an active lifestyle, this study was not powered to detect significant differences.

Conflict of Interest: None declared

P454-T

SOFT TISSUE BODY COMPOSITION AND HIP BONE MINERAL DENSITY IN HIP-FRACTURE WOMEN

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Soft tissue body composition has been shown to affect bone mineral density (BMD), but the relative roles of lean and fat compartments have not been definitively elucidated. Our aim was to investigate the relationship between femoral bone mineral density (BMD) and both appendicular lean mass (aLM) and fat mass (FM) in a sample of elderly women with hip fracture. We assessed 293 of 325 hip-fracture women admitted consecutively to a rehabilitation hospital. Soft tissue body composition and BMD were assessed by dual-energy x-ray absorptiometry (DXA), 23.2 ± 7.7 (mean ± SD) days after fracture occurrence. BMD was measured at the unfractured femur. We calculated aLM as the sum of LM in arms and legs. Because metal implants (prostheses and nails) affect the regional assessment of body composition, aLM was corrected by substituting LM in unfractured leg for LM in fractured leg: corrected aLM = (LM in unfractured leg × 2) + LM in arms. We used two approaches to adjust corrected aLM for body size: corrected aLM divided by height squared (aLM/ht2), and corrected aLM adjusted for height and FM (residuals). Both FM and aLM were significantly correlated with femoral BMD. However the correlation coefficients for aLM were lower than for FM, they further decreased after adjustment for height squared, and were no longer significant after correction for both height and FM (residuals). When FM, aLM/ht2, age, and time spent between fracture occurrence and DXA assessment were included together as the independent variables in a regression model, FM was the only independent variable significantly associated with BMD. The coefficients of partial correlation ranged from 0.414 to 0.647 depending on the femoral region of BMD assessment ($p < 0.001$). The lack of a significant relationship between aLM and femoral BMD in our patients casts doubts on the role of sarcopenia (i.e., the clinical condition of having abnormally low levels of muscle mass) in the genesis of osteoporosis in the elderly. In the same patients, FM emerged as a pivotal determinant of femoral BMD.

Conflict of Interest: None declared

P455-S

OSTEOPOROSIS STUDY IN COMPLETE SPINAL CORD INJURED MEN

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AIM: To compare changes in bone parameters in spinal cord injured (SCI) men and able-bodied controls and to assess the influence of positive and negative factors in SCI men in osteoporosis.

METHODS: We studied 24 men: 16 complete SCI in chronic stage (>1.5 yrs) separated in group A (n = 8, T4–T7 neurological level of injury (NLoI)) and group B (n = 8, T8–T12 NLoI) in comparison with 8 healthy men as control group of similar age, height, and weight. Mean age in all cases 32.3 yrs. All subjects were examined by peripheral quantitative computed tomography system pQCT XCT 3000, Stratec, Germany) in distal epiphyses and midshafts of the tibia. We calculated bone parameters (BMDtrb, BMD tot, SSIPol 2, BMDcort, SSIPol 3, THIcort, Bone/Muscle ratio)and studied the influence of positive and negative factors (age at injury, duration of paralysis (DoP), spasticity, pharmacological treatment, ambulation and daily activities)on bone structures.

RESULTS – CONCLUSIONS: In group A (mean age: 26.8 yrs, DoP: 6.3 yrs) and in group B (mean age: 37yrs., DoP: 4.3 yrs) all bone parameters (except BMD cort)were statisticant signifficant. No significant relationships were found between the intensity of bone loss and any of positive and negative factors.

Table:

PQCT	Param.	Control Group C	Parapl. group A	Parapl. group B	Differ. C vs.A	Differ. C vs.B	ANOVA p value
Tibia Slices	subject	8	8	8			
4%	BMDtrb	264 ± 39	110 ± 60	134 ± 68	-58.3%	-49%	< 0.0005
	BMDtot	342 ± 42	181 ± 49	188 ± 65	-46.9%	-45%	< 0.0005
14% (x1000)	SSIPol2	2.1 ± 0.1	1.8 ± 0.4	1.6 ± 0.2	-14.5%	-24.5%	0.009
38% (x1000)	BMDcort	1.1 ± 0.1	1 ± 0.2	1 ± 0.4	-1.8%	-4.6%	0.03
(x1000)	SSIPol3	2.3 ± 0.1	1.8 ± 0.2	1.9 ± 0.1	-19%	-17%	0.003
	THIcort	6.4 ± 0.4	5.1 ± 1	5.3 ± 0.7	-19,8%	-17%	0.002
66%	Bone/Mu	5.9 ± 0.7	10 ± 4.9	15 ± 5.2	82%	159%	0.001

Conflict of Interest: None declared

P456-M

DIETARY PROTEIN INFLUENCES INTESTINAL CALCIUM ABSORPTION, PTH PRODUCTION AND PAMIDRONATE EFFECTS

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We demonstrated a decrease in bone mass and in bone strength, and an anabolic effect of bisphosphonates in rats fed an isocaloric low protein diet. Whether changes in calcium (Ca) metabolism and/or calciotropic hormones are involved is not known. To test this hypothesis, we investigated whether intestinal Ca absorption, parathyroid gland response to serum Ca and parathyroid hormone (PTH) production could be influenced by dietary protein intake. In 6 month-old rats, intestinal Ca absorption was assessed over a period of 72 hours. Modulation of calcemia was induced by a single subcutaneous injection of pamidronate (0.6 mg/kg) and blood samples were collected at days 0, 4 and 7. Long-term effect was investigated 8 weeks after administration of pamidronate (0.6 mg/kg/day) during 5 days. PTH, IGF-I and osteocalcin were determined. Values are means ± SEM, *$p < 0.05$ vs. controls, °$p < 0.05$ vs. low casein as evaluated by ANOVA. Fractional intestinal Ca absorption (% of the dietary Ca intake) was decreased in rats fed a low protein diet as compared to control (16.5 ± 2.6* vs. 27.4 ± 3.3). This was associated with increased PTH in rats fed a low protein diet as compared to controls (431 ± 114* vs. 182 ± 31 pg/ml). Four days after a single injection of pamidronate, PTH (expressed in % of controls) was increased by +144.6 ± 53.6 and +43.0 ± 27.7° in rats fed a low and normal protein diet, respectively. At day 7, the values were +52.2 ± 26.4 and +4.7 ± 14.7 in the same groups. Table represents the results obtained 8 weeks after pamidronate treatment.

Reduced protein intake was associated with a decreased intestinal Ca absorption which in turn induced secondary hyperparathyroidism. This could contribute to bone loss observed under low protein diet. Secondary hyperparathyroidism induced by pamidronate was more pronounced in rats fed a low protein diet. Chronic increase of PTH levels together with inhibition of bone resorption by pamidronate could be a possible explanation for the positive bone balance and improved mechanical properties under low protein diet and bisphosphonate.

Table: Results obtained 8 weeks after pamidronate treatment

	Normal protein + vehicle	Normal protein + pamidronate	Low protein + vehicle	Low protein + pamidronate
PTH (pg/ml)	169 ± 9	155 ± 18	264 ± 56*	452 ± 69*°
IGF-1 (ng/ml)	681 ± 53	698 ± 39	525 ± 37*	518 ± 27*
Osteocalcin (μg/l)	12.3 ± 0.5	9.4 ± 0.4*	11.9 ± 0.7	8.6 ± 0.4*°

Conflict of Interest: None declared

P457-T

THE EFFECTS OF 6 MONTHS GROWTH HORMONE SUPPLEMENTATION ON BIOCHEMICAL MARKERS OF BONE RESORPTION IN OSTEOPOROTIC WOMEN

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Fourteen post-menopausal osteoporotic women [average age 63.4yr, range 52–79yr] were commenced on 0.2 mg GH/day for 4 weeks, after which the dose was increased by increments of 0.1mg/day every two weeks until the serum concentration of insulin-like growth factor1 [IGF1] was within ±1 SD of the median value for a woman aged 45. Fasting serum samples were collected pre and 6 m post GH supplementation and stored at −70°C until analysed. A panel of biochemical markers covering osteoclast activity and collagen breakdown were used to assess bone resorption: tartrate resistant acid phosphatase 5b [TRACP], cathepsin K [cathK], carboxy terminal cross-linking telopeptide of type 1 collagen [CTX-MMP], carboxy [CTX] and amino [NTX] terminal telopeptides, . We also measured the RANKL decoy receptor osteoprotegrin [OPG] and IGF1. All the assays had interassay CVs of < 10 % over the measurable range. Results are expressed as mean ± SD. IGF1 increased from 14.2 ± 4.1nmol/L to 24.6 ± 8.0 [$p > 0.001$] at 6 m of GH therapy, TRACP increased from 5.4 ± 1.8 to 6.7 ± 2.0 U/L, NTX from 36 ± 15 to 118 ± 51ng/L. Of the two C- terminal peptides CTX increased from 0.79 ± 0.34 to 1.21 ± 0.37mcg/L whilst CTX-MMP increased from 5.0 ± 1.7 to 7.6 ± 2.4 mcg/L, the ratio CTX-MMP/CTX decreased from 7.1 ± 3.4 to 6.6 ± 2.3 [ns]. CathK decreased from 12.3 ± 15.6 to 9.7 ± 15.2 pmol/L; no significant change was seen in OPG, 4.1 ± 1.0 pmol/L to 3.8 ± 0.9 pmol/L. After 6m the change in IGF1 correlates with that for TRACP, CTX, CTX-MMP [$p < 0.05$] and OPG [$p < 0.01$]; the change in CTX also correlates with that for TRACP and CTX-MMP [$p < 0.05$]. The decrease in cathK correlates with the increase in NTX[$p < 0.05$] whereas the change in the ratio CTX-MMP/CTX shows a negative correlation with that for OPG [$p < 0.05$]. After 6 m of GH therapy there was an increase in osteoclast numbers as shown by the increase in TRACP and an increase in bone resorption. The decrease in cathK showed no correlation with the ratio CTX-MMP/CTX but correlated with the increase in NTX. Even though there was no significant change in OPG, from the correlation data it appears that the change in individuals is influenced by the increase in IGF1. All the changes reported are directly or indirectly under the influence of increased IGF1 as a result of GH supplementation.

Conflict of Interest: None declared

P458-S

TRANSVERSE ELEMENTS OF VERTEBRAL TRABECULAR BONE ARE SACRIFICIAL ELEMENTS ALLOWING DISSIPATION OF ENERGY

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The aim of this study was to investigate the mechanical relationships of longitudinal and transverse elements of vertebral trabecular bone.

T12/L1 vertebral bodies from 6 individuals and the L4/L5 vertebral bodies from another 7 individuals were collected postmortem (mean age 69.3 ± 11.2 years). Cubes of trabecular bone of approximately 10 × 10 × 10 mm were cut from the central part of each vertebral body. Cubes from each T12/L1 and L4/L5 pair were assigned to either superoinferior (SI) or anteroposterior (AP) testing groups. Initially, all samples were tested to failure (uncompromised) by uni-axial compression according to their SI or AP allocation. Cubes were then re-tested (compromised) in the orthogonal direction. Following mechanical testing, apparent ultimate failure stresses (UFS) and apparent elastic moduli (E) were computed.

No significant difference in architectural parameters was found between males and females or between SI and AP test groups. UFS and E were significantly greater in the uncompromised SI tests than in the uncompromised AP tests (by approximately 76 % & 84 %, respectively). UFS and E were significantly greater in the compromised SI tests compared to compromised AP tests (by approximately 77 % & 89 %, respectively), similar to the relationship in uncompromised sample tests. No significant differences in mean UFS or mean E were found between uncompromised and compromised samples ($P = 0.50$ and $P = 0.49$, respectively) tested in the SI direction. In AP tests no significant difference ($P = 0.32$) in mean UFS was found between uncompromised and compromised samples, however a significant difference ($P = 0.03$) was observed for mean E. Interestingly, in both uncompromised and compromised tests the relative magnitude difference in strength was greater between SI and AP strengths (76% & 77%, respectively) compared to uncompromised and compromised strengths for both SI and AP tests (9% & 15%, respectively).

These results show that anisotropic strength differences between longitudinal and transverse elements of the vertebral body are greater than strength difference resulting from compromised orthogonal structures. Thus, transverse elements may have a significant role in the dissipation of energy during trauma. In effect, they may act as sacrificial elements allowing dissipation of energy away from the principal longitudinal elements, thereby minimizing damage to the SI elements, analogous to the crumple zones in modern vehicles.

Conflict of Interest: NL Fazzalari, Eli Lilly, Procter and Gamble, Grant Research Support

P459-M

EVALUATION OF SERUM OSTEOPROTEGERIN MEASUREMENT AS MARKER OF CARDIOVASCULAR RISK IN POSTMENOPAUSAL WOMEN

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Introduction: Recently, osteoprotegerin (OPG), a key factor in bone remodelling, was implicated in human atherogenesis. Some prospective studies have reported a relationship between OPG and both fatal stroke and cardiovascular mortality. Cross-sectional studies have also demonstrated a relationship between OPG levels and severe coronary atherosclerosis, mainly in male subjects. However, the role of OPG as a cardiovascular risk factor in postmenopausal women with osteoporosis is still unclear. Objectives: To determine the relationship between serum concentrations of OPG and a cardiovascular risk score using clinical y biochemical markers in women with postmenopausal osteoporosis. Subjects and methods: We selected 126 postmenopausal women (mean age 63 ± 7 years) and anthropometric parameters and serum OPG (OPG ELISA, BIO-MEDICA-GRUPPE Wien, Austria) were determined. The participants were classified using a cardiovascular risk score performed according to a point system taking into account the presence of the following: established coronary hearth disease (CHD - 4 points), arterial disease of the legs (4 points), age of at least 70 years (2 points), diabetes mellitus (3 points), smoking (1 point), arterial hypertension (1 point) and hyperlipidemia (1 point). Results: Our study population could not be considered as having a high risk profile for cardiovascular disease (mean score: 1, 7). Only 12 percent of the patients had a high risk of cardiovascular disease (score > 4). OPG was correlated with cardiovascular risk score (Spearman rank correlation coefficients: 0.2, $p = 0.04$). OPG was also related to diabetes mellitus ($p = 0.05$) and sports/leisure activities (0, 2, or > 2 hours per week). Patients with a low risk of cardiovascular disease (score < 1) had OPG serum levels lower than the rest of the patients. Conclusion: The measurement of serum OPG might contribute to the better stratification of cardiovascular risk in postmenopausal women. Future studies will evaluate the role of serum OPG as a prognostic marker of cardiovascular events.

Conflict of Interest: None declared

P460-T

BONE MICROARCHITECTURAL CHANGES IN RHEUMATOID ARTHRITIS ASSESSED BY 3D MICRO-CT

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Aim: To assess the microstructural implications of rheumatoid arthritis (RA) in a set of previously performed human transiliac bone biopsies from steroid-free patients using μ-CT and 3D structure analysis.

Methods: 14 transiliac bone biopsies from steroid-free RA patients were obtained. None of them had other diseases or was taking any medication known to affect bone mineral metabolism. Complete medical history and laboratory tests including rheumatoid factor (RF) were performed. Bone specimens were embedded in methyl methacrylate and μ-CT imaging was performed in a eXplore Locus SP μ-CT specimen scanner. Acquisition parameters: 80 kVp/80 μA, FOV = 2 × 2cm^2 , 360° acquisition mode, 720 views, exp. time 1700 ms., voxel resolution 28μm. Selection of 3D region of interest (ROI) in trabecular bone was performed using a cylindrical ROI aligned parallel to the outer cortical and using an spline-drawn ROI interpolated from selected image slices. Cortical bone was avoided, and bone tissue was segmented from bone marrow by automatic thresholding. Stereological parameters BV/TV, Tb.Th, Tb.Sp, Tb.N and Euler volume were automatically generated using parallel-plate algorithms. Comparison between patients and 14 age and sex matched controls was performed using t-test for unpaired samples. Statistical significance was set at $p < 0.05$.

Results: 14 RA specimens (10 women, 4 men) were included. Patient data (mean ± SD): age: 59.8 ± 11 years; Steinbrocker's index: 2.21 ± 0.5; RA duration: 42.2 ± 27.8 months; 71.4% were positive for RF. Correlation for all stereological parameters between both ROI selection methods was excellent ($r2 = 0.83$–0.91). BV/TV in RA was significantly lower than controls ($p < 0.05$). Tb.Th in controls was higher than in RA patients, especially with spline ROI due to inclusion of thicker peripheral trabeculae ($p = 0.06$). RA had a significantly higher Tb.Sp than controls ($p < 0.05$). Controls had higher Tb.N than RA patients, but difference was significantly only in the spline-drawn ROI ($p = 0.02$). Euler volume was significantly higher in controls ($p < 0.01$), showing a moderate correlation with Tb.N in the spline ROI ($r2 = 0.69$).

Conclusions: RA patients have a poor trabecular bone quality with low network connectivity. Selection of a spline-drawn ROI is a more accurate

method as mimetizes histomorphometry. 3D μ-CT is a powerful tool when assesing bone trabecular microstructure.
Conflict of Interest: None declared

P461-S

BONE MICROARCHITECTURAL ASSESSMENT IN MALE IDIOPATHIC OSTEOPOROSIS BY 3D MICRO-CT

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Aim: To assess the microstructural implications of male idiopathic osteoporosis (OP) in a set of previously performed human bone specimens using μ-CT and 3D structure analysis.

Methods: 14 transiliac bone specimens from male patients with idiopathic normocalciuric OP were obtained. Exclusion criteria were existence of disorders and drug therapies potentially affecting bone metabolism as well as hypercalciuria. BMD in lumbar and femoral neck was measured with DEXA scan. Bone specimens were embedded in methyl methacrylate and μ-CT study was performed (eXplore Locus SP μ-CT specimen scanner). Acquisition parameters: 80 kVp/80 μA, 10^{-3}-inch thick aluminum filter, FOV$\approx 2 \times 2$ cm, 360° acquisition mode, 720 views (0.5° incr.), 4 frame averages/view, exposure time 1700 ms and voxel resolution of 28 μm. Selection of 3D region of interest (ROI) in trabecular bone was performed using a cylindrical ROI aligned parallel to the outer cortical surfaces of diameter 50% the distance between the inner endosteal surfaces and using a spline-drawn ROI interpolated from selected slices avoiding cortical bone. Automatic thresholding was used to segment bone tissue from bone marrow. Stereological parameters BV/TV, Tb.Th, Tb.Sp, Tb.N and Euler volume were automatically generated using parallel-plate algorithms. Comparison between patients and 11 age and sex matched controls was performed using t-test for unpaired samples. Statistical significance was set at $p < 0.05$.

Results: Patient data (mean ± SD): age 50.71 ± 10.4 years; serum calcium 9.2 ± 0.5 mg/dL; 25-OH vitamin D 24.06 ± 16.96 ng/mL; PTH-i 39.8 ± 14.9 pg/mL; BMD (g/cm^2): lumbar 0.762 ± 0.26; femoral 0.615 ± 0.21; ward's 0.407 ± 0.16; Troch 0.566 ± 0.26. Excellent correlations were found between both ROI selection methods for all stereological parameters (r2 = 0.70–0.92). BV/TV was significantly higher in controls than in OP males ($p < 0.05$). Tb.Th was higher in controls but differences were not significant ($p = 0.26$). Tb.Sp and Tb.N in both ROI selection methods were significantly higher in controls ($p < 0.05$). Euler volumes were higher in healthy controls, but the differences were not significant ($p = 0.14$)

Conclusions: Male OP patients have a poor trabecular bone quality with low network connectivity. Both ROI selection methods have an excellent correlation but spline-drawn ROI seems a more accurate method as mimetizes stardand histomorphometry. 3D μ-CT is a powerful and faster tool when assesing bone trabecular microstructure.
Conflict of Interest: None declared

P462-M

HOSPITALIZATION AND THE RISK OF FRACTURE IN HEALTHY OLDER ADULTS

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Background: People age 65 or older have 13 million hospitalizations per year in the U.S., staying an average of 6 days. The risk of fracture after hospitalization has not been studied. We hypothesized that long or repeated hospital stays would indicate an increased risk of hip fracture.

Methods: We prospectively recorded hospitalizations and validated fractures in 3075 white and black women and men, aged 70 to 79. We excluded fractures due to trauma, pathologic conditions, stress fractures, and those of unknown cause.

Results: During a mean 6.6 years of >95% complete follow-up, 2030 subjects had hospitalizations, and 387 suffered clinical fractures, including 83 hip fractures. Adjusting for age, race, and gender, any hospitalization resulted in a 2.0-fold increased risk of clinical fracture (95% confidence interval: 1.6 to 2.5). Hospital stays > 3 days indicated a 2.6-fold (95% confidence interval: 1.6 to 4.2) increased risk of hip fracture; shorter stays were not associated with an increased risk (1.3; 0.8 to 2.2). Those hospitalized 3 or more times had a 3.7-fold (1.6 to 8.1) increased risk of hip fracture. We estimated that a 75 year old white woman who stays > 3 days in hospital had a 3.7% (95% confidence interval 0.9 to 6.3) 5 year risk of hip fracture and a 75-year old white man, a 0.5% (95% confidence interval 0.0 to 1.2) 5 year risk of hip fracture.

Conclusion: Older women and men who have long or repeated hospitalizations have a substantially increased risk of hip fracture. Evaluation and treatment to prevent fractures should be considered for elderly patients who stay at least 3 days in the hospital. Long hospital stays often present missed opportunities to prevent fracture.
Conflict of Interest: None declared

P463-T

PIOGLITAZONE TREATMENT SIGNIFICANTLY DECREASED BONE MINERAL DENSITY IN A RANDOMISED PLACEBO-CONTROLLED STUDY IN PATIENTS WITH POLYCYSTIC OVARY SYNDROME

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Background: The in-vivo effect of PPARgamma agonists on bone mineral density (BMD) is largely unknown. In the present study we investigated the effect of pioglitazone treatment on BMD in polycystic ovary syndrome (PCOS).

Methods: Thirty PCOS patients were randomised to 16 weeks of pioglitazone (30 mg/day) or placebo. Before and after intervention, patients underwent measurements of BMD (hip (neck, total), lumbar (L2–L4)), bone metabolic parameters (alkaline phosphatase (ALP) and 25-hydroxyvitaminD (25OHD), endocrine profiles (testosterone, estradiol and insulin) and measurements of body composition (waist circumference, BMI and whole body DXA scan measures). Thirty age and weight-matched females were included as a control group.

Results: Lumbar BMD levels were significantly higher in PCOS patients than in controls, (geometric mean (-2SD + 2SD)) 1.133 (0.974 – 1.318) vs. 1.077 (0.868 – 1.336) g/cm^2, $p < 0.05$.

Pioglitazone treatment was followed by significantly decreased (baseline vs. 16 wks.) lumbar (1.140 (0.964 – 1.348) vs. 1.127 (0.948 – 1.341) g/cm^2) and hip BMD (neck 0.966 (0.767 – 1.217) vs. 0.952 (0.760 – 1.192), total hip 1.080 (0.900 – 1.310) vs. 1.078 (0.885 – 1.314) g/cm^2), all $p < 0.05$. Furthermore, ALP levels significantly decreased (186 (110 – 315) vs. 173 (104 – 288) U/l), $p < 0.05$. Fasting insulin levels were significantly decreased during pioglitazone treatment, whereas no significant changes were observed in testosterone, estradiol, body mass index or fat mass. 25OHD analyses are ongoing.

No significant changes in BMD, ALP or endocrine profiles were observed during placebo.

Conclusion: Pioglitazone treatment was followed by significantly decreased lumbar and hip BMD even following a short treatment period in a small study population.
Conflict of Interest: None declared

P464-S

EXCESS MORTALITY AFTER HIP FRACTURE AMONG POSTMENOPAUSAL WOMEN: EVIDENCE FROM DATA SEARCHES AND LIFE-TABLE ANALYSES FOR LONG-TERM DIFFERENCES IN ABSOLUTE RISK OF DEATH AFTER HIP FRACTURE

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Aims: To determine the magnitude and duration of excess mortality (difference in absolute risk of death) after hip fracture among postmenopausal women.

Materials and Methods: To determine the difference in absolute risk of death (absolute risk difference of mortality) after hip fracture, we applied life-table methods using U.S. age-specific mortality rates for white women, and pooled relative risks for mortality after hip fracture, estimated from cohort studies by standard meta-analytic methods. We selected only cohort studies that reported data on postmenopausal women aged 50 years or older, carried out a life-table analysis, and displayed the survival curves of the hip-fracture group and an age- and sex-matched control group.

Results: Sixteen cohort studies contributed to this analysis. Our results indicate that the estimated excess mortality depends largely on age. At any given age the estimated excess mortality (difference in absolute risk of death = absolute risk difference) after hip fracture among postmenopausal women was always higher than among women from an age- and sex-matched control group. We estimated that a white woman, when sustaining a hip fracture at age 70, for example, has an excess mortality of 3%, 4%, 7%, and 13% at 1, 2, 5, and 10 years after injury, respectively. At age 75, these differences in absolute risk of death are 5%, 6%, 11%, and 17%; at age 80, 8%, 10%, 16%, and 19%; at age 85, 13%, 17%, 21%, and 16%; and at age 90, 21%, 25%, 23%, and 9%, respectively.

Conclusions: Excess mortality among postmenopausal women having sustained a hip fracture depends largely on age. At any given age, however, the excess mortality (absolute risk difference = difference in absolute risk of death) after hip fracture among postmenopausal women is always higher than among women from an age- and sex-matched control group. The impact of a hip fracture on excess mortality among postmenopausal women continues for up to 10 years after injury.

Conflict of Interest: None declared

P465-M

CALCIFIED LARGE ARTERIES, OSTEOPOROSIS& ACUTE STROKE. WHAT IS THE RELATIONSHIP?

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Background: Atherosclerosis and osteoporosis are currently considered unrelated diseases. As age advances, osteoporosis is more frequently found in women than men; atherosclerosis is an illness predominantly affecting men Fujita 1984. A parallel relationship has been noted between spinal osteoporosis and aortic calcification due to atherosclerosis Dent CE, et al. 1968. Osteoporosis and stroke share several risk factors, such as age, smoking, low level of physical activity, and hypertension. Nguyen TV et al. 2000. Thus Low bone mineral density (BMD) and a high risk of stroke may thus be related, but studies on this relationship are sparse. The artery wall contains cells capable of differentiation into osteoblasts, following the same stages of differentiation as occur in bone-derived osteoblasts, Parhami F et al. 1997.

Objectives: To examined the relationship between Calcified large arteries, BMD and acute stroke in hospitalized patients aged > 60 years.

Methods: Seventy-five stroke patients (40 women and 35 men) in addition to sixty-five ages matched control group were included in the study. Careful family history, full clinical exam. Radiological examination for both lumbar & pelvic regions . Routine lab, Lipid profile investigations were done. The atherogenic index was calculated as the ratio of (total cholesterol–HDL cholesterol) to HDL cholesterol. Body mass index (BMI) was calculated for the entire studied group. BMD was measured by using dual-energy x-ray absorptiometry at both distal forearms to avoid fallacies that done by the calcified aorta. BMD measurements of the stroke patients were performed one week after the onset of stroke.

Results: There was a highly significant difference between the stroke patients and their controls as regards Total cholesterol, LDL, HDL and BMD. However in males; no difference was found between the stroke patients and their controls regarding BMD. As regards aortic calcifications, the noncalcified aorta was significantly higher in controls than stroke group. The advanced calcified aorta &moderate one was significantly higher among stoke group than controls, despite the mild aortic calcification show a non significant difference between both groups. Conclusion: High Total cholesterol& LDL, but Low HDL& BMD in addition to aortic calcification whatever moderate or advanced may early predict stroke both in females and males. This may be an important explanation for the increased incidence of hip fracture in stroke patients.

Conflict of Interest: None declared

P466-T

PIOGLITAZONE INDUCES ADIOPOCYTE DIFFERENTIATION IN A MURINE STROMAL CELL LINE, D1

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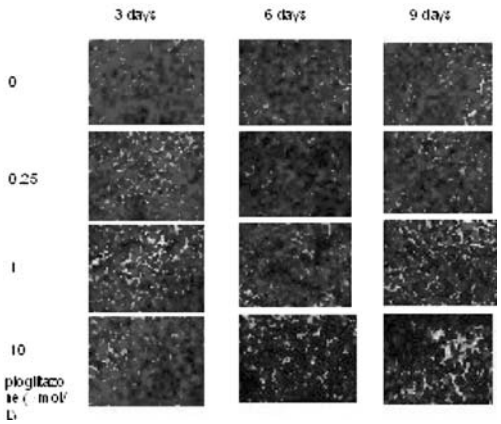
Thiazolidinedione is one of the agonist of PPARγ receptor which has been a medication for diabetic mellitus for years. Treatment with TZDs leads to selective accumulation of subcutaneous adipose tissue. In this study, we tested the adipogenic effect of a TZD, pioglitazone, in murine stromal cells, D1.

METHODS: D1 cells were treated with pioglitazone, 0.25, 1 and 10μmol/L, for 3, 6 and 9 days. The activity of alkaline phosphatase (ALP) was checked. Adipogenesis were analyzed by oil red stain.

RESULT: The activity of ALP decreases significantly after treating with pioglitazone in a dose- and time-dependent pattern. In the 3rd day, pioglitazone, 1 and 10μmol/L but no 0.25μmol/L, mildly increases adipogenesis on oil red stain. In the 6th day, pioglitazone , 0.25 and 1μmol/L, mildly increases adipogenesis on oil red stain. However, Pioglitazone, 10μmol/L, intensively increases adipogenesis on oil red stain. (Fig) Adipogenic effects of pioglitazone expresses in a dose- and time-dependent pattern.

CONCLUSION: We find pioglitazone can decrease the alkaline phosphatase activity and induce adipogenesis in D1 cells. Osteogenesis may therefore be hampered with the activation of PPARγ receptor. As pioglitazone has been a popular medication for DM, the adipogenic effects on stem cells should be close monitored.



Conflict of Interest: None declared

P467-S

WALKING AND MODES OF HABITUAL PHYSICAL ACTIVITY ARE BENEFICIAL FOR BONE AND BODY COMPOSITION IN OLDER WOMEN

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Positive impact of structured exercise on bone health and body composition has been reported in several studies. However, the effects of low-impact and/or habitual physical activity (PA) on bone or body composition are more difficult to elucidate, although they might be a more realistic life-style component, particularly in older women. The aim of this study was to examine the influence of low-impact and habitual PA on bone in postmenopausal women over 3 years. Simultaneously, the interaction between PA and body composition (lean and fat tissue) was evaluated and the compounded effect of the two on bone examined. The participants included 136 (at enrollment) healthy Caucasian women, 68.6(7.1) years (Mean (SD)), BMI = 26.0(3.8) kg/m^2 who were evaluated every 6 months. Body composition and BMD were measured by Lunar DPX-MD. Dietary intake was assessed by 3-day records, while PA was assessed using the Allied Dunbar National Fitness Survey for older adults. The activities assessed were expressed as hrs/week and included: heavy housework, gardening, stair-climbing, walking, and sports/recreational activities. Also, total activity was computed by summing all assessed activities. Results from repeated measures ANCOVA (adjusted for common confounders) revealed subjects with more walking hrs/week had significantly higher BMD of total body (F-ratio 2.2, $p = 0.0418$), femoral shaft (F-ratio 2.1, $p = 0.0488$) and spine (F-ratio 2.2, $p = 0.0442$). To examine whether more active subjects lost less bone over 3 years, the % change in BMD between baseline and 3 years was calculated and subjects were stratified based on the cumulative median for total activity (5.1 hrs/week). ANCOVA, followed by Bonferroni correction revealed subjects with higher total activity increased BMD in total femur and femoral neck compared to those with lower activity ($p = 0.01$). When evaluating the influence of body composition on bone, multiple regression results revealed lean tissue had higher influence on BMD than fat tissue. Various modes of PA were beneficial for body weight and fat, but there was no relationship between PA and lean tissue. In conclusion, walking (1.3 (1.8) hrs/week) was beneficial for BMD of total body, spine and femoral shaft and generally more active subjects increased femoral BMD during 3 years, compared to less active ones. Based on these results, walking and other habitual modes of PA could be beneficial for bone and reduce body weight/fat in older women. Funded by NRI/USDA.

Conflict of Interest: None declared

P468-M

MINERAL WATER AND ALCOHOL CONSUMPTION ARE ASSOCIATED WITH BODY WEIGHT AND BONE, RESPECTIVELY, IN OLDER CROATIAN WOMEN

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Studies on nutrition and life-style modifiers in relation to bone and body composition in older Croatian women are sparse. Our objective was to investigate the association of mineral water (MW), coffee and alcohol consumption and smoking with bone mineral density (BMD) and body weight in 120 healthy Croatian postmenopausal women aged 59.9 ± 7.3 (mean ± SD) y, BMI 26.5 ± 3.8 kg/m^2. Hip and spine BMD was assessed by Lunar-Prodigy. Nutrient assessment from 3-day dietary records was analyzed using the USDA Food Composition Tables and the Croatian National Institute of Public Health Tables. Subjects were also asked to record the consumption of alcohol, coffee, tea and mineral waters (MW) and smoking habits. Forty-two (35%) subjects consumed mineral water on a regular basis averaging 202 ± 196 ml/d that provided about 30.7 ± 32.3 mg/d Ca, 45.7 ± 93.0 mg/d Mg, 4.5 ± 5.3 mg/d K and 194.1 ± 189.5 mg/d of Na. Almost all subjects drank coffee (197 ± 104 ml/d) and 64 (53.3%) subjects consumed alcohol, most often wine, n = 52 (97.1 ± 78.2 ml/d), beer, n = 24 (170.8 ± 126.5 ml/d) and hard liquors, n = 7 (12.4 ± 4.6 ml/d). Only 14 (11.7%) subjects smoked, averaging 13.3 ± 10.6 cigarettes/d. When subjects were divided based on the MW consumption (controlled for age), the difference in weight/BMI was statistically significant: 71.6 kg and 27.1 kg/m2 for non-consumers vs. 67.2 kg and 25.5 kg/m2 for consumers (p = 0.0236 and 0.0176, respectively). There was no relationship between MW consumption and BMD of any skeletal sites, probably because mineral waters typically consumed in Croatia have no considerable amounts of minerals like Ca, Mg, K. Alcohol consumption was positively related with hip and spine BMD in multiple regression models (controlled for years-since-menopause, weight and/or height), while coffee consumption and smoking were not related to BMD or body weight, the latter one probably because only ~12% of subjects smoked. In conclusion, consumption of MW might be beneficial for weight/BMI as it probably replaces other energy-rich drinks. Moderate alcohol consumption was beneficial for BMD of both hip and spine in this population of apparently healthy Croatian women, as was previously shown in other populations. Funded by the Croatian Ministry of Science, Education and Sports.

Conflict of Interest: None declared

P469-T

EFFECT OF PREVALENT VERTEBRAL FRACTURES ON LIFE EXPECTANCY AND FUNCTIONAL PROGNOSIS AFTER HIP FRACTURES IN THE ELDERLY AGED 90 YEARS AND OLDER

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This longitudinal study was conducted to assess the long-term functional outcome of very elderly patients with hip fractures, to determine whether BMD and prevalent vertebral fractures could affect mortality and ambulatory status, and to examine which patient characteristics reported in the literature to be predictive of the mortality and ambulatory status also in these very elderly patients. Seventy-four patients 90 years and older with hip fractures were analyzed and followed up until death or at least 7 years. The mean age was 92.8 years (65 women and 9 men), and all patients were treated surgically and the 7-year survival rate was 12%. All patients had osteoporosis (mean T score, 48%) and 67 of 74 patients (90.5%) had at least one prevalent vertebral fracture on admission, and the mean number of prevalent vertebral fractures was 2.5 ± 1.6 per person. Walking ability at discharge was decreased compared with that before injury and walking ability decreased during 1 year after discharge, but thereafter reached a plateau. The results suggest that the preoperative American Society of Anesthesiologists score (RR = 2.82, 95% CI 1.57–5.08), walking ability (RR = 1.56, 95% CI 1.03–2.34), type of fracture (RR = 2.08, 95% CI 1.05–4.12), type of surgery (RR = 1.98, 95% CI 1.01–3.91), and the number of prevalent vertebral fractures on admission (RR = 1.38, 95% CI 1.11–1.72) are correlated to survival. The degree of dementia (on admission, OR = 28.6, 95% CI 3.73–200.0; after surgery, OR = 22.7, 95% CI 3.03–166.7) and the number of prevalent vertebral fractures at the time of admission (OR = 1.64, 95% CI 1.04–2.59) are the factors associated with the recovery of walking ability after surgery. Thus, the increasing number of prevalent vertebral fractures on admission was correlated with poor recovery of walking and high mortality rate after surgery; strengthening the importance of treatment of osteoporosis for preventing vertebral fractures.

Conflict of Interest: None declared

P470-S

RELATIONSHIP BETWEEN SERUM HSCRP CONCENTRATION AND BIOCHEMICAL BONE TURNOVER MARKERS IN HEALTHY PRE- AND POSTMENOPAUSAL WOMEN

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Although osteoporosis and atherosclerosis seem to be related, the mechanisms are not understood yet. We previously observed that women with higher serum concentrations of high sensitivity C-reactive protein (hsCRP), a strong risk factor for atherosclerosis, had lower bone mineral density (BMD). However, the relationship of hsCRP level with bone turnover rate, an independent risk factor for osteoporotic fracture, is not known. We performed a cross-sectional hospital-based survey in apparently healthy pre- and postmenopausal women (n = 39 and 150, respectively). Urinary N-terminal telopeptide of type I collagen (NTx) and serum bone specific alkaline phosphatase (BALP) were measured using commercially available immunoassay kits. Serum hsCRP concentrations were measured by a particle-enhanced immunoturbidometric method. Both urinary NTx (γ = 0.288, P < 0.001) and serum BALP (γ = 0.260, P < 0.001) were positively correlated with serum hsCRP levels. The significances were persistent even after adjustment for age, body mass index and years since menopause (γ = 0.257, P < 0.001, and γ = 0.163, P = 0.027, respectively). Compared with subjects in the lowest hsCRP quartile (≤ 0.6 mg/l), those in the highest hsCRP quartile (≥ 1.6 mg/l) had significantly higher urinary NTx concentrations (P = 0.001) after adjustment for confounding variables. The difference among the hsCRP quartile groups for serum BALP was a trend for higher BALP concentrations (P = 0.073). These findings suggest that low grade systemic inflammation may be a common linking factor between development of atherosclerosis and increased bone turnover rate.

Conflict of Interest: None declared

P471-M

GENDER DIFFERENCES IN CALCIUM HOMEOSTASIS: A REFLECTION OF AGING?

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Introduction: Total and ionized serum calcium levels are tightly regulated, predominantly by the kidney, intestines and bone through the actions of PTH and 1α-25(OH)$_2$-D$_3$, that are known to change with aging. The aim of our study was to assess the calcium homeostasis during life in men and women.

Methods: Two different study populations were used. First, we studied about 4000 subjects aged > 55 years of the Rotterdam Study of whom total and ionized serum calcium levels were available. The second population was derived from the database of the Clinical Chemistry Laboratory (CCL). People (n = 6500) that attended the hospital between January and November 2005 and whom had total serum calcium and albumin measurements were included. This population was divided in 3 different age groups, > 55 years, 40–50 years and 1–18 years.

Results: A significant decrease in total serum calcium with aging was found in men as well as women in both study populations. This could be explained by the decrease in albumin with aging in both genders. Men had significant lower total serum calcium levels (2.382 vs. 2.392 mmol/l) than women; p < 0.01 after adjustments for age, weight, serum creatinin and albumin. Analyses of the CCL database provided similar results for the > 55 years group, with men having lower total serum calcium levels, 2.326 vs. 2.359 mmol/l; p < 0.001 after adjustments for age and albumin. Interestingly, no gender differences in serum calcium were observed in the 2 younger age groups.

In the Rotterdam study, ionized serum calcium levels were available and also these were significantly lower in men than in women (1.283 vs. 1.289 mmol/l, p < 0.01; after adjustment for age, weight, serum creatinin and albumin). In both genders, ionized serum calcium levels decreased with aging, p < 0.001 after adjustments for weight and serum creatinin. 1α-25(OH)$_2$-D$_3$ levels in the Rotterdam study were significantly higher in men than in women (111.5 pmol/l vs. 107.6 pmol/l, p < 0.01), and decreased significantly with aging in both genders.

Conclusion: In older men and women total and ionized serum calcium levels decreased with aging. Furthermore, we demonstrated a yet unidentified age-related gender difference in total as well as ionized serum calcium levels. The differences in calcium homeostasis control between males and females became apparent after menopause, suggesting a role for hormonal factors, such as estradiol or gonadotrophins, that strongly change after menopause.

Conflict of Interest: None declared

P472-T

CHANGES IN BONE TURNOVER MAKERS FOLLOWING GLUCOCORTICOID THERAPY FOR EXACERBATIONS OF INFLAMMATORY BOWEL DISEASE

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We previously observed that inflammatory bowel disease (IBD) patients receiving an eight week reducing course of glucocorticoids (GCs) for disease exacerbations undergo rapid bone loss. To investigate the relative contribution of

disease activity and GC therapy to bone loss, we examined bone markers in 49 IBD patients, comprising 18 patients about to commence GC therapy following a disease exacerbation, and 31 patients with inactive disease. We used multivariable regression analysis to study the relationship between disease activity as reflected by Crohn's/Ulcerative Colitis clinical activity scores, bone resorption (serum CTX) and bone formation (PINP) markers. There was no association between either CTX or PINP and disease activity, suggesting IBD does not directly influence skeletal metabolism. Next, we examined the effect of GC therapy on bone metabolism in IBD, by comparing CTX and PINP levels immediately before, and one week after, commencement of GC therapy in the 18 patients with active disease. GC therapy increased CTX by 60%, but decreased PINP by 31% (see Table below), suggesting profound uncoupling of bone formation from resorption. Finally, we explored the role of interactions between GC therapy and IBD disease activity, by comparing changes in biomarkers in response to GC treatment across tertiles of disease activity. Broadly similar changes were observed in serum CTX and PINP, irrespective of disease activity tertile:

See table of statistical analysis.

We conclude that GC therapy given for exacerbations of inflammatory bowel disease causes rapid and profound uncoupling of bone turnover, which we assume underlies the bone loss previously observed in this context. There was little evidence for an interaction with current levels of disease activity, suggesting that other factors contribute to individual variations in response.

Table: Data for Pre-GC versus Post-GC

Tertile	CTX (ng/mL) Pre GC	Post GC	PINP (ng/mL) Pre GC	Post GC
1st	0.18 +/- 0.05	0.29 +/- 0.06	36.5 +/- 4.3	27.2 +/- 3.3*
2nd	0.23 +/- 0.09	0.37+/- 0.07*	46.7 +/- 7.8	30.7 +/- 4.6*
3rd	0.18 +/- 0.01	0.32 +/- 0.09	45.2 +/- 7.2	31.3 +/- 3.3*
Combined	0.20 +/- 0.03	0.32+/- 0.04*	42.8 +/- 3.8	29.7 +/- 2.1*

Mean +/- SEM *P < 0.05 by paired Student's t-test

Conflict of Interest: None declared

P473-S

EARLY REDUCTION OF HIP FRACTURES DEMONSTRATES RISEDRONATE IS MORE COST-EFFECTIVE THAN GENERIC ALENDRONATE: THE CASE OF GERMANY

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OBJECTIVES: Reduction of the risk of hip fractures has not been evaluated in head-to-head randomized clinical trials. However, recent results from claims databases have shown differential short-term efficacy between bisphosphonates on hip fractures. The objective of this analysis was to assess the cost-effectiveness and health utility of risedronate compared to generic alendronate in clinical practice.

METHODS: A validated Markov model of osteoporosis (Tosteson, 2001) was used to estimate the impact of therapy on hip fractures, costs, and quality adjusted life years (QALYs). The analysis included women aged 75 years treated with once-a-week dosing of risedronate or once-a-week dosing of alendronate over 1 year. The model further simulated downstream costs and QALYs for a 10-year period. Country-specific data included general population mortality, fracture costs, and annual drug costs (Actonel plus Calcium 547.76; Generic Alendronate 411.23). Costs and outcomes were discounted at 3%. A differential hip fracture relative risk reduction of 43% was applied to risedronate vs. alendronate (Silverman SL, et al., Osteoporosis International, 2006).

RESULTS: In a cohort of 1,000 women with 1 year of treatment the model predicted a difference of 2.5 hip fractures, 1.88 more QALYs and a cost savings of 103,275 for risedronate compared to generic alendronate. If extrapolated to a German female osteoporotic population of 75 years and older, this analysis suggests that 1,227 additional hip fractures can be avoided and 50.7 million saved by treating patients with risedronate rather than generic alendronate. These results do not reflect the potential added benefit of calcium included with risedronate in Actonel plus Calcium.

CONCLUSIONS: Based on "real world" data the analysis favors the adoption of risedronate therapy for the treatment of postmenopausal women 75+ with osteoporosis.

Conflict of Interest: HP Kruse: Procter & Gamble, consultant M Pasquale: Procter & Gamble, employee W Moehrke: Procter & Gamble, employee

P474-M

BODY COMPOSITION AND ANTHROPOMETRICS FACTORS AS DETERMINANTS OF PHALANGEAL BONE ULTRASOUND IN WOMEN

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Background: Body composition is known to be related with measures of bone mineral density (BMD). However, the relationship between segmental body composition and peripheral bone mineral density measurements are limited. The aim of this study is to examine the effect of body compartment's fat mass and lean body mass (in trunk and arms) on phalangeal bone ultrasound parameters.

Method: Data from a sample of 377 spanish women between 23–82 years old were divided according to their gonadal status (pre, peri and postmenopausal women). Fat and lean body mass were determined using a Tanita BC 418 bioimpedance assay system, that can show separate body mass readings for the right arm, left arm, trunk, right leg and left leg. We evaluated bone status using an ultrasound device that measures the amplitude-dependent speed of sound (Ad-SoS) measured at the proximal phalanx of the nondominant hand (DBM Sonic Bone Profiler. Igea, Carpi, Italy).

Results: Stepwise regression analysis showed that body mass index (BMI) and age negatively, and trunk lean mass positively, were determinants of Ad-SoS in overall group of women ($p < 0.0001$). BMI was negatively determinant of Ad-SoS in premenopausal women ($p < 0.01$), while that in perimenopausal women height were positively determinant ($p < 0.0001$). In postmenopausal women, lean mass (positively) and BMI (negatively) were determinants ($p < 0.0001$).

Conclusion: The strong influence of BMI and its components, previously reported for Ad-SoS, persists, even considering as variables the segmental body composition by bioimpedance analysis.

Conflict of Interest: None declared

P475-T

EFFECTS OF ANABOLIC AND ANTI-CATABOLIC AGENTS ON MANDIBULAR BONE IN OVARIECTOMIZED RATS

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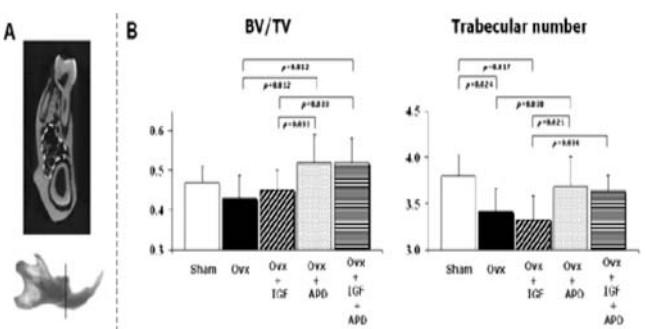
Background. Bone loss induced by ovariectomy (OVX) affects less the mandible than other skeletal sites. The mandible is of membranous origin in contrast to most of the axial and peripheral skeleton, and it is subjected to very heavy abrupt forces during mastication. Whether this modifies its response to osteotropic agents is not known. Aims. We investigated the effect of IGF-I (anabolic) and pamidronate (APD, anti-catabolic) on BMD (DXA) and trabecular micro-architecture (micro-CT) of mandibular alveolar bone in OVX rats. Methods. 44 4-month-old female Sprague-Dawley rats underwent transabdominal OVX or sham operation (sham). After 9 weeks OVX animals were randomly allocated into 4 groups. Two of them received IGF-I by osmotic minipumps implanted subcutaneously. The two other OVX groups and the sham group received the vehicle alone. Then, one group which received IGF-I and one OVX control group received subcutaneous injections of pamidronate (APD). The sham and the other OVX groups received the vehicle. Results. IGF and APD increased BMD in OVX animals ($p < 0.05$ and $p < 0.01$), while OVX did not influence mandibular BMD. Trabecular micro-architecture was not influenced by IGF. However, APD increased BV/TV ($p < 0.05$), and trabecular thickness ($p < 0.01$) and number ($p < 0.05$). OVX decreased trabecular number and spacing ($p < 0.05$ and $p < 0.05$). IGF increased cortical thickness ($p < 0.05$) which may explain the concurrent BMD increase. Conclusions. Mandibular trabecular bone responds favourably after OVX to APD but not to IGF-I, while cortical bone seems to respond favourably only to the latter. These results confirm that the mandible responds differently than other skeletal sites to osteotropic agents, possibly due to functional, morphological, and embryological differences.



Conflict of Interest: None declared

P476-S

DIFFERENCES IN THE NORMAL REFERENCE VALUES FOR THE BIOCHEMICAL BONE MARKERS IN GREEK PREMENOPAUSAL WOMEN

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Aim: Determining the rate of bone turnover is a valuable tool and is widely used in everyday clinical practise. However, the normal values for the premenopausal rate of bone turnover (biochemical bone markers) has never been quantified in Greek population. Therefore, the values used are those defined by the reagent kits which are based on different population groups.

Material and Methods: One hundret Greek premenopausal woman between the ages of 30 to 54 years were examined and written consent was received prior to blood sampling. Women who had diseases affecting bone metabolism were excluded. The values of the following bone markers were determined from the blood serum: osteocalsin (Roche Diagnostics GmbH, Mannheim), bone alkaline phosphatase (Quidel Corporation, San Diego, CA USA), PINP (Roche Diagnostics GmbH, Mannheim), serum NTX (Osteomark NTx Serum, Wampole laboratories NJ USA) and serum CTX (Roche Diagnostics GmbH, Mannheim). The results then underwent statistical analysis. The reproducibility was 5–8%.

Results: According to our study the normal values for premenopausal women were:

1) Osteocalsin: 3, 56 to 33.8 ng/ml (μ.o: 18, 68, SD: 7, 56. 2) Bone Alkaline Phosphatase: 4, 52 to 27, 08 U/L (μ.o: 15, 80, SD: 5, 64). 3) PINP: 12, 01 to 58, 49 μg/l (M.O: 35, 25, SD: 11, 62). 4) NTX: 5, 18 to 21, 98 ng/ml (M.O: 13, 58, SD: 4, 20). 5) CTX: < 0, 531ng/ml, (μ.o: 0, 265, SD: 0, 133).

Conclusions: 1. According to our results reference values for the biochemical markers that determine bone turnover in Greek women have now been evaluated. 2. It is now possible to determine whether Greek women have a normal , high or low bone turnover following the menopause. 3. The values of osteocalsin and bone alkaline phosphatase in the Greek population differ significantly to those given by the manufacturing companies accompanying the reagent kits whilst smaller, less significant differences were seen with the rest of the parameters

Conflict of Interest: None declared

P477-M

DO MOTHERNAL ANTECEDENTS OF FRAGILE PERIPHERAL FRACTURES INFLUENCE YOUNG WOMEŃS BONE MASS?

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INTRODUCTION: Colles fracture is often accompanied by decreased bone mineral density (BMD) and a higher risk of later fractures. It is usually the earliest osteoporotic fracture found in young women and this plays an important role in the family history of osteoporotic disease.

AIMS: To evaluate whether the daughters of women with Colles fractures reach or not a normal peak of bone mass, and if the maternal antecedent of more than onea osteoporotic fracture has a greater repercussion on their bone mass.

SUBJECTS AND METHOD: We have studied 176 healthy women between 20–40 years old. 95 had antecedents of Colles fracture (cases) and 81did not (controls). Medical history, general analytical, bone marker turnover and measurement of bone mass (DXA-Lunar) at hip (femoral neck (FN), Wards triangle (WT), and total hip (TH)) and lumbar spine (LS) were performedon everyone. SPSS 14.0 for Windows program was used for statistical study.

RESULTS: the BMD peak in daughters of women with Colles fractures does not differ significantly tothe control group in all locations:

Controls: LS 1.25 ± 0.12 gHA/cm^2, FN 1.00 ± 0.14 gHA/cm^2, WT 0.93 ± 0.15 gHA/cm^2 and TH 1.00 ± 0.17 gHA/cm^2.

Cases: LS 1.20 ± 0.11 gHA/cm^2, FN 0.98 ± 0.13 gHA/cm^2, WT 0.87 ± 0.16 gHA/cm^2 and TH 0.99 ± 0.14 gHA/cm^2. However, a high percentage of cases was BMD Z –score low than -0.5 (36.8% in LS 40 % in FN, 29.5 % in WT and 36.8 % in TH).

These daughters of mothers with another fracture in addition to the Colles fracture had significantly lower BMD than those of mothers only having Colles fracture (0.93 ± 0.11 gHA/cm^{2}opposite to 0.99 ± 0.12 gHA/cm^2 in FN, $p = 0.018$, and 0.82 ± 0.10 gHA/cm^2 opposite to 0.88 ± 0.15 gHA/cm^2 in TW, $p = 0.05$, respectively) and than control group (0.99 ± 0.14 gHA/ cm^2, $p = 0.013$). We could verified in daughters of mothers with Coolleśs fracture that the maternal antecedent of more than one fracture increased the risk of lower BMD in FN, expressed as Z-score ($p = 0.018$, OR = 2.75 and IC = 95 %, 1.17–6.41)

CONCLUSIONS: The daughters of women with Colles fractures have a similar bone mass peak in all central skeletal locations to the control group but if their mothers present another fracture associated to that of Colles, they have a greater risk of lower BMD in femoral neck.

Conflict of Interest: None declared

P478-T

THE EFFICACY OF TERIPARATIDE (TPTD) IN REDUCING BACK PAIN IN PATIENTS AFFECTED BY SEVERE OSTEOPOROSIS WITH VERTEBRAL COMPRESSION FRACTURES (VCFS) AT THE EVOLUTION IN PSEUDOSTEOARTHITIS

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The efficacy of TPTD in reducing back pain in patients affected by severe OP with vertebral compression fractures (VCFs) is well documented; it's also known the antalgic effect of vertebroplastic procedures.

Aim of the study: 1) evaluate the evolution of back pain in plurifracturated pat. with severe OP treated with TPTD via subcutaneous injection at the dosage of 20 mcg/die, in association with calcium and vitamin D 2) verify, in case of persistence of pain, the necessity of having recourse to vertebroplastic procedures. We studied 75 female patients, mean age 74 years. Back pain was due to recent (approximately 30 days) VCFs in 57 pat., in 18 pat. to non recent VCFs (at least 6 months); right now only 10 patients concluded the 18 months expected treatment.

All patients undergone: Lumbar spine X-rays (at the time of the fracture) with evaluation of the Spinal Deformity Index (SDI) – basal dosage of: calcium, ALP, PTH, Vitamin D and 24 hours calciuria – dosage of calcium, ALP and calciuria after 3, 6, 18 months – lumbar spine X-rays, spinal and femural BMD at the end of the study. Back pain was evaluated using a visual analogic scale (VAS) at 0, 1, 3, 6, 12, 18 months with the following score: 3VAS 10–7)2(6–4)1(3–1).

At basal the patients with recent VCFs (57 pat.) and those with non recent VCFs had a score of 3 and 2 respectively.

Results:

–reduction of pain in all the patients
–rapid and significant reduction of pain after one month treatment in the group of 57 pat. with recent VCFs
–significant reduction of pain after 3 months treatment in the group of 18 pat. with non recent VCFs
–no SDI change in 10 pat.who completed the 18 months treatment
–after 6 months treatment 1 was theVAS pain score for all the patients
–no patient had to recourse to vertebroplastic procedures to reduce pain

In this study the analgesic effect of TPTD was demonstrated in both recent and non recent VCFs.

Maybe this is attributable to the osteoinductor effect of the TPTD; the rapid and significant reduction of pain is probably explainable with the acceleration of the healing fractures process.

It's presumable that the persistence of pain in non recent VCFs is due to the pseudosteoarthitis evolution; in these cases, in addition of vertebroplastic procedures, TPTD can have a rational use.

Conflict of Interest: None declared

P479-S

FHS IS RELATED TO BONE METABOLISM IN EARLY POSTMENOPAUSAL WOMEN

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Objective: We examined serum and urine in recent postmenopausal women to determine the relation of FSH level, to calcium, phosphorus and biochemical bone markers.

Subjects, material and methods: 176 women aged from 50 to 55 years (mean $52,7 \pm 1,6$ yr) were randomly selected in the province of Albacete (Spain). All women were postmenopausal, from 6 to 36 months (mean 19.0 ± 9.7), and they did not have any diseases and were not taking any drugs known to affect bone metabolism. Calcium (Ca), phosphorus (P), intact osteocalcin (OC) and CTx were measured in serum. DPD, NTx, and calcium (Ca/Cr) were measured in fasting 2 h morning urine. FSH was measured in serum and the values were distributed in quartiles (Q) for the comparison to the other variables.

Results: Table 1.

Conclusions: There is a direct correlation of early postmenopausal FSH to increasing some bone metabolism parameters, but it is significant only for serum calcium and serum CTx. Changes in these parameters may reflect differences in the rate of bone loss.

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Table: Bone metabolism parameters and FSH

FSH	s-Ca mg/dL	s-P mg/dL	Ca/Cr mg/mg	OC ng/mL	s-CTX pM/L	DPD nM/mM	u-NTX nM/mM
Q1	9.2 ± 0.4	3.4 ± 0.5	0.16 ± 0.	19.8 ± 9	4162 ± 21	9.9 ± 3.8	72.6 ± 37
Q4	9.4 ± 0.3	3.6 ± 0.5	0.19 ± 0.	22.3 ± 7	4992 ± 16	10.1 ± 2.	79 ± 25.6
P	0.005	N.S.	N.S.	N.S.	0.048	N.S.	N.S.

Conflict of Interest: None declared

P480-M

LOW TSH LEVEL AND BONE IN IODINE DEFICIENCY REGION

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Objectives. In 1997–1998 epidemological investigations showed, that West Siberia was iodine deficiency region according to WHO/ UNICEF/ ICCID criteria. So, in this territory thyroid pathology is widespread. In modern endocrinology predisposal low TSH level role on osteoporosis development is discussed.

Aims. To study osteoporosis (OP) frequency in Graves disease (GD), subclinical hyperthyroidism (SHT) and patients recieving supressive levothyroxine doses (LT4) in iodine deficiency region.

Materials and methods. We inspected 92 patients, including 46 persons with GD, 25 with SHT and 21patient, recieving supressive doses of LT4 – tab 1. In age and gender groups were the same. Comparison group consisted of 159 practically healthy euthyroid inhabitants of West Siberia. BMD was inspected in ultradistal (UD) and mediodistal (MD) forearm parts by DEXA on DEXA-Scan DX-10 (Direx Medical Co., Israel) according to WHO recommendations (1994). The low traumatic fractures (LTF) in the nearest anamnesis were studied.

Results. Maximal low BMD frequency was registered in GD group (82,6%, including 30,4% OP). In SHT patients osteopenic syndrome was found in 80% (OP in 24%). 76,2% of LT4 patients had low BMD and 9,5% had OP ($p > 0{,}06$). Low traumatic fractures were marked in 10,9% 12% and 14% correspondingly ($p = 0{,}927$). In CG fractures were registered in 5% ($p = 0{,}547$), OP in 8,8% ($p < 0{,}02$). The most low BMD was registered in GD patients ($p > 0{,}1$).

Conclusion. So, low TSH level is assosiated with high osteoporosis frequency in West Siberia inhabitants. We showed, that BMD is independt of thyroid hormones level, but depend on TSH.

Table: BMD and TSH in patients groups

	SHT	LT4	GD	CG	p
TSH* mUI/l	0,20	0,19	0,13	–	0,127
fT4* pmol/l	23,4	–	34,8	–	0,000
T-criteri UD, SD	−1,50	−1,48	−1,61	−0,61	0,000
T-criteri MD, SD	−1,45	−1,25	−1,79	−0,77	0,000

* - mediana

Conflict of Interest: None declared

P481-T

RELATION BETWEEN DIARY PRODUCTS AND TOTAL CALCIUM INTAKE IN A COHORT OF SPANISH POSTMENOPAUSAL WOMEN

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Calcium intake (CI) is important to treat and prevent osteoporosis (OP). However it is difficult to know accurately what the calcium intake of each patient is. Diary products (DP) are so far the most important source of calcium in developed countries.

We have evaluated the DP intake and total CI in a cohort of postmenopausal women attending to perform a bone densitometry.

Material and methods: 504 consecutive postmenopausal women were asked for a validated Spanish weekly calcium questionnaire *. Three groups were establish according total CI (group 1: less than 500 mg/day, group 2: 501–1000 mg/day and group 3: 1001–1500 mg/day). Bone densitometry was performed in all participants with a HOLOGIC QDR 4500 SL at lumbar spine, femoral neck and total hip. Spearman correlation between CI and DP intake, ANOVA and chi squared were used to analyze the results using a SPSS 12.0 package

Results: Mean of age was 60.3 + 8.3, (mean + SD), 62.4% had a OP defined by WHO criteria. Total CI was 1.011 + 338 mg/day and DP 736 + 308 mg/day (72.7% of CI). When correlation between CI and DP was analyzed by groups, we found there was a significant correlation when CI in the group 2 $r = 0.641$ $p = 0.0001$ and group 3 $r = 0,751$ $p = 0.0001$ but not in the group 1 The minimum DP intake in the group 3

was 408 mg/day, therefore in the group 1 and group 2 70% and 13.4% respectively of women had DP intake under 408 mg/day. According BMD results there was not differences in the presence of OP by groups: group 1 no OP 1.4%/ OP 2.5%; group 2 no OP 51.8%/ OP 5.7% ; group 3 no OP 46.8%/ OP 42.7%

Conclusion: In a validated weekly questionnaire of calcium intake, correlation between DP and CI was significant when CI is higher than 500 mg/day. A possible cut-off for DP to assume that CI is higher that 1000 mg/day would be 408 mg/day.

*Orozco P. Aten Primaria 2004; 33: 237–43

Conflict of Interest: None declared

P482-S

PREVALENCE OF DISEASES AND USE OF DRUGS THAT AFFECT BONE IN POSTMENOPAUSAL WOMEN ATTENDED IN A PRIMARY CARE CENTER OF SPAIN. THE CAMARGO COHORT STUDY

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Aims: To determine the prevalence of disease and use of drugs that could have an influence on bone metabolism in a population of postmenopausal women from Cantabria, Spain.

Methods: We present preliminary results of the first 394 postmenopausal women included in the Camargo Cohort Study, a community-based study designed to evaluate the prevalence of metabolic bone diseases and disorders of mineral metabolism, as well as the prevalence of fractures and risk factors for osteoporosis and fragility fractures, in postmenopausal women and men older than 50 attended in a primary care center in Spain. The women studied were 63 ± 9 years old, and were recruited in a primary care center of Camargo, a large semi-urban Spanish city. Demographic, anthropometrics, clinical variables (including drugs and diseases that could affect bone metabolism) were collected.

Results: Seven percent of women have previous diagnosis of urolithiasis, six percent has been diagnosed of cancer, three percent of hyperthyroidism or chronic obstructive lung disease, and two percent of rheumatoid arthritis. Twenty four percent of women were receiving treatment with thiazides, 23% were taking selective serotonin reuptake inhibitors (SSRI), and 16% statins. Oral calcium and vitamin D supplements were administered in 21% and 17% of the women, respectively. Six percent of women were receiving thyroid hormone treatment. Five percent and two percent were taking inhaled or systemic glucocorticoids, respectively.

Conclusion: Two potentially bone protective drugs (thiazides and statins) are among those most commonly used. Glucocorticoid and thyroid hormone treatment, as well as urolithiasis, showed prevalence values around six per cent. However, according to SSRI use, depression could be the most frequent risk factor.

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Conflict of Interest: None declared

P483-M

PREVALENCE OF OSTEOPOROSIS IN POSTMENOPAUSAL WOMEN ATTENDED IN A PRIMARY CARE CENTER OF SPAIN. THE CAMARGO COHORT STUDY

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Aims: To determine the prevalence of osteoporosis in a population of postmenopausal women from Cantabria, Spain.

Methods: We present preliminary results of the first 169 postmenopausal women included in the Camargo Cohort Study, a community-based study designed to evaluate the prevalence of metabolic bone diseases and disorders of mineral metabolism, as well as the prevalence of fractures and risk factors for osteoporosis and fragility fractures, in postmenopausal women and men older

than 50 attended in a primary care center of Spain. Women were recruited in a primary care center of Camargo, a large semi-urban Spanish city. Demographic, anthropometrics, clinical, and densitometric (DXA in lumbar spine [LS], femoral neck [FN,] and total hip [TH], Hologic QDR 4.500) variables were collected.

Results: The mean (±SD) age of subjects was 63±9 years. Mean value for height was 156±58 cm, and weight 70±11 kgm. Results of bone mineral density (BMD) are shown in the table.

Twenty eight percent of postmenopausal women had osteoporosis (T <-2.5) at either the spine, the femoral neck or the total hip. In particular, 19% showed osteoporotic values at the spine, 17% at the femoral neck and 2% at the total hip. Osteopenia was seen in 60% of the women (46% in LS, 59% in FN, and 43% in TH). Only a quarter of the osteoporotic patients had been previously diagnosed of this disease.

Conclusions: Prevalence of osteoporosis among Spanish postmenopausal women is similar to other European countries. Only a small proportion of these patients were aware of this diagnosis.

This study has been supported by a grant from the ''Fondo de Investigación Sanitaria'', Ministerio de Sanidad y Consumo, Spain (FIS: PI05 0125)

Table:

	LS	FN	TH
BMD (g/cm^2)	0.930±0.142	0.738±0.112	0.879±0.124
T-score	-1.35±1.29	-1.56±1.14	-0.80±1.04

Conflict of Interest: None declared

P484-T

THE INCIDENCE OF SECONDARY HYPERPARATHYROIDISM AMONG POSTMENOPAUSAL WOMEN SUFFERING FROM END-STAGE KNEE OSTEOARTHRITIS. INTERMEDIATE RESULTS

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Background/aims. Aim of this study was the assessment of the incidence of secondary hyperparathyroidism (SH) among otherwise 'healthy' and symptoms' free post-menopausal women suffering from primary, end-stage knee osteoarthritis. These are the intermediate results of this study, as the latter is expected to finish with the enrollment of 300 patients in total. Methods. Between November 2004 and November 2006, two hundred and fifty-eight women suffering from end-stage knee osteoarthritis (Outerbridge Grade IV) were enrolled in this prospective cohort study. None of them had suffered any osteoporotic fracture, received any anti-osteoporosis treatment or suffered from any disease interfering with their bone mass/quality. The serum levels of Intact-Parathyroid Hormone (I-PTH), Calcium (Ca) and Phosphorus (P) were evaluated. Their renal function was also assessed. Results. The patients' mean age was 70.13 years (range: 49–81). The years that had passed since their menopause ranged from 6 to 32 (mean of 18.9 years). The patients were divided into three groups according to their age: Group A (n=43) age <64 years, Group B (n=150) age 65–74 years and Group C (n=65) age >75. The overall incidence of SH in all three groups was 36.82% (95/258 patients). One Group B patient was found to have diminished I-PTH value. Her Ca and P values were within normal range. Another Group B patient suffered from Primary Hyperparathyroidism. Group C patients were most likely to suffer from SH (27/65 patients or 41.53%). The PTH values of Group A patients were normal in 28 out of 43 patients (65.11%) and of Group B in 95 out of 150 patients (63.33%). Conclusion. It seems that Secondary Hyperparathyroidism appears to be a 'silent' epidemic among elderly post-menopausal women. According to this study's results, women 75 years-old and older are most likely to suffer from SH. One of the main reasons for that may well be the insufficient calcium and/or vitamin D intake. It is our belief that anti-osteoporosis diagnosis and treatment modalities should be focused on this group of patients. Furthermore, there is emerging significant evidence that there might be a liaison between osteoarthritis and I-PTH elevated values, without being able at the moment to determine whether the cause of these abnormal I-PTH values are the osteoarthritic changes at the cartilage and subchondral bone or vice versa.

Conflict of Interest: None declared

P485-S

BONE HISTOMORPHOMETRY IN MALE IDIOPATHIC OSTEOPOROSIS; INFLUENCE OF BODY COMPOSITION, SEX HORMONES AND INSULIN-LIKE GROWTH FACTOR-1 ON BONE FORMATION

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Impaired bone formation and low levels of sex hormones and insulin-like growth factor-1(IGF-1) have been reported in male idiopathic osteoporosis. We have evaluated bone histomorphometry parameters in transiliacal bone biopsies after tetracycline labelling from 50 eugonadal men with idiopathic osteoporosis. Their median age was 53 years (range 29–73) and bone density below 2,5 SD of the young male adult mean at the lumbar spine and/or femoral neck. Previous fracture was reported in 82 % of the men. Bone density and body composition were analyzed by DXA. Biochemical evaluation included sex hormones, IGF-1 and IGF-binding protein-1 (IGFBP-1) and bone markers. The bone volume (BV/TV and BS/TV) was significantly decreased compared to healthy age-matched men and women. Final erosion depth was similar in osteoporotic men and normal men but decreased compared to women. The reconstructed wall thickness was similar in osteoporotic men and normal men. Men with idiopathic osteoporosis had a significantly thinner osteoid layer compared to control men. The osteoid thickness was positively correlated to bone mineral density at the lumbar spine. Osteoid thickness and mineral apposition rate were positively correlated to body composition; BMI, body weight, total body fat, lean body mass and to serum free estradiol-index (estradiol/SHBG) and to the ratio IGF-1 to IGFBP-1. Bone formation rate was positively correlated to osteoid thickness, free estradiol-index and weight. We found no significant correlations between biochemical bone markers and bone histomorphometry parameters.

Men with idiopathic osteoporosis show some signs of compromised bone formation. Bone resorption was not increased. Low body weight and low body fat mass were associated with a more severe osteoporosis. The results are in agreement with epidemiological data and emphasize the importance of nutritional factors in male idiopathic osteoporosis.

Conflict of Interest: None declared

P486-M

A LOW LEVEL OF VITAMIN D IS A STRONG RISK FACTOR FOR HIP FRACTURES IN OLDER WOMEN IN NORTHERN SWEDEN – THE UFO STUDY

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It has been suggested that the higher incidence of hip fractures found in northern Sweden compared to the southern part is explained by a relative vitamin D deficiency, secondary to the limited sunlight exposure during winter months. Our aim was therefore to investigate whether serum levels of 25-hydroxy vitamin D (25OHD) is associated with the risk of incident hip fractures in women, participating in the Umeå Fracture and Osteoporosis study (UFO). The UFO study is a nested case–control study, investigating associations between serum markers, lifestyle data and osteoporotic fracture and is based on the Northern Sweden Health and Disease Study (NSHDS), consisting of blood samples and lifestyle data from around 100.000 subjects. The NSHDS cohort was coanalyzed with a prospective fracture database, capturing all osteoporotic fractures occurring in the county during 1993–2004. Eighty-three women were identified from these cohorts as having donated blood samples before they sustained a hip fracture. Each fracture case was compared with two controls identified from the same cohort and matched for age and month of sample collection; yielding a total cohort of 249 women (87% postmenopausal) with a mean age at the time for sample collection of 60.4 years (range 49.4–69.8). The mean antedating time (time from blood sampling to fracture occurrence), was 5.8±3.5 years and the mean follow up period was 8.8±2.8 years. Serum 25 OHD was measured by high-performance liquid chromatography. In our population, 1.6% had a serum 25OHD below 30 nmol/l and 16.1% had a value below 50 nmol/. Multivariate Cox proportional-hazards regression models were used for analysis of the risk of fracture, with adjustments for age, BMI, menopausal status and use of HRT. The overall adjusted relative risk of sustaining a hip fracture was 2.7 (95% confidence interval, 1.3–5.5) in subjects with a serum 25OHD below 50 nmol/l. Since age was strongly correlated with vitamin D levels, the results were also presented separately for persons aged 49–60 years at baseline (n=130) and for persons above 60 years at baseline (n=119). In the oldest age group, the HR of sustaining a hip fracture was 3.5 (95% confidence interval, 1.2–10.7) for the group of individuals with a serum 25OHD below 50 nmol/l, whereas no significant association was found in the youngest age group. In summary, a serum 25OHD below 50 nmol/l seems to be a strong and independent risk factor for hip fractures in women over 60 years.

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P487-T

RISK FACTORS FOR OSTEOPOROTIC FRACTURES IN BRAZILIAN MEN AND WOMEN – THE BRAZILIAN OSTEOPOROSIS STUDY (BRAZOS)

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Background/ Aims: Osteoporotic fractures are an important public health problem in worldwide. Several European, North American and Asian studies have demonstrated the identification of risk factors has a relevant role to early diagnosis and for treating people with higher risk for osteoporosis. However, in Brazil and Latin America there are fewer papers on this issue. The purpose of the present study was to evaluate the risk factors for osteoporotic fractures in Brazilian men and women older than 40 years. Patients and Methods: A total of 2420 subjects (1695 women) were enrolled in the BRAZilian Osteoporosis Study – BRAZOS, undertaken in 120 cities across 5 regions (North, Northeast, Central, Southeast and South) of the country, and included people from five categories of economical level (from A to E). This study evaluated aspects on lifestyle, fractures, dietary intake, physical activity, falls, quality of life, willingness to pay and knowledge on osteoporosis by quantitative and personal research that it was applied face-to-face way. The sampling was based in the census data from IBGE (Brazilian Institute of Statistics and Geographic) 2000 and PNAD (National Research of Home Sampling) 2003 and it was calculated according probabilistic and representative sample of Brazilian population. The coefficient of variation is 2.2% with 95% confidence interval. The BRAZOS is the first epidemiological study performed in a representative sampling of the Brazilian population. Results: The subjects were classified as White (50%), Mixed Race (35%), Black (13%), Asian (1%) and Indian (1%). Mean height and weight for men and women was 1.68 m and 1.57 m and 74 and 66 kg, respectively. The majority of men (56%) and women (51%) had BMI > 25.1 kg/ m2. Menopause's mean age was 47 years and 39% of them were postmenopausal and 15% was taking hormone therapy currently. Osteoporotic fracture (forearm, femur, ribs, spine and humerus) were the main skeletal sites reported. and osteoporosis were reported by 5.29% and 6% of this population, respectively. Conclusions: Our results have demonstrated that main risk factors for osteoporotic fractures in Brazilian men and women were age, sedentary, familiar history of hip fracture, smoking and low intake of calcium and vitamin D. This study suggests that a better comprehension of risk factors in our population could be an important tool to identify men and women with higher risk for osteoporotic fractures.

Conflict of Interest: Grants: Wyeth Health Consumer

P488-S

DIETARY VITAMIN D IN BRAZILIAN POPULATION

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Dietary vitamin D is an important component of vitamin D status. Several studies have reported low vitamin D intake, mainly in elderly and in countries were vitamin D fortification is not mandatory. The Food and Nutrition Board, in 1997, established the level of Adequate Intake (AI) for men and women at 5 mg/d for < 50y , from 51 to 70 y at 10mg/d and from 71y and older at 15 mg/d. Furthermore, they recommended that vitamin D intake should be evaluated throughout lifespan by geographical and racial variables. The purpose of the present study was to evaluate vitamin D intake in a representative sample of Brazilian men and women, older than 40 y. This study was part of the BRAZilian Osteoporosis Study (BRAZOS), undertaken in 120 cities across the 5 regions (North, Northeast, Central, Southeast and South) of the country, and enrolled people from 5 categories of economical level. A total of 2400 people were enrolled in the study. Dietary intake of 1000 (one hundred) men and women, 70% women and 30% men, were evaluated by one 24 h dietary record. For the nutrient analysis the Nutrition Data System software (Minneapolis, MN 2005) was used. The dietary records were calculated by a trained dietitian. The use of supplements was not considered in the present analysis. The mean dietary vitamin D intake in all participants was 2.2 ± 1,2 mg/d. No differences were observed considering gender and age. People from North region presented significantly higher mean vitamin D – 2.8 ± 2.5 mg/d, compared to the all other regions (2.1 ± 1.8mg/d in South; 1.8 ± 1.7mg/d in Southeast, 2.2 ± 1.8 mg/d in Central, 2.2 ± 1.8mg/d in Northeast, $p < 0.05$). This observation could be explained by the higher fish consumption presented by the people from North region. A positive correlation was observed between dietary vitamin D and calcium ($r = 0.53$ $P < 0.0001$). Mean calcium intake was 404 ± 263 mg/d. Although not significant, Central and Southeast regions presented higher mean calcium intakes (458 ± 289 mg/d and 418 ± 264 mg/d, respectively). The present study demonstrated an important dietary inadequacy in our population. Dietary vitamin D was under recommended values for age in all regions and economical levels. Also, calcium intake above the preconized value by the Food and Nutrition Board (1200 mg/d). Consequently, our observations suggests that an improvement in vitamin D and calcium intake should be recommended routinely for people older than 40 years.

Conflict of Interest: Grants: Wyeth Health Consumer

P489-M

USE OF ANTIPSYCHOTICS AND RISK OF HIP/FEMUR FRACTURES: A POPULATION BASED CASE–CONTROL STUDY

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Introduction: Side-effects of antipsychotics include extrapyramidal symptoms and increased prolactin levels. These effects may lead to falling or osteoporosis, which are associated with hip/femur fractures. In general, these side-effects are stronger in conventional antipsychotics, compared to atypical antipsychotics. However, the association between different categories of antipsychotics and fracture risk has not been studied.

Objective: To evaluate the association between use of antipsychotics and risk of hip/femur fracture.

Methods: A case–control study (n = 6,763 cases) was conducted using data from the Dutch PHARMO Record Linkage System. Cases were adult patients with a first fracture of the hip or femur. The date of the fracture was the index date. Up to 4 control patients were matched to each case by year of birth, gender and region and had the same index date. Current use of antipsychotics (a prescription in the month before the index date) was compared to never use. We adjusted our analyses for 20 general risk factors for fractures, with a focus on central nervous system medications. Smoothing spline plots were used to visualize the association between recency of use, cumulative duration of use and risk of hip/femur fracture.

Results: Current users of antipsychotics had a significantly increased risk of hip/femur fracture compared to never users (adjusted (adj.) OR 1.68; 95% CI, 1.43–1.99). Among current users, the highest risk estimates were found for patients with 5–8 weeks of treatment (adj. OR 2.30; 95% CI, 1.65–3.20). Fracture risk remained increased after >6 months of antipsychotic use. We found a significant difference between users of conventional (adj. OR 1.76; 95% CI, 1.48–2.08) and atypical antipsychotics (adj. OR 0.83; 95% CI, 0.42–1.65). Smoothing spline plots of recency of exposure and duration of use supported our findings from categorical analysis.

Conclusions: Current use of conventional, but not atypical antipsychotics was associated with increased risk of hip/femur fracture, particularly after 5–8 weeks of treatment. Our results support a possible fall-related, and osteoporosis-related increased risk of hip/femur fracture among users of antipsychotics. Hip/femur fracture risk assessment may be considered for elderly who use conventional antipsychotics.

Conflict of Interest: None declared

P490-T

EVALUATION OF VALIDITY OF IOF'S ONE-MINUTE OSTEOPOROSIS RISK TEST FOR POSTMENOPAUSAL WOMEN

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Osteoporosis and its complications are considered "the silent epidemic of XXI century". They differ from other diseases of locomotor apparatus in an almost complete lack of clinical manifestations up to the occurrence of fracture. 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 33 postmenopausal women aged 50–69 years (mean age 59,0 ±1,4). 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. We created additional index of sum total (the answer "yes" was awarded 2 points, "no" – 1 point).

Results. Among examined women osteoporosis was revealed in 51,5% of cases, low bone mass – in 30,3%, normal parameters of bone – in 18,2%. Parameters of ultrasound bone densitometry were as follows: SOS = 1525 ± 5 m/s, BUA = 107,5 ± 2,1 dB/MHz, SI = 77,8 ± 2,7, - range = 0,39 ± 0,24, Z-range = 0,73 ± 0,22. Significant correlation was found between index of sum total and the BUA ($r = -0,37$, $p = 0,034$) which characterizes the quality of bone, 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 ($r = 0,45$; $p = 0,09$), BUA ($r = 0,36$; $p = 0,038$), SI ($r = 0,42$; $p = 0,014$), - range ($r = 0,42$; $p = 0,015$), Z- range ($r = -0,27$; $p = 0,14$).

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 question 4 (''Have you lost more than 3 cm (just over 1 inch) in height?). Research is continuing.

Conflict of Interest: None declared

P491-S

INFLUENCE OF ORCHECTOMY ON BONE MINERAL DENSITY IN MALE RATS OF REPRODUCTIVE AGE

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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.

There were inspected 16 male rats of reproductive age, ''Vistar'' line. 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 (RC).

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 (Tab). The index was calculated according to the formula: Δ BMD (%) = (Δ BMD/BMD ref.) x 100.

Annotation: ± m; CG – animals of control group; RC – 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.

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.

Table: Dynamics of BMD and BMC in male rats

Group	BMD ref.	B	B	BMD ref.	B	B
CG	0,1 ± 0,002	0,02 ± 0,01	19,33 ± 9,8	9,65 ± 0,26	2,44 ± 0,28	25,88 ± 3,4
ORC	0,1 ± 0,002	−0,003	−2,87 ± 2,4	11,6 ± 0,31	−0,3 ± 0,31	−2,4 ± 2,65
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

Conflict of Interest: None declared

P492-M

DIETARY INTAKE OF FOLATE BUT NOT VITAMIN B12 IS ASSOCIATED WITH INCREASED BONE MINERAL DENSITY 5-YEARS AFTER THE MENOPAUSE: RESULTS FROM A 10 YEAR FOLLOW-UP STUDY IN EARLY POSTMENOPAUSAL WOMEN

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Background: Folate and vitamin B12 may affect bone directly or through and effect on plasma homocysteine levels. Previously, positive association has been found between plasma levels and bone mineral density (BMD) and risk of fracture and a 2-year RCT has demonstrated an antifracture effect of combined treatment with folate and B12. However, there is no data on whether dietary intake of folate and vitamin B12 affects bone.

Aim: To investigated whether intake of folate and vitamin B12 affects BMD and fracture risk

Design: In a population-based cohort of 2,016 perimenopausal women (The Danish Osteoporosis Prevention Study (DOPS)), we studied associations between intakes and BMD at baseline and after 5-years of follow-up. Moreover, associations between intakes and 5- and 10-years changes in BMD as well as risk of fracture were studied. At baseline and at the 5-year visit, we used a 4- or 7-day food record to assess current daily intakes of energy, vitamins, and minerals. A dietician used food models and photographs during a 15-min. validation-interview to evaluate serving sizes and cooking habits.

Results: Intakes of folate and vitamin B12 were 417 (290–494) microgram/day and 4.98 (3.83–6.62) microgram/day, respectively, i.e., slightly above the intake recommended by the FAO. At year 5, but not at baseline, cross-sectional analyses showed positive correlations between daily intake of folate and BMD at the femoral neck (beta = 0.027; 95% CI, 0.008–0.046, p = 0.005). However, no associations were found between intake and changes in BMD. During the 10-years of follow-up, 360 subjects sustained a fracture. Compared with 1440 controls, logistic regression analyses revealed no difference in intake of folate or vitamin B12 between cases and controls.

Conclusion: Although dietary intake of folate and vitamin B12 was not consistently associated with BMD or fracture risk, our data do suggest that a high dietary intake of folate may exert positive effects on BMD.

Conflict of Interest: None declared

P493-T

AROMATASE GENE POLYMORPHISMS ARE ASSOCIATED WITH HIP FRACTURE RISK

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Background. Some common polymorphisms of the aromatase and estrogen receptor (ER) genes which are associated to gene transcription in vitro influence bone mineral density in postmenopausal women (Riancho et al, Eur J Endocrinol 2006). The objective of this study was to determine if they are associated to hip fractures, the most ominous consequence of osteoporotic bone fragility.

Subjects and methods. We studied 578 postmenopausal women aged 60–98 years, including 331 with hip fractures (age 80 ± 8 yr) and 247 controls without clinical fractures (age 75 ± 8 yr). A C/G polymorphism of the aromatase gene and a T/C polymorphism of the ER gene (rs1062033 and rs2234693, respectively) were studied with Taqman assays.

Results. In the whole study group, the aromatase and ER allelic frequency distributions were similar in fracture and control women. However, a significant interaction between both polymorphisms was observed. In women with TC/CC genotypes of ER, the aromatase gene polymorphism was not significantly associated to fracture risk. However, in women bearing TT alleles of the ER (reported to be transcribed less actively), aromatase alleles were associated to fracture risk. Thus, the CC aromatase genotype was present in 36% of women with fractures and only in 16% of controls (p = 0.004). The unadjusted odds ratio was 3.0 (95% confidence interval 1.4–6.4; p = 0.005), and the age-adjusted odds ratio was 4.0 (1.7–9.3; p = 0.001). In the subgroup of women up to 85 years of age, the odds ratio was 5.8 (2.2–15.6; p = 0.0005).

Conclusions. These two frequent polymorphisms of the aromatase and ER genes, which have been previously shown to be associated with bone mineral density, appear to influence the risk of hip fractures.

Supported by a grant from FIS 04/0028

Conflict of Interest: None declared

P494-S

THE AFFECT OF AGE ON VITAMIN D LEVEL: SIGNIFICANT DIFFERENCES BETWEEN EUROPE AND NORTH AMERICA

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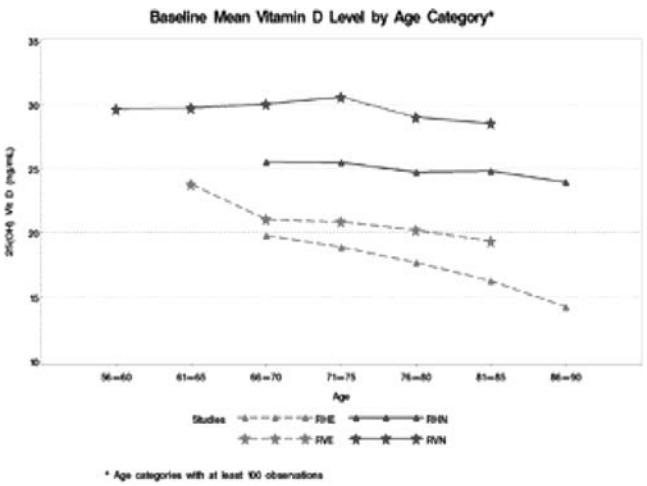
Clinically relevant vitamin D (Vit-D) deficiency has been widely documented among seniors. There is also a strong belief that Vit-D deficiency might be more common in Europe (EU) than in North America (NA). In this research, the affect of age on Vit-D levels was examined for North American and European patients and the mean Vit-D levels between the two continents were compared. The data for all randomized patients from the VERT and HIP risedronate trials were included in this analysis. These patients were all postmenopausal and had either a relevant vertebral fracture, low BMD at baseline or were over 80 years old with at least one clinical risk factor for osteoporosis. Baseline Vit-D (25-OH-D3) levels were used to examine the affect of age as well as possible difference between the two continents. A total of 12642 patients were included in the analysis. Patient's ages ranged from 38 to 100 years. 57% of the patients had at least one vertebral fracture. The mean femoral neck T-score was -2.6 and lumbar spine T-score was -2.6. Results from the regression model showed that the mean Vit-D level in EU was 8.2 ng/mL lower than the mean Vit-D level in NA ($p < 0.0001$). For every decade increase in age, the average Vit-D level decreased by 2.5ng/mL in EU ($p < 0.0001$). The inverse relationship between age and Vit-D level was not statistically significantly in NA ($p > 0.1$). The overall Vit-D levels in EU were significantly lower than in NA. The negative association between age and Vit-D level was found in EU, but not in NA.



Conflict of Interest: JD Ringe, Procter & Gamble Pharmaceuticals, consultant X Zhou, Other, Employee Procter & Gamble Pharmaceuticals AB Klemes: other Employee Procter & Gamble Pharmaceuticals

P495-M

STUDY OF OPG/ RANKL SYSTEM IN HUMAN PRIMARY OSTEOBLAST CULTURES IN PATIENTS WITH OSTEOPOROSIS. INFLUENCE OF ESTRADIOL AND VITAMIN D

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The RANKL is a powerful physiological instigator of the osteoclastogenesis. Its action is inhibited by osteoprotegerin (OPG). Treatment with OPG decreases bone remodelling in postmenopausic women.

AIMS: To study the secretion and expression of OPG and RANKL in human primary osteoblast cultures (hOB) from patients with osteoporosis (OP) and to compare with persons without osteoporosis (OA). To evaluate the influence of 17-beta-estradiol (E2) and 1-alpha-25(OH)2D3 (VD) on the OPG/ RANKL system in hOB on both groups studied.

M & M : Primary hOB cells were isolated from bone fragments taken from patients who had undergone surgery (8 OP, 9 control) Ethical approval had been obtained from the local Research Ethic Committee. Briefly, trabecular bone was cut into pieces (1–2 mm) and thoroughly rinsed with PBS. Cells were incubated in a humidified CO2 incubator at 37°C. In all the cultures, > 85% of the cells showed intense staining for alkaline phosphatase activity. Tripan blue was used to screen out dead cells after harvesting. We quantified the OPG protein levels (ELISA) in basal conditions and after incubation with E2 (10^{-7}M) and/or VD (10^{-8}M) during 24h, as well as, the mRNA of OPG, RANKL by RT-PCR semi-quantitative. The results were analyzed statistically by U-Mann-Whitney and coefficient of Spearman's correlation, considering the level of significance as $p < 0.005$.

RESULTS: The mean age of the patients with OP was older than the control group (82 ± 2 v.s 73 ± 2, $p = 0.024$). The cellular confluence took place later in OP patients and though this related with age ($r = 0.502$, $p = 0.04$), after the adjustment for this variable it continued being more extensive in OP than in the OA group (34 ± 2 v.s. 19 ± 2 days, respectively, $p = 0.002$). In OP patients, the OPG protein secretion was greater than OA group in all situations ($p < 0.05$). OPG expression was comparable between both groups, except after stimulus with VD where it was higher in OA v.s. OP ($p = 0.017$). The RANKL expression and the RANKL/OPG ratio tended to be higher in OP v.s. OA, and they reached a significant difference after treatment with E2 + VD ($p = 0.009$ and $p = 0.021$, respectively). We did not observe significant modifications after the E2 and VD treatment.

In conclusion, the hOB cultures from patients with OP present slower growth than OA, higher OPG secretion and a tendency to an increase in the RANKL/OPG ratio. These findings could explain, at least partly, the greater activity of the bone metabolism in osteoporitic patients.

Conflict of Interest: None declared

P496-T

MENOPAUSE-RELATED CHANGES IN THE TRABECULAR TEXTURE OF THE VERTEBRAE, MEASURED BY HIGH RESOLUTION COMPUTED TOMOGRAPHY

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Menopause-related Changes in the Trabecular Texture of the Vertebrae, measured by High Resolution Computed Tomography (HRCT). Capiglioni R, Montangero VE, Aude F, Luchini R, Roldán EJA . Inst. Tecnol. Especiales., Univ. Abierta Interamericana, Rosario; Dpt. Investig. MusculoEsqueléticas, IDNEU, Buenos Aires.

Since the trabecular bone mineral density (BMD) does not always reflect the degree of strength, other variables are used to complement the diagnosis such as the vertebral pattern or texture by means of HRCT. This study aims at the possibility of quantifying age-related changes in the trabecular texture of the vertebrae in osteoporotic women. Volumetric BMD (vBMD) and texture of the spine were measured in 40 postmenopausal (pm) women assigned to either of 2 groups: 20 early pm women (< 6 ysm, age 51.1 ± 2.9 years) and 20 late pm women (> 6 ysm, age 63.4 ± 2.1 years). All L1 and L2 vertebras were studied with QCT and HRCT. To analyze the HRCT images, the trabecular bone was identified according to thresholds, regional growth and skeletonization stages. In the processed images, the trabecular bone fraction (TB/TV), the trabecular thickness (Tb Th), the trabecular space (Tb sp), and the sizes of mean (xH) and maximum (mH) holes were determined. These texture indexes were correlated with the age and vBMD by QCT. With the exception of Tb Th, all the other texture parameters were associated with age and vBMD ($0.64 < r < 0.80$), $p < 0.001$. Among all studied parameters, the xH, mH and trabecular vBMD showed the best correlation with age. The mean difference between both groups was of 30.0% for xH and 21.5% for mH, with $p < 0.001$. The mean difference between both groups for vBMD was of 16.4%, $p < 0.001$. The texture indexes provided by HRCT showed age-related changes comparable to the trabecular vBMD. These results support the potential use of this technique for the follow-up of age- or disease-related bone changes, not only according to densitometric but to inner structural variables.

Conflict of Interest: Roldan, E, Gador S.A., Scientific Director

P497-S

SPINE VARIATIONS IN HEALTHY ADULT WOMEN ANALYZED BY DIGITAL VERTEBRAL MORPHOMETRY (DVM)

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Spine Variations in Healthy Adult Women Analyzed by Digital Vertebral Morphometry (DVM). Capiglioni R, Montangero V, Aude F, Roldán EJA. Dep. Investig. Músculo-Esqueléticas (IDNEU) – Buenos Aires.

Vertebral (V) fractures are diagnosed subjectively and are, therefore, prone to observer bias. Normal DVM parameters and indexes of aging effects in healthy women are described here. Two age groups were constituted: G1 aged 38–59 years (n = 55), and G2 aged 60–87 years (n = 65). Frontal and lateral x-rays of dorsal and lumbar V were taken, and assessed using DVM. On the lateral x-rays, 6 points were used to determine the anterior (AH), media (MH) and posterior (PH) heights of the V body from T4 to L5, and on the frontal radiographs the lateral heights were used to discard deformations. It was observed that the PH increased progressively from T4 to L2 (L3 stable) and decreased in L4–L5. The AH and MH increased from T4 to L5. The wedge index (WI) was greater in T6–T8; the biconcavity index (BI) was greater in T7; the compression index (CI) was greater in T8. The mean AH increase was of 1.13mm from T4–T8 and of 10.9mm from T8–L3. The PH increased 2.1mm from T4–T8 and 8.9mm from T8–L3. The V area increased 6.6cm^2 in T4–L3 (from 5.4 to 12.0cm^2). The relation AH/PH was of 5% for medial dorsal V, almost cero in L2–L3 and positive for L4–L5 because the AH was bigger than the PH. The greater AH/PH reduction was observed in T7 (5.2%). Values around -2DS showed that the reduction percent may be almost of 0.4% in some dorsal V. The comparison between G1 and G2 showed no significant change in height. When comparing the V Curvature angle (VCa) between both groups, WI – BI – CI significantly increased in T8 to T10 in G2, in all V. The G2 showed a more significantly increase of the V body area in T4, L1 and L2, and greater V area in all dorsal V than in G1. The results express a significant increase of the wedge index in the postmenopausal period after 70, being DVM an objective method.

Conflict of Interest: Roldán EJA, Gador S.A., Scientific Director

P498-M

BONE MINERAL DENSITY AND INCIDENCE OF HIP FRACTURES – SECULAR TRENDS OVER A DECADE

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Introduction: Osteoporosis and its consequence of hip fracture represent a major public health problem among elderly women. Although the incidence of hip fractures has been reported to dramatically increase worldwide during the last decades, its not clear whether these findings are the result of a deterioration in bone mineral density (BMD) and/or an increasing proportion of elderly women in the population. This study was designed to characterize secular trends in BMD and the incidence of hip fractures within an urban and rural female population during the last decade.

Methods: BMD at the distal radius was assessed in a population-based sample of urban and rural women aged 50 to 80 years in 1988 (n = 257 vs. 180) and 1998 (n = 171 vs. 119) by single-photon absorptiometry. Analysis of covariance adjusting for age was used to compare the BMD between the cohorts. Additionally, all hip fractures sustained in the same target population aged 50 years or more between 1987 and 2002 were registered, and the incidence of hip fractures were calculated by use of demographic data. Age-adjustment was done by direct standardisation with the mean population between 1987 and 1988 as the standard population. Time-trend analysis was done by linear regression analysis.

Results: There was no significant difference in the age-adjusted BMD between urban and rural women in 1988 (450 mg/cm^2; 95%CI, 442 to 458, vs. 465, 453 to 481) or in 1998 (445, 435 to 455 vs. 463, 449 to 477). The overall trend in the incidence of hip fracture during the study period was increasing among urban women (4.1 hip fractures per 100,000 women per year; 95%CI, −0.6 to 8.8), whereas the incidence was found to decrease among rural women over the same period (−6.8, −12.0 to –1.2). However, after age-adjustment the overall trend was decreasing in both the urban and rural women during the study period (−4.5, −9.0 to 0.04 vs. −8.0, −13.6 to −2.3).

Conclusions: These data suggests that the discrepancies in the crude incidence of hip fractures between urban and rural women is more likely to be attributable to an increasing proportion of elderly women among the urban population, than to discrepancies in secular deterioration in BMD.

Conflict of Interest: None declared

P499-T

BONE MINERAL DENSITY, BONE TURNOVER MARKERS IN TYPE 1 DIABETIC WOMEN

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Background and Aims: Although osteopenia is reported as a complication of type 1 diabetes mellitus (DM), its frequency and severity remain unclear, and studies of bone mineral density (BMD), bone turnover markers in type 1 diabetics women have yielded conflicting results.

Materials and Methods: In the study were included 92 women with type 1 DM (mean age 32,31 + 0,72 yrs, duration of disease 11,92 + 0,64 yrs, HbA1c 8,7 + 1,05 %). 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 30 healthy matched controls.

Results: Osteopenia was revealed in 45 % of the diabetic women (T-score: −1,46 + 0,43 vs −1,24 + 0,65 in controls), predominantly in the femour neck (32%). The levels of serum ALP (87,23 + 34,25 IU/l) and osteocalcin (18 + 6,7 ng/ml) were statisticly ($p < 0,05$) lower in the patients with osteopenia than in those without osteopenia (110,31 + 23,15 U/l and 27 + 5,7 ng/ml accordingly), that suggested lowering bone formation. In the patients with osteopenia mean CTX (512,75 + 40,9 pg/ml) was statisticly higher ($p < 0,01$) than in those without osteopenia (452,75 + 32,12 pg/ml). There were no differences in the mean levels of HbA1c between the diabetic patients with and without osteopenia (8,12 + 0,98% vs 7,97 + 1,12%).

Conclusions: The data confirme the high prevalence of osteopenia in type 1 diabetics women and demonstrate that at least in type 1 DM women, osteopenia is the consequence of a lowered bone formation with a predominance of bone resorption over formation.

Conflict of Interest: None declared

P500-S

BONE MINERAL DENSITY AND CALCIUM–PHOSPHORUS METABOLISM IN PATIENTS WITH UNDERWEIGHT

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Underweight is associated with increased risk of low bone mass.

The aim of our study was to determine whether decreased body mass index affects bone mineral density and calcium–phosphorus metabolism.

Materials and methods: We have investigated 28 underweighted females (BMI < 18.0 kg/sm^2) under age 30. We measured body mass index (BMI), serum ionized calcium, phosphorus, parathyroid hormone (PTH) values, osteocalcin (OC) from osteosynthesis markers and bone mineral density (BMD) with ultrasound bone densitometry.

Results: In 67.8% of investigated patients we revealed osteopenia, the mean value of T-score was −2.1 ± 0.01. Ca + + values were at the lower level of the norm (1.02 ± 0.01 mmol/l), phosphorus was at the upper level of the norm (4.8 ± 0.01 mg/dl). PTH was at the upper level of the norm (59.3 ± 0.01 pg/ml). Decreased level of bone formation marker – OC was detected (lower level of the norm 7.7 ± 0.02 ng/ml).

Conclusions: Decreased body mass index affects bone mass and calcium–phosphorus metabolism.

Maintaining bone mass in patients with low BMI deserves special attention.

Conflict of Interest: None declared

P501-M

INCREASED MORTALITY IN PATIENTS WITH A HIP FRACTURE – CAUSE OR CONSEQUENCE?

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Background: Patients with a hip fracture have a significant excess mortality. However, it remains unclear if the mortality is linked to the pre-morbid conditions (e.g. cardiovascular diseases) leading to the hip fracture through falls or to complications to the fracture (e.g. lung infections and pulmonary embolism).

Aim: To investigate the causes of mortality after a hip fracture.

Material and methods: All subjects with a hip fracture in Denmark between 1977 and 2001 compared to three age- and gender matched subjects from the general population. Co-morbidity at the time of fracture and causes of death were evaluated.

Results: A total of 169,145 fracture cases were compared to 524,010 controls. The cases had a much higher prevalence of co-morbidity than the controls. The mortality rate was twice as high in fracture cases compared to controls (HR = 2.26, 95% CI: 2.24–2.27). Adjusting for confounders only changed the excess mortality risk little. The mortality after the hip fracture was divided into two categories: an excess mortality of 19% within the first year following the fracture (relative survival = 0.81), and an excess mortality of 1.8% per year (relative survival 0.982) for every year following the fracture. The major causes of the excess mortality were due to factors linked to the accident that caused the fracture, and to a smaller extent to infections. Older hip fracture patients were more likely to die from trauma related factors than younger patients with hip fractures.

Conclusions: Patients with a hip fracture have a pronounced excess mortality risk. The major cause was linked to factors associated with the accident that caused the fracture. Prevention of hip-fracture related mortality may perhaps best aim at preventing the falls and other accidents that lead to the hip fracture.

Conflict of Interest: None declared

P502-T

DISCREPANCIES IN BONE MINERAL DENSITY AND FRACTURE RISK IN PATIENTS WITH TYPE 1 AND TYPE 2 DIABETES – A META-ANALYSIS

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Aim: To study fracture risk and changes in BMD in patients with type 1 (T1D) and type 2 (T2D) diabetes.

Methods: The Pubmed (1951 – December 16, 2005), Embase (1974 – December 16, 2005), and ISI Web of Science (1945 – December 16, 2005) were searched using the terms ''diabetes'' and ''fracture'', and ''diabetes'' and ''bone mineral''. This produced 80 references.

Results: Hip fracture risk was increased in T1D (RR = 6.94, 95% CI: 3.25–14.78, 5 studies) and T2D (1.38, 95% CI: 1.25–1.53, 8 studies) compared to subjects without diabetes. The increase in relative hip fracture risk was significantly higher in T1D than in T2D. BMD Z-score was decreased in the spine (mean ± SEM −0.22 ± 0.01) and hip (-0.37 ± 0.16) in T1D and increased in the spine (0.41 ± 0.01) and hip (0.27 ± 0.01) in T2D. A meta-regression showed that body mass index (BMI) was a major determinant for BMD in both the spine and hip. Glycated haemoglobin (HbA1C) was not linked to BMD. The increase in fracture risk was higher and BMD lower in patients with complications to diabetes such as retinopathy, neuropathy, nephropathy, and arteriosclarosis, but the number of studies was limited.

Conclusions; Hip fracture risk is increased in both T1D and T2D, whereas BMD is increased in T2D and decreased in T1D. A common factor such as complications may explain the increase in fracture risk, whereas BMI may

ameliorate the increase in fracture risk by increasing BMD in T2D. Osteoporosis and fractures may represent a hitherto overlooked complication of diabetes.
Conflict of Interest: None declared

P503-S

HIGH PREVALENCE BUT INSUFFICIENT RECOGNITION AND UNDER-TREATMENT OF VERTEBRAL FRACTURES IN ELDERLY PATIENTS

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Introduction: The risk of osteoporotic vertebral fractures increases with age. Vertebral fractures can cause substantial morbidity in elderly patients and are also good predictors of new vertebral and nonvertebral fractures. Although vertebral fractures can be easily diagnosed on a chest x-ray, they often remain unreported and (thus) subsequently untreated. (Osteoporos Int 2000;11: 577–82, Arch Intern Med 2005;165: 905–9)

Objective: To investigate the prevalence of vertebral fractures on chest x-rays, diagnosis of osteoporosis and treatment among elderly patients attending the geriatric diagnostic day- clinic.

Methods: Retrospectively, 103 subsequent patients attending the geriatric diagnostic day-clinic

in the Slotervaart Hospital, a teaching hospital, were included. Chest x-rays are made routinely in the day-clinic. Vertebral fractures were scored on the lateral chest x-ray using Genant's semi-quantative method. (J Bone Miner Res 1993;8: 1137–48). The x-ray reports were checked for descriptions of osteoporotic deformities: vertebral fractures, increased kyphosis, wedge-shaped vertebrae, or vertebral height loss. Treatment of osteoporosis was recorded from the patients' medical records.

Results: In the group of the 103 included patients (mean age 81 years, 64% female) a total of 51 patients had at least a grade 1 vertebral fracture (table)

In the 27 patients with at least a moderate (grade 2 or 3) vertebral fracture, the radiologist described osteoporotic deformities in 9 patients (33%) in only 4 case an actual vertebral fracture was reported. Only 5 of these 27 patients (19%) were using anti-osteoporotic drugs at discharge from the day-clinic.

Conclusion: In this cross-sectional study we found that there is a very high prevalence (50%) of vertebral fractures among patients attending the geriatric diagnostic day-clinic.

Remarkably, even the moderate and severe fractures (grade 2 and 3) were only diagnosed in 33% of these cases and only 20% of the patients with a moderate or severe fracture were treated.

Table:

	Prevalence	Precentage
No vertebral fracture	52	50%
Grade 1 (20–25%)	24	23%
Grade 2 (25–40%)	17	17%
Grade 3 (> 40%)	10	10%
Total vertebral fractures	51	50%

Conflict of Interest: None declared

P504-M

WHICH IS THE BEST ROI TO DETECT AGE-RELATED BMD CHANGES?

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Patients and methods. We analysed 498 women (n = 498) in Bratislava (BA) population aged 21 to 90. We measured bone mineral density (BMD) in the area of proximal femur with one densitometric instrument DXA Osteocore II, France, applying T-score values in three standard regions of interest: Neck (ROI1), Ward´s area (ROI2), Global trochanter (ROI3). Results: The osteoporosis incidence in Bratislava female population, according to T-score values measured in ROI1 is 2.40%, in ROI2 16.34% and and ROI3 3.83% . Following the division of women into ten-year intervals, the statistically significant sample averages of T-score values were decreasing in relation to age only for ROI2. The osteoporosis incidence in age intervals was rising with age for ROI2. For ROI1 the number of osteoporotic patients in the 61 to 70-year interval was lower than in the 41 to 50-year interval, and for ROI3 the number of osteoporotic patients in the 51 to 60-year interval was lower than in the 41 to 50-year interval. In addition to the abovementioned intervals, T-score values decreased in relation to age also in ROI1 and ROI3 . According to the analysis of variance, the age category explains 9.6% of the overall variability of T-score values for ROI1, 24.7% for ROI2 and 11.70% for ROI3 . Conclusion: The osteoporosis incidence percentage in BA population is lower for ROI1 and ROI3 and higher for ROI2 in comparison with the average percentage in European population. As in ROI2 (Ward's area) only the trabecular part of the bone is measured and in both ROI1 and ROI3 the trabecular as well as cortical parts of the bone are measured, the ROI2 reflects best the age-related BMD changes. It is due to the fact that the trabecular bone is eight times more active metabolically than the cortical bone, and so it is more sensitive than the cortical bone for the determination of BMD changes. In ROI1 and ROI3 the relation was distorted by an accidental selection of a higher number of osteoarthritic patients in the 61 to 70-year interval for ROI1 , and in the 51 to 60-year interval for ROI3 . The age of patients is a significant risk factor. Bone mass decreases with age independently of the period of other risk factors activities, and also independently of the fact whether the patient suffers from primary or secondary osteoporosis (osteopenia). The development and application of densitometric methods measuring the trabecular bone excludes the distorting factor of osteoarthrosis.
Conflict of Interest: None declared

P505-T

A STATISTICAL ATLAS OF THE PROXIMAL FEMUR

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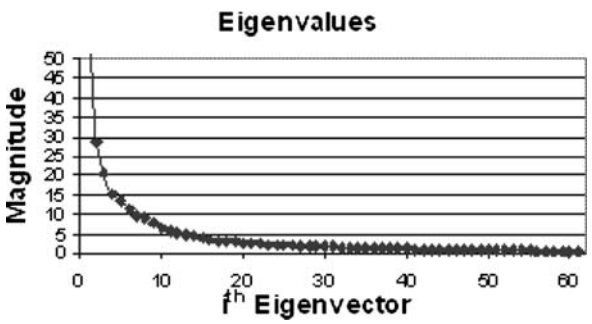
Reconstruction of the three dimensional volume of an anatomic region or organ from a limited number of views has a number of useful applications in medicine. One promising method employs a statistical atlas containing substantially all of the individual variation of the anatomy of interest. If the variation of statistical atlas can be well described by a limited number of parameters, computational complexity, time and resources can be significantly reduced.

We hypothesized that the 3D anthropometric variation of the proximal femur can be adequately explained by a limited number of normal modes.

Sixty-two Caucasian male femurs were acquired with multi-slice CT (1 mm^3 resolution) and segmented to separate bone and soft tissue. To model the data, a tetrahedral mesh (2.5 mm between vertices) with associated density functions for each tetrahedron was derived. Principal component analysis (PCA) was then performed on the tetrahedral mesh to obtain a statistical model. The model consists of a mean-shape with associated eigenvectors (modes of variation). The eigenvalue of each eigenvector measures the amount of variation the eigenvector explains.

As expected, the eigenvalues declined approximately exponentially, indicating that most of the individual anatomical variation is captured by the 10 to 20 eigenvectors with the largest eigenvalues.

A statistical atlas of the proximal femur was constructed using a tetrahedral mesh to model the geometry and density of the anatomy. The variation was substantially captured with a small number of eigenvectors. Further investigations will focus on the use of the atlas to reconstruct the proximal femur from several two dimensional views of the anatomy and compare this to CT volumetric reconstruction of the same femur.



Conflict of Interest: OM Ahmad, Hologic, Grant Research Support K Engelke, Hologic, Grant Research Support KE Wilson, Hologic, Employee K Ramamurthi, Hologic, Employee RH Taylor, Hologic, Grant Research Support

P506-S

LOW ESTIMATES OF PRAL (NUTRITIONAL ACID LOAD) CORRELATES WITH BONE ULTRASOUND MEASUREMENTS IN ELDERLY FRACTURED WOMEN

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Background: There is growing evidence that the Western diet may be a risk factor for osteoporosis through excess acid intake, which can be estimated by PRAL* (potential renal acid load). It's an estimate of the production of endogenous acid that exceeds the level of alkali produced by the ingested food. The more negative the PRAL, the more alkaline the diet. Fruit and vegetable intake may balance the excess acidity by providing potassium.-High PRAL estimates were associated with lower BUA in a large cross-sectional population study **. Objective: The aim of our study was to assess the possible relationship between PRAL and bone ultrasound (QUS) measurements at the heel (bone ultrasound attenuation-BUA and stiffness index-SI). Design: As part of on going study, we assessed PRAL and QUS in 401 elderly Swiss ambulatory women, who had already had a QUS 5 years before (SEMOF study). Dietary intake was assessed with a validated food frequency questionnaire, which enabled calculation of PRAL***. QUS was done with Achilles, Lunar Corporation as this approach was predictive of fracture risk in elderly women. We identified 2 subgroups: 256 women (mean age 80.6 yrs, BMI 24.6kg/m2, BUA 96.8) who had reported a fracture in their life-time and the remaining 145 (mean age 79.9 yrs, BMI 25.5kg/m2, BUA 101.7) with no fracture. Fractured women had significantly lower BUA, SI, higher 5 year loss of SI, lower BMI, MNA (mini nutritional assessment), but no difference in nutrient intakes. Results: The whole group ($n = 401$) and the sub-group of non fractured women showed no significant correlations between nutrient intake, PRAL and BUA. However, in the sub-group of fractured women, lower PRAL estimates ($p = 0.023$) and potassium intake ($p = 0.033$) were significantly correlated to higher BUA. As expected, BUA also showed a significant positive correlation with calcium ($p = 0.016$), BMI ($p < 0.001$) and MNA ($p = 0.019$) Conclusion: High nutritional acid load was correlated with lower BUA in fractured women, but the effect of the association was relatively low compared to age and BMI. This is an additional risk factor which might be relevant in patients with an already high risk of fracture.

*) PRAL (mEq/d) = 0.49 x protein + 0.037 xP – 0.021 xK – 0.026 xMg – 0.013 xCa (Remer, T., Manz, F. 1995. J Am Diet Assoc. 95, 791–7).

**) Welch AA et al. 6th Int. Symp. Nutrit. Aspects of Osteopor. Lausanne 2006.

***) Wynn Dumartheray, E. et al. J Hum Nutr Dietet. 2006. 19: 321–330.

Conflict of Interest: None declared

P507-M

OSTEOPOROSIS AND OSTEOARTHRITIS: IS OSTEOPOROSIS A PREDICTOR OF THE INCIDENCE OF OSTEOARTHRITIS, OR VICE-VERSA?

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Background/aims: To clarify the causality between osteoporosis (OP) and osteoarthritis (OA), both of which are major causes of disability in the elderly requiring urgent strategies for prevention.

Methods: A population-based epidemiological study was conducted in Miyama village, Wakayama, Japan. A cohort of 1543 inhabitants aged 40 to 79 years (716 men, 827 women) was established. From these individuals, a subcohort of 400 participants was recruited and divided into 4 groups of 50 men and 50 women each, stratified by age decade according to birth-year decade (1910–19, 1920–29, 1930–39, 1940–49). Initial bone mineral density (BMD) and radiographic examinations were performed in 1990 (baseline study). Dual energy X-ray absorptiometry (DXA: Lunar DPX) was used to measure BMD, providing antero-posterior images at lumbar vertebrae L2–4 and the proximal femur. BMD measurements were repeated on the same participants after 3, 7 and 10 years. Presence of OP was diagnosed using WHO criteria. Baseline radiographic examination provided antero-posterior and lateral images of thoracolumbar vertebrae Th5–L5. Radiographic examination was performed again after 10 years. Presence of OA was defined as Kellgren-Lawrence grade $> = 3$ for the highest score of the whole lumbar spine.

Results: Evaluation of radiographic surveys after 10 years was completed for 299 of the 400 participants (137 men, 162 women; 74.8%). Cumulative incidences of lumbar OA over 10 years for subjects in their 40s, 50s, 60s and 70s were 15.6%, 24.1%, 24.2% and 34.4% for men, and 27.1%, 32.6%, 38.5% and 58.3% for women, respectively. Logistic regression analysis was performed using incidence of OA over 10 years (1: yes; 0: no) as an objective factor and baseline prevalence of OP at the femoral neck (1: OP; 0: osteopenia or normal range) as an explanatory factor after adjustment for age. The prevalence of OP was not significantly associated with the incidence of OA in men and women (men: odds ratio (OR), 0.72; 95% confidence interval (CI), 0.29–1.79; $P = 0.48$; women: OR 0.95; 95% CI, 0.37–2.41; $P = 0.91$). Similarly, prevalence of OA was unrelated to the incidence of OP over 10 years.

Conclusions: Presence of OP does not predict future incidence of OA or vise-versa.

Conflict of Interest: None declared

Satellite Symposia

Companies supporting satellite symposia were entitled to submit abstracts for publication.

Clinical Progress in Osteoporosis: Annual Bone Health Management (Supported by Novartis)

SS01

DEVELOPMENT OF LONG-ACTING BISPHOSPHONATES FOR ANNUAL THERAPY

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The global burden of osteoporotic fractures is increasing despite currently available treatments. Fractures are an important cause of disability among postmenopausal women, and estimated annual medical care costs associated with osteoporosis are ≥ $18 billion in the United States. Oral bisphosphonates inhibit osteoclast-mediated bone resorption and reduce vertebral fracture risk, however, about half of patients prescribed daily or weekly oral bisphosphonate treatment are not compliant by one year. Poor compliance has been shown to compromise fracture efficacy and increase medical costs.

Zoledronic acid (ZOL) 5 mg was developed as a once-yearly IV, in the hope it would provide real-world effectiveness for osteoporosis treatment and unsurpassed hip and vertebral fracture protection. ZOL is the most potent bisphosphonate currently prescribed, with the highest affinity for hydroxyapatite and strongest inhibitory activity on FPP synthase *in vitro*. Adult ovariectomised rats studies have shown that after a single ZOL IV injection at the equivalent dose of 100 μg/kg and even at 5 times this dose (500 μg/kg), there is prolonged antiresorptive efficacy after 32 weeks with reduced bone turnover, resulting in improved mechanical properties of bones and no impairment of mineralisation. Clinical trials in post-menopausal osteoporotic women have shown that a once-yearly IV dose of ZOL 5 mg resulted in a rapid and sustained reduction in bone turnover within the expected range for bisphosphonates.

Evidence in rats and humans suggests chronic exposure to the bisphosphonate alendronate reduces the bone anabolic response to parathyroid hormone (PTH). Chronic subcutaneous, but not single intravenous, administration of bisphosphonates (ZOL, ibandronate, risedronate and alendronate), resulted in a significant reduction of the anabolic response to PTH. Although osteoblasts are not generally considered to be a target for bisphosphonate inhibition, repetitive uptake of small quantities into osteoblasts and lining cells by fluid endocytosis may lead to accumulation of intracellular bisphosphonate concentrations capable of interference with the mevalonate pathway. This in turn may impair the initial anabolic response to PTH, namely the activation of bone lining cells, a process which requires a functional cytoskeleton to induce the shape change of osteoblasts. Due to the inefficient uptake mechanism for bisphosphonates into osteoblasts, single high doses do not impair their function.

SS02

EFFECT OF ANNUAL BISPHOSPHONATE THERAPY ON HIP, SPINE, AND NON-SPINE FRACTURES

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INTRODUCTION: Intravenous (IV) zoledronic acid (ZOL), given as a single 5 mg infusion per year, has been shown to decrease bone turnover and improve bone density. These effects are sustained in full for 12 months after a single 15-minute infusion. This study evaluated the effect of once yearly ZOL 5 mg to decrease the risk of vertebral, hip and other types of fractures in postmenopausal women with osteoporosis.

METHODS: The HORIZON-Pivotal Fracture Trial was a three-year, randomised, double-blind, placebo-controlled trial which included 7736 women from 239 clinical centres in 27 countries. Patients were placed into one of two strata based on their concomitant osteoporosis medication use, at the time of or before randomisation. Stratum I patients were not taking any osteoporosis medications at baseline or had sufficient washout of previous osteoporosis therapy and Stratum II patients were either currently taking or had recently stopped taking ≥ one osteoporosis medication. In both strata, patients were randomised to either a single 15-minute IV administration of ZOL 5 mg or placebo at baseline, month 12, and month 24. Patients were given Calcium 1000–1500 mg/day and vitamin D 400–1200 IU/day.

Post-menopausal women (65–89 years) were eligible for inclusion if their femoral neck T-score was ≤ -2.5 or ≤ -1.5 with two mild or one moderate prevalent vertebral fracture.

The primary endpoints were new morphometric fractures (Stratum I) over 3 years and hip fracture (both strata). Secondary efficacy fracture endpoints in-

cluded all non-vertebral, clinical vertebral, and any clinical fracture. Other secondary endpoints included changes in total hip, femoral neck and lumbar spine bone mineral density, changes in markers of bone resorption (serum C-telopeptide of type I collagen) and formation (bone-specific alkaline phosphatase, and N-terminal propeptide of type I collagen).

RESULTS: All primary and secondary efficacy endpoints were met including significant reduction in the risk of morphometric vertebral, hip, non-vertebral, any clinical, and clinical vertebral fractures. ZOL 5 mg also produced significant improvements in bone mineral density and significant reduction in the markers of bone metabolism. ZOL 5 mg showed a favourable safety and tolerability profile.

CONCLUSION: The anti-fracture efficacy demonstrates the effectiveness for ZOL 5 mg as a potential treatment for post-menopausal osteoporosis.

SS03

VERSATILITY OF THE ANNUAL BONE HEALTH MODEL: A CLOSER LOOK AT EFFICACY AMONG KEY PATIENT SUBGROUPS

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INTRODUCTION: The HORIZON-Pivotal Fracture Trial was a randomised, double-blind, placebo-controlled study to evaluate the efficacy and safety of zoledronic acid (ZOL) 5 mg in the treatment of osteoporosis in postmenopausal women at high risk of fragility fractures (women with femoral neck BMD T-score ≤ -2.5 or ≤ -1.5 with 2 mild or 1 moderate vertebral fracture at baseline).

METHODS: Participants in Stratum 1 were not taking any osteoporosis medications at baseline or had sufficient washout of previous osteoporosis therapy, those in Stratum 2 were permitted to maintain osteoporosis medications (except bisphosphonates) prior to randomisation or during follow-up. 7736 patients were randomly assigned to receive either ZOL 5 mg or placebo by intravenous infusion at randomisation, Year 1, and Year 2. All patients received supplemental calcium and vitamin D.

RESULTS: Of the randomised patients, 22 did not receive any study drug, 7714 received the first infusion, 6926 received the second, and 6297 received the third.

The study was preplanned to investigate subgroups: (a) baseline vertebral fracture stratus (0, 1, ≥2), (b) femoral neck T-score ($\leq -2{,}5$, > -2.5), (c) age (< 70, 70–75, ≥75 years), (d) prior bisphosphonate use, (e) region (N. America/Oceania, Latin America, W Europe, Asia, E. Europe). For these subgroups, the efficacy variables assessed were: proportion of patients with = 1 new morphometric fracture over 36 months, time to first hip fracture, time to first clinical fracture.

Due to the anticipated low incidence of hip fractures and any type of fracture overall, the study was not powered to a priori to evaluate differences across subgroups, but to explore the magnitude and trend of efficacy benefits.

This pivotal clinical trial achieved positive results in all primary and secondary endpoints.

CONCLUSIONS: The subgroup analyses provided evidence for the consistency of the treatment efficacy of ZOL 5 mg across postmenopausal osteoporosis patients with different degrees of disease severity, in different age groups and in different regions of the world. Additionally, the study provided evidence of the first-ever anti-fracture efficacy against both vertebral and hip fractures in an adjunctive therapy additional to non-bisphosphonate therapies already proven to reduce the risk of vertebral fractures.