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Abstracts

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I001

The Assessment of Fracture Risk: A Global Perspective

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For many years, the assessment of fracture risk and osteoporosis has been considered almost synonymous with bone mineral density (BMD) measurement. Indeed, diagnostic criteria for osteoporosis, based on absolute or relative BMD, were in practice often used as therapeutic thresholds. Whereas this had the merit of simplicity, we have now more knowledge about other risk factors associated with fracture risk and how these can be incorporated into an overall assessment of fracture risk.

When integrating risk factors, one has first to consider the expression of risk. A single and easy to use metric is useful. The *T* score has served this purpose in the past, but there is a growing consensus that the assessment should concentrate on fracture risk rather than on a biological variable, a similar evolution as, for example, in the field of cardiology, where CVD risk is the outcome of interest rather than blood pressure, although it remains an important intermediate.

Fracture risk can be expressed as a risk relative to other individuals of the same age, gender, ethnicity, or location in the world. For most purposes, the value of interest will not be the remaining lifetime risk but the absolute risk in the foreseeable future, i.e., the absolute risk in the 5 to 10 years ahead, as this is the timeframe for which an intervention will be considered.

There are many potential clinical indicators for assessing this risk, but to be of practical value, they should be important risk factors, at least partially independent from each other, and sufficiently prevalent in the population considered. Several examples will be discussed, including body weight, previous fracture, family history, and lifestyle parameters. Finally, the integration of these risk factors with the assessment of bone (BMD, ultrasound or other) will be discussed.

I002

Combination Therapy for Osteoporosis

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Most standard therapies for osteoporosis reduce bone resorption and increase bone mineral density (BMD) modestly. Antiresorptive agents increase the mineralization of pre-existing bone matrix but do not increase bone formation or the amount of true bone tissue. Thus, they do not cure osteoporosis. Because single-agent antiresorptive therapy has, at best, modest effects on BMD, alternative strategies have been entertained.

Several studies have examined the effects of combinations of antiresorptive agents on BMD including bisphosphonates plus hormone therapy (HT) and bisphosphonates plus selective estrogen receptor modulators. Regardless of whether the two antiresorptive agents are started simultaneously or a bisphosphonate is added to ongoing HT, the incremental increases in BMD with two antiresorptive agents are small. It is unknown whether such additional increases in BMD will reduce fracture rates below those seen with single antiresorptive agents. In fact, it is theoretically possible that additional suppression of bone resorption may have adverse effects on bone strength and it is nearly certain that side effects will be more common with two antiresorptive agents than with one. Thus, combination antiresorptive therapy is generally not recommended.

Recently, parathyroid hormone (PTH) became available to treat both men and women with osteoporosis. Unlike antiresorptive agents, PTH administration increases bone formation and bone resorption. Because once-daily PTH increases bone formation more than it increases bone resorption, it increases BMD substantially and it causes the production of actual new bone. Because PTH also increases bone resorption, however, it would seem likely that combining PTH with an antiresorptive agent would increase BMD more than with either agent alone. When PTH is added to long-term continuous HT, BMD increases more than with continued HT alone. It is not clear from those observations, however, whether combination therapy is superior to PTH alone. Two recent studies have compared

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the effects of alendronate (ALN) alone, PTH alone, and PTH plus ALN on BMD in osteoporotic subjects. Surprisingly, both studies suggest that PTH monotherapy increases BMD of the spine more than does combination therapy or ALN alone. It is unknown whether these differences in BMD will be accompanied by differences in fracture rates. Nonetheless, at the present time, it seems prudent to use PTH alone rather than in combination with an antiresorptive agent.

I003

Rab GTPAses and the Control of Membrane Traffic in Bone Cells

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Small GTPases of the Rab family are key regulators of membrane traffic. Each cell type expresses a characteristic set of Rabs to control ubiquitous and specialised trafficking pathways. In bone cells, the importance of Rabs has been highlighted by several recent studies. Firstly, the discovery that biphosphonate drugs act by inhibiting protein prenylation, and consequently Rab function. Secondly, the realisation that understanding the molecular mechanisms of bone resorption in osteoclasts will be critical to find new therapeutic avenues to fight common bone diseases. I will discuss the importance of Rabs as regulators of membrane traffic and their involvement in disease, with a focus on osteoclasts.

I004

Bone Dynamics and Vesicular Trafficking

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Throughout life, the skeleton is continuously remodeled. Bone formation by osteoblasts and bone resorption by osteoclasts are processes that are completely dependent on vesicular trafficking. Signals, generated at the plasma membrane by growth factors, cell–cell and cell–extracellular matrix adhesion, converge to modulate their intracellular trafficking machinery to ensure correct and timely delivery of cargo and proteins.

In vivo, bone-depositing osteoblasts form a continuous cell layer on top of the newly deposited matrix. Here, extracellular-matrix proteins are secreted in a polarized fashion, i.e., away from neighboring capillaries and towards the existing bone surface. Enzymes involved in mineralization, specifically alkaline phosphatase, are localized to the basolateral plasma membrane highlighting polarized protein delivery. The exact mechanism responsible for this polarized delivery in osteoblasts has not been elucidated.

Since polarized trafficking depends on targeting information present both on the transport vesicle and the target membrane, the establishment of cell–cell junctions and their spatial arrangement provide important cues for the organization of polarized trafficking. In this light, we have shown that osteoblastic cells form functional tight junction-like structures in culture. Moreover, these cells contain a selected repertoire of proteins known to be essential for secretion. Via means of high-resolution immunolocalization studies, we provide evidence that in migratory osteoblasts, t-SNAREs and secreted matrix proteins are preferentially accumulated at the leading edge. A similar localization was also observed for lysosomal-associated proteins, linking for the first time lysosomes and their enzymatic content with the process of bone formation. Understanding the vectorial nature of the specific functions undertaken by osteoblasts and their regulation will be essential if stem cell maturation and function is to be controlled for therapy of osteoporosis and other bone diseases or for optimizing tissue engineering.

I005

Membrane Trafficking and Podosomes

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Focal delivery/exposure of matrix metalloproteases (MMP) is crucial for extracellular matrix (ECM) remodeling events during physiological and pathological processes alike. There are many mechanisms through which this occurs. For instance, in vivo, ECM degradation is confined to the immediate pericellular environment via the membrane type MMP (MT-MMP) which together with other proteins act as receptors/activators for soluble MMP. Clear molecular links between the MT-MMP and cytoskeleton proteins form the basis for their localization to limited districts of the plasma membrane (e.g., leading edge). In addition, secretory traffic is known to be polarized towards sites of active membrane reorganization, for instance, towards the leading lamella in wound-edge fibroblasts. It is now well-known that in vitro, ECM degradation by invasive cells occurs at specialized plasma-membrane structures (invadopodia or invasive podosomes) where a number of key proteins are concentrated, including regulatory cytoskeletal proteins, tyrosine kinases, integrins, and an MT-MMP. This implies that focal ECM degradation involves a tight coordination between trafficking processes, signaling events and cytoskeletal rearrangements.

Novel data will be presented concerning the regulation of the invadopodia/podosome machinery and the focalized targeting of MMP activity. In detail, morphofunctional studies will be presented describing (1) the regulatory cytoskeleton cascade controlling invadopodia structure; (2) the relationship between invadopodial protrusions and the ECM; and (3) the molecular and structural basis for polarized secretion towards the sites of ECM degradation (i.e., invadopodia and podosomes).

I006

PET and Bone Metastases

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The use of 18FDG PET in the evaluation and management of patients with malignancy continues to increase. However, its role in the identification of bone metastases is far from clear. FDG has the advantage of demonstrating all metastatic sites, and in the skeleton, it is assumed that its uptake is directly into tumor cells. It is probable that for breast and lung carcinoma, FDG PET has similar sensitivity, and improved specificity, when compared with the isotope bone scan although there is conflicting evidence with several papers suggesting that it is less sensitive than conventional imaging in breast cancer. There is convincing evidence that for prostate cancer, FDG PET is less sensitive than the bone scan and this may well be tumor specific. There is very little data relating to lymphoma, but FDG PET seems to perform better than the bone scan and there is increasing evidence relating to the valuable role of FDG PET in myeloma presumably because FDG is identifying marrow-based disease at an early stage. There are, however, several other important variables which should be considered. The morphology of the metastasis itself appears to be relevant. At least in breast cancer, different patterns of FDG uptake have been shown in sclerotic, lytic, or lesions with a mixed pattern. Furthermore, the precise localization of a metastasis in the skeleton may be important with regard to the extent of the metabolic response induced. Previous treatment is highly relevant and it has been found that while the majority of untreated bone metastases are PET positive and have a lytic pattern on CT, following treatment, incongruent CT positive/PET negative lesions are significantly more prevalent and these are generally blastic which presumably reflects a direct effect of treatment. Finally, the aggressiveness of the tumor itself may be relevant. The most important question, however, is irrespective of whether a lesion is seen on X-ray, CT, or bone scan and irrespective of lytic or blastic morphology, is, if the FDG PET study is negative, what is the clinical relevance of that lesion?

I007

Anatomical and Functional CT Imaging of Bone

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With recent advances in genetics and molecular medicine, there is a strong need for quantitative imaging of three-dimensional (3D) biological structures. A number of new microstructural imaging modalities have been put forward recently allowing phenotypic quantification with high precision and accuracy in humans and animals; especially in the mouse. Although biomedical imaging technology is now readily available, few attempts have been made to expand the capabilities of these systems by adding not only quantitative but also functional analysis tools as an integrative part of biomedical information technology.

In this lecture, new strategies for advanced quantification of bone and its structure–function relationship will be presented. Engineering endpoints as an integral part of the emerging field of quantitative biology have become an important factor for success in basic research and the development of novel therapeutic strategies in skeletal biology and orthopedic practice. The approaches that are used for such quantification employ hierarchical bioimaging and visualization of bone as well as biomechanical testing and simulation techniques. A field of special interest is image-guided failure assessment of porous microstructures as a means to provide functional imaging. We have developed a technique called micro-compression to non-destructively monitor failure initiation and propagation as well as damage accumulation in three-dimensional porous matrices and tissue engineered constructs. Today, these methods are successfully employed for the quantitative assessment of structure–function relationships in tissue healing, growth, and adaptation and are now also often used for precise phenotypic characterization of tissue response in mammalian genetics, gene therapy, and molecular biology.

In conclusion, hierarchical bioimaging in combination with biocomputational approaches are well suited to investigate structure–function relationships of bone and can be used to improve our understanding of skeletal pathologies and to reduce bone failure. This will ultimately lead to better patient care and an increase quality of life.

I008

Quantitative MRI of Trabecular and Cortical Bone Architecture

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Second only to mass density, the structural arrangement of bone – be it trabecular or cortical – is the strongest determinant of bone mechanical competence and fracture resistance. Until recently, however, there were no modalities able to provide architectural information noninvasively. This lecture intends to critically examine the potential role of micro-MRI to provide detailed architectural information on scale, topology, and anisotropy of the trabecular network and examines the technical requirements for this modality to become a clinically practical tool. The skeletal locations at which the in vivo virtual bone biopsy (VBB) can be practiced include the distal radius, distal and proximal tibia and calcaneus, which enable images to be acquired at a

resolution allowing retrieval of the trabecular network at an image voxel size at which structural parameters can be reliably measured. The demands placed on measurements of cortical parameters are somewhat less stringent and most skeletal sites are amenable to cortical analysis. The extraction of structural information in the limited spatial resolution achievable in vivo demands image-processing methods not yet commercially available. The reproducibility achievable with these techniques hinges on the reproducible positioning and volume selection, as well as means to control for patient motion and/or motion correction during/after the 10- to 15-min scan. Recent work in the speaker's laboratory suggests that micro-MRI-based structural parameters better discriminate patients with osteoporotic fractures from their unfractured peers than does BMD. The data further indicate that one of the hallmarks of postmenopausal osteoporosis – the conversion of trabecular plates to rods, and their eventual disruption – is corroborated, for the first time, noninvasively. Another recent study highlights the structural changes attendant to both trabecular and cortical bone in late-stage renal disease. Lastly, of particular interest is whether the VBB is able to detect structural changes in response to treatment with anticatabolic or anabolic drugs. Examples reviewed include early postmenopausal women receiving estrogen/progesterone as well as hypogonadal men treated with testosterone. In the latter study, large increases in plate-to-rod ratio and decreased topological erosion index suggest an anabolic effect in this group of patients.

I009

New Insights Into Osteoclast Diseases

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Osteoclast diseases have illustrated key processes necessary for osteoclast formation and function and helped to clarify which of these are non-redundant in vivo. The genetic basis for many diseases is now elucidated. Diseases in which osteoclasts do not resorb bone adequately, such as the various types of osteopetrosis, result mainly from deficiencies in the ability of osteoclasts to produce or secrete protons effectively, whereas pycnodysostosis, a milder form of osteopetrosis, is caused by mutations in the gene for Cathepsin K, resulting in deficient collagen breakdown. Diseases of osteoclast overactivity, such as Familiar Expansile Osteolysis, Expansile Skeletal Hyperphosphatasia and early onset Paget's disease and Juvenile Paget's disease are caused by mutations in the genes for RANK, and osteoprotegerin, critical genes in osteoclast differentiation, activation, and survival. In late onset, Paget's disease mutations in the gene for Sequestosome-1/p62 are found. These appear to result in loss of interaction of p62 with ubiquitin and ultimately interfere with protein degradation in the proteasome. Further genes are yet to be found in this disorder. Work is now underway to better understand how the mutated proteins lead to abnormal osteoclast function and to correlate genotype with phenotype in clinical disease. Mutated RANK proteins are expressed in cells, including osteoclasts, to investigate their cellular distribution and interactions with signaling molecules and these studies are complemented by immunocytochemical studies on patient material. Osteoclasts are generated in vitro from patients with known genetic defects to analyze phenotypical and functional defects. Transgenic approaches are used to create animal models for human osteoclast diseases, in particular for the diseases of osteoclast overactivity. In the osteopetrotic disorders, spontaneous, or engineered mouse models for the diseases already exist and in some cases (gl/gl mouse and oc/oc mouse) formed the basis for identification of the human genes in the first place. Examples of the histopathology of the various conditions will be presented to illustrate the cellular abnormalities resulting from the genetic defects, but also to highlight areas where further research should be done. Osteoclast diseases have illustrated several essential and, in some cases, unexpected or unknown, pathways in osteoclast physiology and will continue to pose many fundamental questions on osteoclast biology.

I010

The Role of NFAT in Osteoclast Differentiation

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Elucidating the regulatory mechanism of osteoclast differentiation is critical for understanding bone metabolism in health and disease. Signaling through receptor activator of NF-\kappaB (RANK) and c-Fms (M-CSF receptor) is indispensable for the induction of osteoclast differentiation. Although several molecules activated by RANKL have been shown to be essential for osteoclastogenesis, it has been unclear how RANKL specifically activates this process.

To explore the specific molecular mechanism underlying osteoclast differentiation, we performed a genome-wide screening of genes induced by RANKL. We identified that the transcription factor nuclear factor of activated T cells c1 (NFATc1) is selectively induced by RANKL. *NFATc1*-deficient embryonic stem cells fail to differentiate into osteoclasts in response to RANKL stimulation, and the ectopic expression of NFATc1 causes the precursor cells to undergo efficient differentiation without RANKL signaling. Thus, NFATc1 represent a master switch regulator for the terminal differentiation of osteoclasts, functioning downstream of RANKL signaling. Recent progress in the regulation of NFATs during osteoclastogenesis will be discussed in the lecture.

Induction and activation of NFATc1 is regulated by calcium-dependent phosphatase calcineurin, but it remains unknown

how calcium signaling is induced during this process. In the course of our investigation into the possible calcium-mobilizing receptors in osteoclast precursor cells, we found that immunoreceptor tyrosine-based activation motif (ITAM) signaling mediated by dual membrane adaptors, Fc receptor (FcR) common\gamma subunit (FcR\gamma) and DNAX activating protein (DAP12) is essential for RANKL induction of osteoclast differentiation. FcR\xgamma and DAP12 associate with multiple immunoreceptors such as OSCAR and TREM-2 and activate calcium signals leading to the induction of NFATc1. These results indicate that RANKL-induced osteoclast differentiation is finely regulated through costimulatory signals provided by multiple immunoreceptors and that RANKL and M-CSF are not sufficient to activate the signals required for osteoclast differentiation.

I011

Bone Formation: Of Mice and Men

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Bone formation affects the level of bone mass at maturity, bone structure, material properties, and changes in skeletal character during adulthood. While medical and environmental factors influence bone formation, genetic factors are undoubtedly important as well. For instance, during growth and development, bone formation results in considerable heterogeneity in skeletal character among individuals, and bone mass, size, biomechanical properties, and material properties are highly heritable. A wide variety of genes are likely involved. Among others, polymorphisms in the vitamin D receptor, type 1 collagen and estrogen receptor genes probably affect bone at least in part via variation in bone formation. Because of the enormous knowledge of mouse genetics, the ability to study large numbers of animals using invasive techniques, and the molecular technologies available (knockouts, transgenics, etc.), mouse models can be tremendously useful in identifying genes that affect bone formation and the mechanisms by which they act. One example is the progress made on the role of lipoxygenase pathways in the control of bone mass and stem cell function. Using classical genetic approaches, the mouse 15-lipoxygenase gene (*alox15*) was identified as being a regulator of bone mass, potentially via the effects of lipid metabolites on ppar gamma activity and the differentiation of mesenchymal stem cells along adipocyte vs. bone cell pathways. In parallel, ppar gamma has been shown to be a critical determinant of osteoblast function in vitro, and human epidemiological studies have implicated the human 12-lipoxegenase gene (a homologue of mouse *alox15*) in the determination of bone mass. Since dietary arachidonic and linoleic acids are substrates for lipoxygenase genes, it would be expected that there are environmental–genetic interactions that affect these pathways. In fact, interactions have been noted between lipoxygenase gene polymorphisms and dietary intakes of fatty acids in the control of atherosclerosis in humans. In sum, bone formation is determined in part by genetic influences. The discovery of the genes involved will be of considerable interest in the understanding of bone physiology and the development of novel therapeutic approaches.

I012

Bone Anabolics: The BMP Pathway

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A strong need exists for bone anabolic agents effective orally for osteoporosis treatment and locally for treating bone defects. Recent reviews list possible peptide anabolic agents including BMP, GH, IGF-1, PTH, and low-molecular-weight anabolic compounds such as Fluoride, Strontium, and Estrogen-like compounds. Although most peptides are unsuitable for systemic delivery, PTH recently received approval for intravenous use in the treatment of end stage osteoporosis and BMP2 which stimulates ectopic bone formation, received approval for local use in repairing bone defects. Due to the cost and stability of peptide therapies, the search for low-cost, low-molecular weight anabolic agents recently focused on the BMP pathway as a possible target for modulating bone formation.

Low-molecular-weight anabolic agents which positively affect the BMP pathway have been discovered using high-throughput screening methods. A number of classes of these compounds active on the BMP pathway have been identified including the statins of which 9 published family members exist. These compounds stimulate bone formation by multiple mechanisms one of which is by up-regulating the BMP2 protein production. Further evaluation of the mechanism of action of these compounds led to the discovery of a more potent family of anabolic agents, distinctly different from the statins, known as proteasome inhibitors of which more than 30 published family members exist. Recent work suggests these agents may act not only on BMP itself but at multiple sites in a positive bone anabolic cycle inclusive of the BMP pathway. Further study of this positive anabolic cycle has clarified new and possibly more selective and potent compounds for the use as bone anabolic agents.

I013

Aggrecan Knock-In Mice Resistant to Proteolysis in the Interglobular Domain: Implications for the Growth Plate

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The growth plate is a transitional region of cartilage and highly diversified chondrocytes that controls long bone formation. The composition of growth plate cartilage changes markedly from the epiphysis to the metaphysis, notably with the loss of type II collagen, concomitant with an increase in MMP-13, type X collagen and the C-propeptide of type II collagen. In contrast, the fate of aggrecan in the growth plate is not clear: there is biosynthesis and loss of aggrecan from hypertrophic cartilage, but the mechanism of loss is unknown. All matrix metalloproteinases (MMPs) cleave aggrecan between amino acids N_{341} and F_{342} in the proteinase-sensitive interglobular domain (IGD) and MMPs in the growth plate are thought to have a role in aggrecanolysis. We have generated genetically-modified mice with a mutation that renders the aggrecan IGD resistant to cleavage at the major MMP cleavage site. Compared with MMP null mice in which ablation of any one MMP may be compensated for by another, the mice in this study resist cleavage by all members of the MMP family at a single key site in the IGD. The mice develop normally with no skeletal abnormalities. Similarly, mice with aggrecan resistant to proteolysis by "aggrecanases", or the ADAMTS family of enzymes, also develop normally. The mutant mice do not accumulate aggrecan and there is no significant compensatory proteolysis occurring at alternate sites in the IGD. This is surprising given the environment of the growth plate with its active MMPs and exceptionally high concentration of substrate (aggrecan). Our studies reveal that cleavage by MMPs and aggrecanases in this key region is not a predominant mechanism for removing aggrecan from growth plate cartilage. The results challenge the widely-held tenet that loss of aggrecan from the growth plate is mediated by MMPs and lend support to emerging hypotheses implicating non-proteolytic mechanisms of aggrecanolysis in epiphyseal cartilage.

I014
Rescue of Achondroplasia

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Natriuretic peptide family consists of three endogenous ligands: ANP, BNP, and CNP. They exert their biological actions through two subtypes of particulate guanylyl cyclase: GC-A for ANP and BNP, and GC-B for CNP. Although ANP·BNP/GC-A system has been known as an important regulator in cardiovascular system, recent studies have elucidated that the CNP/GC-B system is a pivotal stimulator of endochondral bone growth. CNP strongly stimulates the longitudinal growth of explanted fetal mouse bones in organ culture, and CNP or GC-B knockout mice exhibit short stature due to their disturbed endochondral ossification. We examined the effect of activation of CNP/GC-B system as a novel therapeutic treatment for achondroplasia, the most common form of skeletal dysplasia caused by constitutive active mutation in FGF receptor 3 (FGFR3). Using a murine model of achondroplasia with an activated FGFR3 (G380R mutation) in cartilage (ACH mice), we achieved targeted overexpression of CNP in the cartilage of ACH mice by crossing them with transgenic mice that overexpress CNP in cartilage under the control of type II collagen promoter. The short stature and the shortening of bones of ACH mice were almost rescued by overexpression of CNP in their cartilage. Histological analysis revealed that the narrowing of the growth plates in ACH mice was almost recovered by CNP. Overexpressed CNP recovered the decreased extracellular space and the decreased production of extracellular matrix in achondroplastic growth plate, whereas the decrease in proliferation of achondroplastic chondrocytes was not changed. CNP and its second messenger, cGMP, inhibited the MAPK pathway of FGFR3 signaling dose-dependently, and MEK inhibitors, PD98059 and U0126, increased the extracellular matrix synthesis in achondroplastic growth plate without affecting the proliferation of achondroplastic chondrocytes and elongated explanted fetal achondroplastic bones. Therefore, CNP restored the narrowing of achondroplastic growth plates by correcting the decreased extracellular matrix synthesis of chondrocytes through inhibition of the MAPK pathway of FGF signaling and recovered the shortening of achondroplastic bones.

In addition to growth hormone therapy and distraction osteogenesis, activation of CNP/GC-B system in cartilage would serve a quite novel therapeutic strategy for achondroplasia.

I015
Indian Hedgehog Signaling During Chondrocyte Differentiation

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During embryonic development most bones of the skeleton are formed by endochondral ossification, a multistep process during which a cartilage template is successively replaced by bone tissue. Chondrocytes in the cartilage anlagen undergo several differentiation stages into hypertrophic cells which are subsequently replaced by bone. The secreted growth factor Indian hedgehog (Ihh) is expressed in a distinct population of chondrocytes. Ihh interacts with a second secreted molecule, Parathyroid Hormone related Protein (PTHrP), in a negative feedback mechanism to regulate the onset of hypertrophic differentiation. In addition Ihh has been shown to regulate chondrocyte proliferation and the ossification of the cartilage anlagen independent of PTHrP.

We have set out to analyze how Ihh interacts with other signaling system in regulating bone development and have

integrated the Ihh/PTHrP system with that of BMPs and FGFs into a common control system regulating several stages of chondrocyte differentiation. We found that BMP signals antagonize the role of Fgfs in a mouse model for achondroplasia.
To analyze how the Ihh signal is propagated in the cartilage anlagen, we have investigated a mouse line carrying a hypomorphic allele of Ext1, a glycosytransferase necessary for the synthesis of heparan sulfates (HS). We found that HS negatively regulates Ihh signaling in the developing limbs. Our data strongly indicate that Ihh signaling is acting as a long range morphogen directly inducing the expression of PTHrP. In addition misregulation of the Ihh/PTHrP system might be the molecular origin of Hereditary Multiple Exostosis, a human inherited disorder characterized by the development of benign bone tumors.

I016
Homocysteine and Bone
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In the general population, a mildly elevated plasma level of homocysteine is a common condition. Hyperhomocysteinemia is recognized as a major risk factor for atherosclerotic and tromboembolic disease as well as for cognitive impairment, including that seen in Alzheimer disease. Recently, an elevated circulating homocysteine levels was also identified as a new and potentially modifiable risk factor for osteoporotic fractures. Epidemiologiocal studies showed that a relative high homocysteine level predicts a higher fracture risk, independent of other known risk for osteoporotic fractures. Recently, Sato et al. provided additional evidence that an elevated homocysteine level might indeed cause more brittle bones. In a 2-year randomized placebo-controlled study in Japanese stroke patients, treatment with folate/vitamin B12 (resulting in reduced homocysteine levels) resulted in a 5 times lower risk of hip fractures in the treated group compared to the placebo group. This fracture risk reduction remained after adjustment for condition also associated with hyperhomocysteinemia, like cardiovascular events, dementia, and falls. The reduced fracture risk seen by Sato et al. could not be explained by metacarpal BMD, which might suggests that bone quality rather than bone quantity explains the difference. Also in a large epidemiological study, no relationship was observed between homocysteine and femoral neck BMD.
A possible mechanism underlying the deleterious effect of homocysteine on bone might involve inhibition of collagen cross linking by high homocysteine concentrations. However, in vivo evidence for this hypothesis is limited, especially under conditions of mildly elevated homocysteine levels. Another way to prove a causal relationship between increased homocysteine levels and fracture risk is by studying Mendelian randomization. This approach was recently successfully applied for the relationship between homocysteine and stroke.
Taken together, evidence for a relation between homocysteine and brittle bones is accumulating, but final proof of causality will have to come from the elucidation of the biological mechanism underlying this relationship.

I017
Calcium, Vitamin D and Bone
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There is no clear consensus on the optimal calcium intake or vitamin D status for bone health in adults. The role of calcium and vitamin D supplementation in the prevention and treatment of osteoporosis also remains uncertain. Chapuy's study shows that calcium and vitamin D decreases hip fractures in elderly institutionalized women, whereas the work of Dawson–Hughes and Larsen suggests that it may also reduce non-vertebral fractures in community-dwelling older people. The results of the anti-fracture studies of vitamin D alone are equivocal, with fracture reduction reported by Heinkinheimo and Trivedi, but no decrease observed by Lips or Meyer. Nevertheless, vitamin D may decrease the risk of falls in older people.
The results of other large studies of calcium and/or vitamin D supplementation in the UK are now emerging, which challenge existing views on the prevention of falls and fractures in older people. The Wessex Fracture Prevention Trial compared the effect of annual IM vitamin D 300,000 IU or placebo in 9440 community-dwelling men and women aged over 75 [Anderson et al., J. Bone Miner. Res. 2004; 19 (Suppl.1): S57]. This showed no reduction in either falls or fractures with IM vitamin D over 3 years. The MRC RECORD Study is a randomized trial of oral calcium (1000 mg daily), vitamin D (800 IU daily), both or placebo on the secondary prevention of low trauma fractures in 5292 men and women aged over 70. During the 24 to 62 months' follow-up, there was no difference between the treatment groups in all reported fractures, radiologically confirmed fractures, hip fractures, or falls. The Northern and Yorkshire Region Study is a pragmatic 'open' trial in 3314 women aged over 70 with a risk factor for hip fracture (any prior fracture, body weight <58 kg, smoker, family history of hip fracture, fair or poor self-reported health). Participants were randomized to receive oral calcium (1000 mg daily) and vitamin D (800 IU daily), together with an information leaflet on diet and falls prevention, whereas the control group received the leaflet alone. After a median follow-up of 25 months, there was no reduction in fracture or falls in women randomized to receive calcium and vitamin D. Although calcium and vitamin D may

decrease the risk of fractures in institutionalized elderly women, the results of these studies suggest that supplementation does not reduce fracture risk in older people living in the community, where vitamin D deficiency is less common.

I018

GI and Bone

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Bone resorption exhibits marked circadian variation. Previously, gender, age, PTH, cortisol, blindness, and skeletal unloading have been excluded as causes. In contrast, food intake has been established to induce a reduction in bone resorption. Thus, the morning decrease in bone resorption seen in fed individuals is inhibited in fasting individuals. Conversely, bone formation does not exhibit clinically relevant circadian variation. Therefore, an endogenous factor involved in the handling of food may possess the ability to uncouple bone resorption and formation and may be central in calcium homeostasis.

A standardized intake of glucose, protein, and fat leads to a decrease in bone resorption similarly as breakfast does. That the decrease in bone resorption after breakfast is significant at 1 h and fully expressed at 3 h indicates presence of a fast-acting factor. Based on insulin and glucagon stimulation tests as well as insulin clamp studies, insulin was excluded as a key factor. Because of the fast effect of food intake upon bone turnover, incretin hormones, which are further upstream for insulin, were studied. Acute human intervention studies ruled out GIP and GLP-1 as mediators. By contrast, in 60 healthy postmenopausal women randomized to placebo or GLP-2, doses from 100 to 800 μg lead to an acute dose-related suppression of bone resorption as evaluated by S-CTX and U-DPD/Creatinine. Thus, GLP-2 may be important in acute regulation of bone resorption. This is supported by data showing that GLP-2 given late at night significantly inhibits the nocturnal rise in bone resorption in a randomized placebo-controlled study of 81 healthy postmenopausal women.

Another important aspect of the influence of food upon bone turnover is that variability in bone turnover markers is significantly lower in fasting as compared to fed individuals. Thus, assessment of bone markers in a clinical setting should always be performed in fasting individuals.

In conclusion, the diurnal variation in bone resorption is due to food intake whereas fasting leads to a continuous high level in bone resorption. These events may be essential in calcium homeostasis. Insulin, GIP, and GLP-1 are excluded as important factors for the mechanism of food-induced inhibition of bone resorption, whereas GLP-2 may be a key component. A clinical consequence of the variability of food intake upon bone turnover markers is that samples for such markers should be collected in fasting individuals.

I019

New Insights into Nutrition and the Skeleton

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The identification of dietary risk factors for osteoporosis is particularly important from the standpoint of prevention, as has been true for calcium and vitamin D. Nevertheless, the bulk of attention to dietary risk factors for osteoporosis has focused on these two nutrients. Other macro- and micronutrients, as well as dietary patterns may also contribute to bone health. This talk will highlight several dietary factors studied in the Framingham Osteoporosis Study that shed light on less well-recognized pathways involved with bone health.

Silicon (Si) has been shown to affect the organic matrix of bone and cartilage, such that a reduction in matrix components has been documented with Si deficiency. Si is also a major ion of osteogenic cells, particularly in the mitochondria. Silicon has beneficial effects not only on the organic matrix of bone, but also on mineralization of the matrix. As a major element of most diets, it circulates in plasma at concentrations as high as zinc and iron; and is excreted in the urine at levels as high as calcium. Major sources of silicon in the diet include cereals, cereal products, certain fruits and vegetables and beer. It is likely that many people in the community have low intakes of silicon, especially those who do not use whole grains or alcoholic beverages like beer. The Framingham Osteoporosis Study has shown positive associations between dietary silicon intake and bone density.

Cofactors in reactions associated with 1-carbon metabolism, such as vitamin B12 and folate, have recently been implicated as playing a role in skeletal health possibly through effects on homocysteine levels, or possibly through direct effects on bone cells. Results from the Framingham Osteoporosis Study will be presented highlighting a possible role for both folate and vitamin B12 on bone density.

Finally, because nutrients travel together in foods, associations between single nutrients and bone may actually be due to the more complex mixture of dietary components. For this reason, the study of dietary patterns may be advantageous to study how the diet influences bone. Using cluster analysis of dietary patterns of food intake, the Framingham Osteoporosis Study was able to demonstrate a protective effect of certain dietary patterns.

In conclusion, these few studies of novel dietary influences on the skeleton suggest that the prevention of bone loss

through diet is complex, involving many nutrients and food intake patterns.

I020

Thyroid and Bone

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Thyroid hormone (T3) plays a key role in skeletal development and bone maintenance. Both hypothyroidism and thyrotoxicosis in childhood result in disorders of bone maturation and cause short stature, while adult thyrotoxicosis accelerates bone turnover and is associated with a 2- to 3-fold increased fracture risk. T3 receptors (TR)\alpha and \beta are both expressed in osteoblasts and chondrocytes but TR\alpha is expressed at 12-fold higher concentrations. To investigate mechanisms of T3 action in bone, we analyzed TR-mutant mice. TR\alpha-null (TR\alpha0/0) mice are euthyroid but display growth retardation resulting from delayed endochondral ossification. Reduced mineralized bone deposition is seen in young animals. In contrast, TR\beta-null (TR\beta−/−) mice have resistance to thyroid hormone (RTH) with increased circulating thyroid hormones. These mice also display short stature but this is due to advanced ossification with accelerated narrowing of the growth plate and increased mineralized bone deposition in young animals. The phenotypes were verified by examining TR\alpha1PV and TR\betaPV mutant mice, which harbor a point mutation (PV) resulting in a frameshift in the TR coding region. PV-mutant TRs cannot bind T3 or transactivate TR-target genes and they act as potent dominant-negative inhibitors of wild-type TR function. TR\alpha1PV mice are severely growth retarded with grossly delayed ossification despite normal circulating T4 and T3 levels, while TR\betaPV mice have severe RTH with 12- to 15-fold elevated thyroid hormone concentrations and display advanced ossification with increased bone mineralization. Thus, young TR\alpha0/0 and TR\alpha1PV mice exhibit a typical hypothyroid phenotype in bone, whereas TR\beta−/− and TR\betaPV mice display skeletal thyrotoxicosis. The skeletal effects of TR\alpha mutation result from disruption of TR\alpha action in bone, whereas effects of TR\beta mutation result from thyrotoxicosis (induced by disruption of TR\beta action in the pituitary) and are mediated by over-stimulation of TR\alpha in bone. Preliminary analysis of bone structure in TR\alpha and TR\beta mutant mice has revealed that adult TR\alpha mutants have increased bone mass with an increased trabecular number and thickness relative to wild-type. In contrast, adult TR\beta mutants display osteoporosis with reduced trabecular number and thickness. These studies indicate that normal TR\alpha function is essential for skeletal development and growth and for normal bone turnover and preservation of bone mass in adulthood.

I021

Regulation of GP130 Action by the SOCS Family of Proteins

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Cytokines that signal via gp130 regulate a myriad of normal biological processes and are implicated in disease pathology. The SOCS family of proteins are attractive candidates as physiological regulators of gp130 signaling. SOCS1 and SOCS3 are highly homologous and the expression of both is induced by cytokines that act through gp130, including IL-6. SOCS1 interacts directly with JAK kinases, while SOCS3 has been shown to interact specifically with phosphorylated tyrosine residues within the cytoplasmic tail of activated cytokine receptors including gp130. In overexpression systems, both SOCS1 and SOCS3 can inhibit STAT activation after gp130 activation. We have assessed the role of SOCS1 and SOCS3 in regulation of cytokine signaling utilizing gene targeting technology in mice. Although SOCS1 potently inhibits gp130 signaling when ectopically or overexpressed, mice lacking SOCS1 display little evidence of deregulated gp130 signaling. Rather, Socs1−/− mice succumb to inflammatory disease characterized by deregulated signaling by IFNg and other inflammatory cytokines. In contrast, SOCS3 has emerged as a critical physiological regulator of gp130 signaling. Mice engineered to lack SOCS3 die at midgestation of placental failure caused by deregulation of LIF signaling. To examine the role of SOCS3 in adult mice, a conditional gene targeting strategy was pursued. We have generated mice in which either hematopoietic or hepatic cells have no functional Socs3 alleles. Following either in vitro or in vivo stimulation of SOCS3-deficient cells with IL-6, STAT3 phosphorylation was both increased and prolonged and cellular responses were amplified, including IL-6-mediated inhibition of macrophage proliferation. Microarray analysis confirmed that altered IL-6-mediated gp130 signaling resulted in aberrant target gene transcription and suggested that SOCS3 is crucial in controlling both quantitative and qualitative aspects of gp130 signaling. Together, these data demonstrate that SOCS3 is a key physiological negative regulator of biological responses to cytokines that signal via gp130. While, to date, our animal models of SOCS3 function have focused on the placenta, liver, and blood forming tissues, the data imply that SOCS3 may also regulate responses in other tissues in which cytokines acting through gp130 play important physiological roles.

I022

Role of the Immune System in Bone Loss

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Under physiologic conditions, the immune and skeletal systems are regulated by families of cytokines and growth factors that work alone or in networks to maintain homeostatic immune system function and regulate skeletal and connective tissue remodeling. These molecules are produced by many different cell types and their receptors are widely distributed, providing the basis for the pleiotropic and diverse activities of these factors. It is not surprising, therefore, that many of the cytokines originally identified as immunomodulatory or proinflammatory, have been shown to play essential roles in regulating bone remodeling. Rheumatoid arthritis (RA) and related animal models of RA have provided useful experimental systems for dissecting the role of the immune system in regulating bone remodeling. In these conditions, there is evidence of focal articular as well as systemic bone loss and several different approaches have been used to establish that osteoclasts are the principal cell type responsible for systemic as well as subchondral and focal bone erosions at the joint margins. In RA, the inflamed synovium produces a variety of proinflammatory and immunomodulatory cytokines with potent osteoclastogenic activity. Immunomodulatory factors that inhibit osteoclast differentiation and activity are also produced in the inflamed synovium but their net effect is overridden by the pro-osteoclastogenic factors. An additional finding in patients with RA is the absence of bone repair. This suggests that the processes that regulate coupling of bone resorption and formation have been disrupted, and that the enhanced focal bone resorption associated with the synovial inflammation is not matched by a compensatory increase in bone formation. It is likely that many of the same cytokines and immune cell products involved in the enhanced osteoclast-mediated bone loss also contribute to the impairment of bone formation. Approaches for inhibiting the proinflammatory and/or immunomodulatory factors have been shown to produce beneficial effects in reducing immune-mediated synovial inflammation in RA. In addition, via effects on osteoclast differentiation and activation, inhibiting the activity of immunomodulatory factors also directly reduces focal articular and systemic bone loss.

I023

Regulation of HSC by Osteoblastic Niche

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The ontogeny of hematopoiesis dynamically changes during embryonic, fetal, and postnatal development, with yolk sac, aorta–gonad–mesonephros (AGM), fetal liver, and bone marrow serving as alternative locations to support ongoing hematopoiesis at different developmental stages. However, the location of hematopoieitc stem cells (HSCs) in bone marrow has been elusive for many years. Recently, we and other groups have identified that a subset of osteoblastic cells lining the bone surface function as the niche to support HSCs. These osteoblastic niche cells express high levels of N-cadherin and \beta-catenin. N-cadherin and \beta-catenin form an adherens complex between HSCs and the niche cells, thus assisting stem cell attachment to the niche. Importantly, the number of HSCs is correlated to a high degree with the number of N-cadherin+ osteoblastic lining cells, indicating the impact of the niche on the homeostatic level of stem cells. Both Wnt/\beta-catenin and BMP signaling pathways play important roles in regulation of bone development and hematopoietic stem cell properties. Therefore, coordination between the Wnt/\beta-catenin and BMP signaling pathways may be required to orchestrate bone development and hematopoiesis.

I024

Skeletal Tissue Repair by Adult Stem Cells

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The use of mesenchymal stem cells (MSCs) as an alternative to mature cells for cell-based tissue repair (e.g., expanded chondrocytes for joint surface repair) is intensively sought because more versatile and amenable to upscaling. In the quest for skeletal progenitors within the joint, we have identified and characterized a population of multipotent MSCs from the adult human synovial membrane (SM), an easily accessible and rapidly self-renewing tissue present in the diarthrodial joints. Culture-expanded SM-MSCs can differentiate at the single cell level to cartilage, bone, adipocytes, and skeletal muscle. We have demonstrated that SM-MSCs can contribute to myofibers and functional satellite cells in a nude mouse model of skeletal muscle regeneration and rescue functional aspects when injected into the mdx mouse model of Duchenne muscular dystrophy. Under specific experimental conditions, SM-MSCs can also form cartilage or bone in vivo.

In a comparative study of adult human MSCs from different sources, we have observed that, under our experimental conditions, SM-MSCs displayed greater chondrogenic potential and lesser osteogenic potential than MSCs from bone marrow or periosteum. Thus, MSCs obtained from different tissues may have distinct indications for skeletal tissue engineering applications.

Ex vivo manipulations of cell populations, such as culture expansion, are associated with phenotypic instability, difficult upscaling and high costs. Circumstantial evidence suggests that the SM might function as a reservoir of stem cells for the repair of those joint tissues, such as articular cartilage and menisci, which have a limited capacity for intrinsic repair. We are currently investigating the niche of MSCs in vivo within the synovial tissue and their contribution to spontaneous joint surface repair in animal models.

The existence of functional stem cells resident within the joint environment represents an opportunity to achieve repair of joint tissues by triggering/enhancing local reparative mechanisms with no need for cell isolation and in vitro manipulations. This in situ tissue engineering approach would circumvent current limitations of the cell-based technologies associated with in vitro expansion, such as genetic and phenotypic instability, disease transmission, high costs, and variability.

I025

Regulation of Osteoblastogenesis and Bone Mass by WNT10B

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Wnts comprise a family of secreted signaling proteins that regulate diverse developmental processes. Activation of Wnt signaling by Wnt10b inhibits differentiation of preadipocytes and blocks adipose tissue development; however, effects of Wnt10b on other mesenchymal lineages have not been defined. To explore the physiological role of Wnt signaling in bone development, we analyzed FABP4-Wnt10b mice, which express the Wnt10b transgene in marrow. Femurs from FABP4-Wnt10b mice have almost four times as much bone in the distal metaphyses, and are mechanically stronger. These mice maintain elevated bone mass at least through 23 months of age. In addition, FABP4-Wnt10b mice are protected from the bone loss characteristic of estrogen deficiency. We used pharmacological and genetic approaches to demonstrate that canonical Wnt signaling stimulates osteoblastogenesis and inhibits adipogenesis of bipotential mesenchymal precursors. Wnt10b shifts cell fate towards the osteoblast lineage by induction of osteoblastogenic transcription factors, Runx2, Dlx5, and osterix, and suppression of adipogenic transcription factors, C/EBPalpha and PPARgamma. One mechanism whereby Wnt10b promotes osteoblastogenesis is by suppressing expression of PPARgamma. Finally, Wnt10b −/− mice have decreased trabecular bone and serum osteocalcin, confirming that Wnt10b is an endogenous regulator of bone formation.

I026

What's New in Phosphate Metabolism?

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Abstract not received.

I027

Calcimimetics in the Treatment of Hyperparathyroidism

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A major therapeutic advance in the treatment of hyperparathyroidism (HPTH) has been the development of effective medical therapies to control parathyroid hormone (PTH) hypersecretion in both uremic secondary and primary HPTH. Cinacalcet, a phenylalkylamine, has been tested in randomized, double-blind, placebo-controlled trials in primary or secondary HPTH or in compassionate use, open-label studies for parathyroid cancer. In patients with chronic renal failure (N = 741), there were statistically significant decreases (43%) in intact PTH levels and (15%) in the calcium (Ca)-phosphate (P) product that persisted for 26 weeks [Block et al., N. Engl. J. Med. 350; 15, 2004]. Cinacalcet was effective in reducing PTH levels in patients with mild, moderate, and severe HPTH classified as PTH levels of 300–500, 501–800, and >800 pg/ml, respectively. Patients with primary HPTH [serum total Ca >10.3 and <12.5 mg/dl and intact PTH values of >45 pg/ml (nl 10–65)] randomized to placebo or cinacalcet (30 to 50 mg twice daily) for 52 weeks experienced rapid reductions in serum Ca that reached the normal range in 73% of cinacalcet-treated patients and in only 5% of placebo-treated patients [Peacock et al., J. Clin. Endo. Metab. 90; 135, 2005]. Serum P also increased from average levels of 2.7 to 3.3 with 52 weeks of cinacalcet therapy—statistically greater than changes in P in the placebo group. Pharmacodynamic responses to cinacalcet in primary HPTH were characterized by rapid declines in PTH nadiring 4 to 8 h post-dose and returning to baseline by 12 h post-dose. Mean pre-dose plasma PTH levels were reduced by 7.6% in cinacalcet-treated patients; however, these levels increased in placebo-treated patients by 7.7%. While BMD by DXA was not statistically different at spine and hip sites after 1 year of therapy, biochemical markers of bone turnover increased modestly (serum and urine N-telopeptide and bone-specific alkaline phosphatase). Adverse events included nausea and vomiting (10–15% of patients). In patients (N = 21) with severe hypercalcemia (serum total Ca > 12.5 mg/dl) due to parathyroid cancer, there was an average 14% decrease in serum Ca (14.5 to 12.4 mg/dl) and an average 16% drop in PTH with 70% of patients experiencing at least a 1 mg/dl decrease in serum Ca [Silverberg et al., JBMR, 19;S103]. This agent offers the potential for medical therapy for hyperparathyroid states, previously only addressed by surgical intervention.

I028

Genetic Basis of Hyperparathyroidism Including Parathyroid Cancer

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Examination of clonal DNA defects and inherited tumor predisposition syndromes have resulted in major strides in the identification of genes that drive the development of parathyroid tumors. The *cyclin D1/PRAD1* and *MEN1*

genes have established roles in the development of common sporadic parathyroid adenomas. *Cyclin D1/PRAD1* was identified as a clonally activated oncogene in parathyroid adenomas and has subsequently been established as a major contributor to human neoplasia including breast cancer and B-cell lymphoma. Cyclin D1 overexpression has been found in 20–40% of sporadic parathyroid adenomas. Cyclin D1 is a key regulator of cyclin-dependent kinases and G1 phase progression in the cell cycle, and the possibility that overexpressed cyclin D1 may also exert oncogenic effects through cdk-independent mechanisms has been raised. Somatic mutation and/or deletion involving both alleles of the *MEN1* tumor suppressor gene have been demonstrated in 12–20% of sporadic parathyroid adenomas. The *MEN1* gene product menin may have a role in transcriptional regulation involving JunD; several other menin-interacting proteins including smad3 in the TGF-b pathway have also been identified using in vitro approaches. In addition, genome-scanning approaches have identified loci which are likely locations for new parathyroid adenoma suppressor genes or oncogenes. While parathyroid adenoma pathogenesis does not seem to involve somatic mutation of the *CASR* gene, decreased CaR expression may be a determinant of biochemical phenotype in adenomas and is targeted by calcimimetic agents. Very different patterns of DNA abnormalities have been found in parathyroid carcinomas as opposed to adenomas, suggesting fundamentally different genetic bases for these conditions which might also be useful diagnostically. Inactivating mutations of the *HRPT2* tumor suppressor gene are remarkably frequent and appear to be central to the pathogenesis of sporadic parathyroid carcinoma. Furthermore, a subset of patients with apparently sporadic parathyroid carcinoma have unsuspected germline mutations in *HRPT2*, recognition of which is important for clinical management. Finally, classic familial predispositions to hyperparathyroidism result from heritable mutations in *MEN1* (MEN1), *RET* (MEN2A), *CASR* (familial hypocalciuric hypercalcemia/neonatal severe HPT), and *HRPT2* (HPT-jaw tumor syndrome), and familial isolated hyperparathyroidism has a mixed genetic basis that in part awaits clarification.

I029

Toward Elucidation of the Role of Altered Wnt Signaling in Myeloma and Development of Corresponding Therapeutic Interventions

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We recently demonstrated that myeloma bone disease is directly related to elevated expression and secretion, by myeloma plasma cells, of the soluble Wnt signaling inhibitor DKK1. Current data suggests that DKK1 can block BMP-2 mediated osteoblast differentiation and that this alters the bone marrow (BM) microenvironment in such a way that it promotes myeloma growth and survival. The mechanisms by which this occurs is unclear, but we hypothesize that this effect is related to shifts in the biology of mesenchymal stem cells (MSC) or so called marrow stromal cells (MSC). The above data suggest that inhibition of osteoblast differentiation through the production of Wnt inhibitors by myeloma plasma cells not only blocks osteoblast differentiation and uncouples bone turnover, but importantly also appears to directly influence myeloma cell growth. It is possible that other therapies that reduce DKK1 effects in the marrow might effectively activate osteoblast differentiation and impact disease progression. Along these lines, we have recently used polyclonal and monoclonal anti-DKK1 antibody therapy to treat primary myeloma grown in a novel SCID-rab mouse model that faithfully recapitulates the disease. Preliminary data suggests that this treatment results in the activation of osteoblasts, inactivation of osteoclasts, and reduction in tumor burden. An update on the progress of these experiments will be reported.

I030

Breast Cancer and Bone

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Breast cancer has a propensity to spread to bone and around 75% of women with advanced breast cancer will develop evidence of skeletal involvement. In recent years, the treatment of bone metastases by radiotherapy and systemic endocrine and cytotoxic drugs has been supplemented by the co-administration of bisphosphonates. These drugs are potent inhibitors of tumor-induced bone resorption that can relieve bone pain and improve the structural integrity of bone. Zoledronic acid and ibandronate are the most potent agents available and prevent 40–50% of the expected skeletal morbidity. It is now clear that the risk of a skeletal complication is related to the rate of bone resorption. Patients with rapid bone resorption (urinary N-telopeptide >100 nmol/mmol creatinine) are at significantly greater risk of an event and thus have potentially more to gain from the administration of a bisphosphonate. Attention is now turning to the development of more rational treatment schedules using biochemical markers of bone metabolism to guide treatment in individual patients. Animal studies and some clinical trials have indicated that the adjuvant use of bisphosphonates may sufficiently alter the bone microenvironment to prevent metastasis in bone. Additionally, direct anticancer effects and synergistic interactions with cytotoxic agents have been demonstrated in cell line systems. Large (n = >3,000) randomized adjuvant trials are ongoing

evaluating either clodronate or zoledronic acid in early breast cancer patients. Finally, bisphosphonates provide a safe and simple treatment for the prevention and reversal of cancer-treatment-induced bone loss. This is an increasingly important complication of cancer treatment given the long survival that many patients now experience and the increasing use of aromatase inhibitors which are known to accelerate bone loss. Despite the clinical utility of bisphosphonates, skeletal morbidity remains a major clinical problem for many patients. New treatments that influence the RANK/RANK ligand/OPG system, antibodies to PTHrP and src inhibitors are all under evaluation. Over the next few years, these agents may come to challenge the role of bisphosphonates or, perhaps more importantly, may form the basis of combination treatments for metastatic bone disease.

OC001

Loop Diuretics Increase Bone Turnover, Decrease Bone Mineral Density, and Increase Fracture Risk

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Background: Loop diuretics (LD) increase the renal calcium excretion, alter the diurnal rhythm of plasma PTH, and may affect fracture risk.
Aim: To study effects of LD on bone metabolism, BMD, and fracture risk.
Methods: Two separate studies were performed: *Study A*: in a double-blinded design, 87 healthy postmenopausal women with osteopenia were randomized to 1 year of treatment with bumetanide 2 mg/day or placebo. BMD, calcitropic hormones, and biochemical bone markers were measured at baseline, after 1 year of treatment, and 6 months after withdrawal of treatment. Calcium (800 mg/day) and vitamin D (10 mg/day) were administrated to all participants during the entire 1.5-year study period. *Study B*: a nationwide population-based pharmaco-epidemiological case-control study with fracture in year 2000 as outcome and use of LD during the previous 5 years as exposure variable.
Results: In the randomized controlled study, bumetanide caused an increase in urinary calcium and plasma PTH levels compared with placebo. After 1 year, BMD in the bumetanide- compared with the placebo-group was significantly decreased by 2% at the total hip and ultradistal forearm, and by 1.4% at the whole body. In addition, bumetanide caused a significant increase (app. +20%) in levels of biochemical markers of bone resorption and formation. Six months after end of treatment, the effects of bumetanide were weakening. In study B, we included 64,699 cases aged 40 years or more that sustained a fracture during year 2000 and 194,111 age- and gender-matched controls. A total of 44,001 subjects used LD. Ever use of LD was associated with a 51% (OR 1.51; 95% CI, 1.48–1.55) increased fracture risk. After confounder adjustment, current use of an accumulated dose less than 500 defined daily dosages (DDD) was associated with an increased risk of any fracture (OR 1.58; 95% CI 1.50–1.66), and an increased risk of fractures at the hip, the spine, and the forearm. However, in subjects older than 65 years fracture risk decreased as number of accumulated DDD increased. Thus, use of more than 1500 DDD was associated with a decreased risk of any fracture (OR 0.91; 95% CI, 0.87–0.95).
Conclusion: Treatment with LD is harmful to bone and should be considered as a risk factor for osteoporosis.

OC002

Baseline Buckling Ratio Derived from Hip Structure Analysis Predicts the Risk of Nonvertebral Fractures

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The purpose of this study was to determine if buckling ratio (BR), an index of cortical bone stability derived from Hip Structural Analysis (HSA) (Beck et al., 1990 and 2000), is able to predict nonvertebral fracture (NVFx) risk. The HSA program analyzes 5-mm cross-sectional regions traversing the proximal femur across the intertrochanter (IT), femoral neck, and femoral shaft. HSA has the ability to measure BMD, cross-sectional area, cross-sectional moment of inertia, section modulus, subperiosteal width and estimated mean cortical thickness. BR is derived as the ratio of the maximum distance from the region center of mass to the outer cortex divided by the estimated mean cortical thickness. A decrease in BR suggests an improvement of local cortical stability (Beck et al. 2001). We investigated the relationship between 3-year NVFx risk and baseline BR in the placebo group of the MORE study (Ettinger et al., 1999). Two analyses were conducted on the three HSA regions of the proximal femur. First, we used an Armitage trend test to determine if NVFx risk was associated with baseline BR tertiles. Second, logistic regression was used to help understand the quantitative nature of the relationship; we modeled 3-year NVFx risk as a function of BR (up to cubic terms). The same analysis strategy was repeated using 3-year hip fracture data. There was a strong relationship between NVFx risk and baseline BR, Table 1. The test for trend was significant ($P < 0.05$) for the IT and shaft BR, and near significant for the neck ($P = 0.07$). The results from the logistic regression analyses confirmed these results, as the main effect of BR was significant for each location. The relationship was linear (on the logit scale), as neither quadratic nor cubic effects were statistically significant. The trend for hip fracture risk was only significant for the IT BR, Table 2. In conclusion, despite the limitations of the estimate, BR derived from HSA may be able to predict the risk of NVFx in postmenopausal women with osteoporosis.

Table 1
Nonvertebral fracture

	First tertile	Second tertile	Third tertile	*P* value
Intertrochanter	23/442 (5.20)	47/444 (10.59)	49/443 (11.06)	0.0023
Femoral neck	35/456 (7.68)	41/461 (8.89)	51/455 (11.21)	0.0658
Femoral shaft	31/443 (7.00)	37/444 (8.33)	51/443 (11.51)	0.0186

Table 2
Hip fracture

	First tertile	Second tertile	Third tertile	*P* value
Intertrochanter	0/442 (0.00)	2/444 (0.45)	6/443 (1.35)	0.0092
Femoral neck	2/456 (0.44)	1/461 (0.22)	5/455 (1.10)	0.1909
Femoral shaft	2/443 (0.45)	1/444 (0.23)	5/443 (1.13)	0.1924

OC003
Utility of MXA for Detecting Prevalent Fractures—Experience from a Large Epidemiological Study

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Low-radiation lateral spine images captured by DXA scanners have a potential for clinical use in detecting vertebral fractures. We have evaluated morphometric X-ray absorptiometry (MXA) in a cohort of elderly women at entry to a large prospective study of fracture risk.

5212 women aged at least 75 years, unselected for osteoporosis, were enrolled to a study of risk factors for fracture. All underwent a comprehensive evaluation of risk factors at baseline, including MXA imaging of the lumbar and thoracic spine (Hologic QDR4500). A visual assessment to detect prevalent vertebral deformities was undertaken by the scanning technicians and the images categorised as normal, equivocal, or abnormal. Measurement of vertebral heights from T4 to L4 were undertaken in all of the equivocal and abnormal images as well as a random sample of the normal images. Prevalent vertebral fractures were determined using the McCloskey algorithm and MXA-specific reference ranges for vertebral height ratios.

MXA scans were undertaken in 5191 women (99.6%). Following visual assessment, 3274 (63%) were classified as normal, 1193 (23%) equivocal and 724 (14%) abnormal. During morphometric assessment in a total of 2109 women, 30 of the equivocal scans and 4 of the abnormal scans were deemed non-evaluable due to poor image quality. None of the normal scans were classified as non-evaluable. The proportion of vertebrae assessable varied from 98.2% at T12 to 57.1% at T4 with more than 92% assessable at levels between T8 and L3. In the random sample of scans from the normal group ($N = 210$), 7(3.3%) were subsequently determined to have prevalent vertebral fractures while the corresponding prevalences in the equivocal and abnormal groups were 26.3% and 76.5%, respectively. In women with all 13 vertebrae assessable, the prevalence of fracture was highest at T12 and L1 (10.1% and 10.8% of vertebrae respectively) and lowest at T4 (0.4%). Prevalent fractures identified by MXA were associated with older age (mean 80.6 vs. 79.3 years, $P < 0.0001$), decreased standing height (mean 154.3 vs. 156.2 cm, $P < 0.0001$), lower hip BMD (0.68 vs. 0.77 g/cm^2, $P < 0.0001$) and increased hip fracture risk (relative risk 2.5, 95% CI 1.8–3.5, $P < 0.0001$).

We conclude that visual assessment of MXA is an effective technique for detecting prevalent vertebral fractures and is feasible in the majority of elderly women. The fractures identified are associated with established clinical correlates of vertebral fractures.

OC004
The RANKL Antagonist OPG-Fc Causes Significant Increases in Cortical Bone Area, BMD, and Bone Strength Index in Male Cynomolgus Monkeys

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OPG-Fc is a RANK Ligand inhibitor that blocks osteoclast differentiation, activation, and survival. OPG-Fc leads to rapid, profound, and sustained suppression of bone resorption and increased bone mineral density (BMD) in rodents, monkeys, and humans. Cortical bone contributes significantly to overall skeletal strength, but tends to be less responsive to antiresorptive therapy when compared to trabecular bone. We therefore examined the effects of OPG-Fc on cortical geometry and BMD in 2- to 3-year-old intact male cynomolgus monkeys. OPG-Fc was injected once weekly for 6 months at 15 mg/kg SC ($n = 5$) or IV ($n = 3$), while control animals ($n = 5$) received PBS. At baseline and after 6 months, the distal radius and proximal tibia were analyzed by pQCT, and serum was collected. The effects of IV and SC OPG were statistically similar, so these groups were merged for analyses. The following comparisons were all statistically significant ($P < 0.05$) versus PBS controls. Bone resorption was dramatically suppressed by OPG-Fc, as evidenced by a 90% suppression of urine N-Telopeptide (NTx) and a 134% increase in serum PTH levels. These biochemical responses were associated with significant improvements in total, trabecular, and cortical volumetric BMD at the distal radius and proximal tibia. OPG-Fc increased cortical thickness by 302% and 77%, cortical area by 268% and 94%, and periosteal circumference by 21% and 14% in the distal radius and proximal tibia, respectively. OPG-Fc treatment also significantly improved both cortical and trabecular BMD at each of these sites, as well as at the midshaft of the radius and tibia. Cross-sectional moment of inertia (CSMI), related to the bone's bending strength, was increased by 288% and 138% in the distal radius and proximal tibia. The product of CSMI and cortical BMD provides a bone strength index (BSI) that correlates well with fracture resistance in preclinical studies. OPG-Fc increased BSI by 356% and 170% in the distal radius and proximal tibia, implying a propor-

tionate increase in the strength of these sites. These results demonstrate that OPG-Fc treatment of male cynomolgus monkeys leads to significant periosteal expansion of cortical bone, suggesting that osteoblast activity is maintained or increased despite the suppression of bone resorption. The ability of OPG to improve both the size and mineral density of cortical bone suggests that RANKL antagonism is a promising approach for improving bone strength.

OC005

Concomitant Teriparatide Plus Raloxifene for the Treatment of Postmenopausal Osteoporosis: Results from a Randomized Placebo-Controlled Trial

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We conducted a 6-month randomized, double-blind, placebo-controlled trial comparing teriparatide [rhPTH(1–34)] 20 μg/day (TPTD20) plus raloxifene 60 mg/day (RLX60) (n = 69) with TPTD20 plus placebo (n = 68) in postmenopausal women who were osteoporosis treatment naïve and received calcium and vitamin D supplementation. The objective of this study was to determine whether concomitant raloxifene therapy would impact the bone activity of teriparatide. Similar significant increases in bone formation (PINP) were shown at 1, 3, and 6 months in both groups (Table). After 1 month, bone resorption (CTX) was not increased from baseline in either group. After 3 months, CTX was significantly increased in the TPTD20 but not in the concomitant group. At 6 months, CTX was significantly increased from baseline in both groups, but the increase in the concomitant group was significantly less than in the TPTD20 group. Mean BMD (±SE) changes from baseline to endpoint in the TPTD20 group were: LS 5.19 ± 0.67% ($P < 0.001$), FN 1.03 ± 0.67% (NS), and TH 0.68 ± 0.59% (NS). In the concomitant group, BMD changes from baseline to endpoint were: LS 6.19 ± 0.65% ($P < 0.001$), FN 2.23 ± 0.64% ($P < 0.001$), and TH 2.31 ± 0.56% ($P < 0.001$). The BMD increase at total hip was significantly ($P = 0.04$) greater in the concomitant group compared to the TPTD20 group. In the TPTD20 group, mean serum calcium levels increased (0.30 ± 0.06 mg/dl, $P < 0.001$) from baseline to endpoint and mean serum phosphate was unchanged. In the concomitant group, mean serum calcium was unchanged and mean serum phosphate decreased (−0.20 ± 0.06 mg/dl, $P < 0.001$). Serum uric acid levels significantly increased versus baseline at study endpoint with TPTD20 (1.28 ± 0.06 mg/dl, $P < 0.001$) and concomitant therapy (0.94 ± 0.11 mg/dl, $P < 0.001$). Therapy in both groups was well tolerated. Compared to TPTD20 therapy, concomitant therapy increased bone formation to a similar degree, decreased bone resorption, and significantly increased total hip BMD. These findings suggest that RLX60 reduces TPTD20-induced stimulation of bone resorption resulting in a more favorable balance of bone remodeling compared with TPTD20 treatment.

Table
Markers of bone turnover (mean ± SE) change from baseline

	Baseline	Mo. 1	Mo. 3	Mo. 6
PINP (μg/l)				
TPTD20-PLA	50 ± 3	39 ± 5†	51 ± 7†	73 ± 12†
TPTD20 + RLX60	59 ± 3*	47 ± 5†	45 ± 7†	65 ± 12†
CTX (pmol/l)				
TPTD20 + PLA	5097 ± 871	−257 ± 281	1805 ± 494†	3704 ± 646†
TPTD20 + RLX60	6313 ± 874	−145 ± 287	648 ± 499	1880 ± 631‡*

*$P < 0.05$ vs.TPTD20, †$P < 0.001$ vs. BL,‡$P < 0.01$ vs. BL.

OC006

NFAT Regulation of the Human Beta 3 Gene in Osteoclast Differentiation

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The transcription factor NFATc1 has been shown to play an essential role in transducing RANKL signals in osteoclast (Oc) differentiation. To date, however, the specific molecular targets of this transcription factor are not known. We previously have shown that expression of the $\alpha_v\beta_3$ integrin is regulated by the β_3 subunit and that genetic ablation of β_3 gene in mice results in Ocs that inefficiently resorb bone. The present studies were undertaken to characterize the regulatory region of the human β_3 integrin gene and to define the potential role of RANKL-induced NFATc1 in regulating the β_3 integrin promoter. Analysis of the 5′ end of the mouse and human β_3 genes revealed considerable sequence homology (>80%) across a region 1 kb upstream of the putative transcription start site (TSS), with multiple conserved transcription factor binding elements present. The region −1242 to +29 (relative to the TSS) was cloned as a luciferase reporter construct as well as a deletion from −1242 to −997, thereby removing conserved NFAT and NFAT:AP-1 sites. Full-length reporter constructs transfected into RAW267.7 cells were highly induced by RANKL, while the deletion construct was unresponsive. Trans-activation by NFATc1 was assessed by co-transfection with human NFATc1. NFATc1 dose-dependently induced the full-length β_3 reporter constructs up to 100-fold. NFATc1 did not trans-activate the deletion construct. These experiments identified the conserved region, −1242 to −997 upstream of the TSS in the human β_3 integrin gene, as the region involved in RANKL and NFATc1 induction. EMSA and competition assays demonstrated direct NFAT binding to two of the

consensus NFAT sites within this region of the promoter. The identity of the shifted complexes was confirmed by supershift experiments with anti-NFAT antibodies. Mutation of both of the NFAT sites in the −1242 to −997 fragment was required to prevent NFAT binding. We generated TAT-dominant-negative (dn) NFATc1 fusion proteins to assess NFAT inhibition in RANKL-induced mouse bone marrow-derived Ocs and found that transduction with the dnNFATc1 protein specifically inhibited \beta$_3$ gene expression. This result was confirmed with the cell-permeable NFATc1 inhibitor 11R-VIVIT. Of note, both treatments were associated with an inhibition of Oc formation. These results confirm the role of NFATc1 signaling pathway in Oc differentiation and establish the \beta$_3$ gene as a direct target of NFAT in RANKL-dependent Oc formation.

OC007

Rac1 Binds Directly with Rab7

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Rabs are small GTPase proteins that control many membrane fusion and vesicular trafficking events in various mammalian cells. Most likely, each Rab protein functions through multiple effectors thus allowing cell and compartment specific actions. Rab7 has been shown to regulate the late steps of the endocytic pathway from early endosomes to late endosomes or from late endosomes to lysosomes. We have shown earlier that in resorbing osteoclasts, Rab7 is involved in formation of the ruffled border which is a late endosomal-like compartment at the plasma membrane. In order to reveal the molecular mechanisms which are important in the ruffled border formation, we used bacterial two hybrid system and rat trabecular bone derived cDNA library to identify specific effectors for Rab7. We used constantly active form of Rab7 as a bait. We identified P21 Ras-related C3 botulinum toxin substrate 1 (Rac1), another small GTPase protein as a new Rab7 interacting protein. Rac1 is known principally for its regulatory role on actin cytoskeleton, cell polarization, and microtubule dynamics. Rac1 has no homology with RILP or Rabring7, which have been reported earlier as other Rab7 effectors in other cellular system. Our pull-down results show that wild type and active GTPase deficient mutant form of Rab7 specifically bind to Rac1. Moreover, we show that this specific interaction doesn't occur with another Small GTPase Rab9 which is also located in late endosomal compartment. More importantly, confocal microscopy images showed that Rab7 colocalizes with Rac1 at ruffled border in the resorbing osteoclasts; however, in non-resorbing cells, Rab7 and Rac1 colocalizes at perinuclear area where the late endosomes and lysosomes are located. These results suggest that Rac1 could act as a downsteam effector of Rab7, thus regulating the late endosomal trafficking in general, and specifically the formation of ruffled border in osteoclasts. As far as we know, this is the first evidence that two small GTPase proteins directly interact with each other. Since Rac1 is known to control actin cytoskeleton directly through its effectors, Rab7–Rac1 interaction may mediate late endosomal trafficking along actin microfilaments to ruffled border.

OC008

Role of BMP Signals in Endochondral Ossification and Fracture Repair; Activation of Osteoclastic Resorption and Remodeling of Bone

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During endochondral bone development, cartilage anlagen is initially formed and replaced by bone. Bone is further remodeled by osteoclastic resorption coupled with osteoblastic formation. In fracture repair, this developmental process is recapitulated. Bone morphogenetic proteins (BMPs) can induce ectopic endochondral ossification, and thus are used for enhancement of fracture healing. In bone, BMP signals appeared to be regulated by a balance between BMPs and their antagonist, noggin. Recent transgenic mouse studies reported that noggin overexpression in mature osteoblasts using osteocalcin promoter developed osteopenia at 4 weeks of age or later, suggesting importance of BMP signals in maintenance of bone quality in postnatal life. To clarify roles of BMP signaling in endochondral bone development, we generated transgenic mice overexpressing noggin in immature osteoblasts under the control of the *Col1a1* promoter sequence. Unforeseen results were markedly reduced osteoclastic bone resorption in transgenic mice compared with that of the wild-type. Numbers of osteoclasts marked by TRAP staining were dramatically decreased from 17.5 days postcoitum to 3 weeks after birth. Dynamic bone histomorphometric assays at 3 weeks old showed that calculated bone resorption rates decreased significantly ($P < 0.05$). Bone formation rate was also significantly decreased. Rates of trabecular bone volume to tissue volume for transgenic mice were 250% of wild-type and cortical widths were 178% of wild-type, indicating more reduction in bone resorption than formation. Transgenic bones were woven and frequently undergo fracture, probably due to insufficient remodeling. Thus, BMPs in bone are regulators of bone remodeling necessary for mechanical strength. We then examined role of BMPs in fracture repair in mouse tibiae. Callus volume was measured using micro-CT. Early application of recombinant BMP2 (rhBMP2) to the fracture site produced large bony callus. Cartilaginous elements in immature callus might be expanded by rhBMP2, serving as a large anlagen which was replaced by bone. On the other hand, late rhBMP2 application caused resorption of bony callus associated with increased number of osteoclasts. Our results suggest that BMPs in bone are not mere osteoblast inducers, but activators of osteoclasts and regulators of bone remodeling during endochondral bone formation. These findings may contribute to figure out ideal strategy for treating fracture using BMPs.

OC009

Overexpression of BMP4 in Bone Increased Osteoclast Number and Caused Osteopenia

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Although genetic experiments revealed that BMPs are involved in skeletal patterning, recent transgenic mouse studies have showed that osteoblast-specific down-regulation of BMP signaling affects bone quality in postnatal life. But it still remains obscure how BMPs play roles in endochondral bone formation, which is the process residing between skeletal patterning and postnatal bone maintenance. Here, we performed cartilage-specific or bone-specific expression of BMP4 during endochondral bone development and analyzed consequent bone formation in transgenic embryos. Cartilage-specific overexpression of BMP4 using the \alpha2(XI) collagen chain gene promoter/enhancer sequences (*Col11a2-Bmp4*) expanded cartilage anlagen as we reported previously. Examination of bone of the mice revealed enlargement of ossification center and thickening of trabecular bone at 16.5 days postcoitum (d.p.c.) and 18.5 d.p.c. On the other hand, overexpression of BMP4 using the \alpha1(I) collagen chain gene promoter sequence (*Col1a1-Bmp4*) showed deformed bone formation. Analysis using micro-CT revealed that trabecular bone was almost absent in marrow cavities of *Col1a1-Bmp4* transgenic mice at 16.5 d.p.c. and 18.5 d.p.c. In situ hybridization analysis showed that BMP4 transgene was expressed in bone. Immunohistochemistry showed increased immunoreactivity against anti-phospho Smads 1/5/8 which recognize only phosphorylated form of these Smads in cells in BMP4 transgenic mouse bone marrow, suggestive of activation of Smads which mediate BMP signals. Histological analysis showed that the relative number of osteoclasts marked by TRAP-staining per bone surface was dramatically increased in *Col1a1-Bmp4* transgenic mice compared with that of wild type mice. Osteoblasts marked by *Col1a1* expression lined on the surface of bony matrix in wild type mouse marrow cavities, whereas those were scattered and did not attached to bony matrix in *Col1a1-Bmp4* transgenic mice. We speculate that BMP4 might overactivate osteoclasts, causing excessive resorption of primary spongiosa and that osteoblats without attaching to bony matrix might fail to produce bone, resulting in severe osteopenia in *Col1a1-Bmp4* transgenic mice. Our results suggest that transient overexpression of BMP4 in cartilage produced large bone, probably being formed by replacing expanded cartilage anlagen and that persistent overexpression of BMP4 in bone caused severe osteopenia.

OC010

Wnt Signaling Regulates Osteoclastogenesis In Vitro

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A growing number of reports implicating Wnt signaling in the regulation of bone mass has prompted widespread interest in the use of Wnt mimetics for the treatment of skeletal disorders. The majority of this work has focused on cells of the osteoblast lineage. In this study, we determined the effects of Wnt signaling on osteoclastogenesis in vitro. Using co-cultures of murine osteoblasts and mononuclear spleen cells, we demonstrated that activation of Wnt signaling by exposure to Wnt3a or the GSK3 β inhibitor LiCl, significantly and dose-dependently inhibited the formation of TRAP-positive multinucleated osteoclasts and resorption of dentine pits, compared to controls. We reasoned that anti-osteoclastogenic effects may be mediated by changes in expression of RANKL by osteoblasts, which is normally required to support osteoclast formation. By Northern and Western analysis, we demonstrated that LiCl completely inhibited RANKL mRNA and protein expression in osteoblastic cells. Overexpression of full-length β-catenin activated TCF-dependent gene transcription and suppressed RANKL promoter activity in cells transfected with a reporter gene containing a 7-kb fragment of the mouse RANKL promoter. In contrast, β-catenin DeltaC695-781, which lacks a transactivation domain, had no effect. Direct effects of Wnt signaling were determined in the absence of osteoblasts using mononuclear cells cultured in the presence of exogenous RANKL and M-CSF. In these cultures, LiCl inhibited mononuclear cell fusion and prevented osteoclast formation. Furthermore, using RT-PCR and immunocytochemistry, we identified expression of Wnt receptors (frizzleds 2, 3, 4, 5, and 6) by mature human osteoclasts and nuclear β-catenin localization in disaggregated rat osteoclasts, consistent with active Wnt signaling. As previous studies have demonstrated an absence of resorptive phenotype in mice lacking the Wnt co-receptor LRP5, we determined expression of the second Wnt co-receptor, LRP6 in human osteoblasts, $CD14^+$ osteoclast progenitors and mature osteoclasts. Although expression of LRP5 was not detected in $CD14^+$ progenitors or mature human osteoclasts, LRP6 was expressed at high levels by these cells and a range of osteoblastic cells (MG63, SaOS-2, TE85, human primary osteoblasts). These data suggest LRP6 mediates a resorptive component of Wnt signaling and highlights a requirement to develop novel therapies that differentially target anabolic and catabolic effects of Wnt signaling in bone.

OC011

BMP-2 Stimulates Osteoblast Differentiation through Activation of Beta-Catenin Signaling in Osteoblasts

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Recent reports suggest that Wnt/β-catenin signaling controls bone development and formation. To determine the specific role of β-catenin in bone formation, we have created bone-specific β-catenin conditional knockout (CKO) mice by two

different approaches. (1) We have deleted one allele of β-catenin gene by breeding β-catenin-loxP mice with CMV-Cre transgenic mice and deleted another allele of β-catenin gene by breeding with Col1-Cre transgenic mice. After analyzing 40 newborn mice and embryos, we found that homozygous β-catenin CKO mice, β-catenin(del/flox);Col1-Cre(±), are embryonic lethal and some of the heterozygous β-catenin KO mice have severe defects in bone development. The animal size is reduced 40–50% and severe losses (30–60%) in bone mineral density and bone volume were observed by micro-CT and histomorphometric analyses. (2) We have created β-catenin CKO mice by breeding β-catenin-loxP mice with Col1-Cre transgenic mice. In this way, both alleles of β-catenin gene were deleted by Col1-driving Cre recombinase. The β-catenin(flox/flox);Col1-Cre(±) CKO mice survived into postnatal stage. The decreases in osteoblast proliferation and differentiation were found in osteoblasts derived from the CKO mice. These results suggest that β-catenin gene dosage is critical for its function and β-catenin plays an essential role in bone development and osteoblast function. Recent studies demonstrate that BMP-2 has synergistic effects with β-catenin to promote osteoblast differentiation. For this reason, we examined the effects of BMP-2 on β-catenin signaling in 2T3 and MC3T3 osteoblasts. Western blot analyses showed that BMP-2 increased protein levels of non-phosphorylated active form of β-catenin in a dose-dependent (10–200 ng/ml) and time-specific (24 h treatment) manner. BMP-2 increased nuclear β-catenin protein levels (Western blot) and induced β-catenin nuclear translocation (immunostaining) in osteoblasts. The effect of BMP-2 on β-catenin activation was mediated through Lrp5. BMP-2 inhibited Kremen-1 and stimulated Lrp5 mRNA expression in osteoblasts. The ability of BMP-2 to stimulate alkaline phosphatase activity, osteoblast marker gene expression and mineralized bone nodule formation was inhibited in β-catenin-deficient osteoblasts. Our findings provide new evidence about the role of β-catenin in bone development and osteoblast function and suggest that β-catenin plays an important role in BMP-2-induced osteoblast differentiation.

OC012

A Regulatory Network of Homeodomain Proteins Supports Osteoblast Differentiation by Regulation of Runx2 and Target Genes

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Homeodomain proteins (HD) are induced in response to BMPs with essential roles in early embryonic development. To address the gap in our knowledge from initial osteogenic BMP signals to the transcriptional mechanisms which promote osteoblast (OB) differentiation, we have examined homeodomain protein regulation of the Runx2 gene, a transcription factor essential for bone formation. The Runx2 gene, which is expressed in mesenchyme and upregulated in osteoblasts, contains at least six homeodomain response elements in the 0.6-kb promoter. We find Msx2 and CDP/Cut classic homeodomain repressor proteins to downregulate Runx2 in both non-osseous and osteoblast lineage cells. In contrast, Dlx3 and Dlx5 strongly activated the endogenous Runx2 gene and increased promoter activity in C3H10T1/2 and NIH3T3 non-osseous cells 5- to 6-fold but not in committed osteoblasts (MC3T3 and ROS 17/2.8 cells) which express constitutive Runx2 levels. Promoter-deletion analysis and gel mobility antibody supershift assays of the Runx2 gene indicate that the multiple homeodomain sites involved in activation reside in the proximal promoter domain from 92 to 388 nucleotides. Using chromatin immunoprecipitation assays, we identified a temporal recruitment of HD proteins and regulatory factors to the Runx2 and osteocalcin gene promoters during osteoblast differentiation that reflected their transcriptional levels (by RNA Pol II association with the genes), as well as the selective expression of HD proteins at different stages of maturation. Msx2 binding in correlates with low Runx2 levels and absence of osteocalcin mRNA, while Dlx3 is transiently associated with the promoters, but Dlx5 increases with Runx2 and osteocalcin expression during OB differentiation. Knockdown of HD proteins by siRNA shows that Dlx3 causes a 70% decrease in Runx2 expression and phenotypic genes inhibit OB differentiation of primary rat calvarial cells. In conclusion, these studies have directly demonstrated that BMP2 induction of Runx2 gene expression involves direct regulation by homeodomain proteins in a region of the promoter in which Smad responsive elements have not been identified. We propose a regulatory network of expressed HD proteins, which selectively bind to promoter elements of the Runx2 bone specific gene at different stages of OB differentiation. This combined regulation supports induction of Runx2 in response to BMP2 in progenitor cells, as well as sustained expression in mature osteoblasts.

OC013

Downregulation of Wnt Signaling by Induction of Dickkopf-1 and -2 Expression During Osteoblast Maturation is Essential for Bone Matrix Mineralization

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In this study, we examined the role of Wnt/β-catenin signaling in bone matrix mineralization using the osteogenic KS483 cell line and murine bone marrow cultures. Continuous presence of LiCl, an intracellular activator of Wnt-signaling, inhibited the formation of mineralized bone nodules, dose-dependently in KS483 and murine bone

marrow cells. Wnt3A only inhibited formation of mineralized bone nodules when it was added early in the differentiation process of KS483 cells, while addition to differentiated KS483 cells or murine bone marrow cells was ineffective. In line with this, Wnt3A efficiently induced β-catenin translocation to the nucleus in undifferentiated, but not in differentiated KS483 cells. In contrast, LiCl induced β-catenin translocation irrespective of differentiation stage. The absence of a Wnt3A response in differentiated KS483 cells was not caused by loss of Frizzled receptor expression. Instead, expression of the Wnt antagonists Dkk-1 and Dkk-2 was induced and peaked during matrix mineralization. We subsequently performed gene knock down experiments using RNA silencing (si). Dkk-1si did not affect cell proliferation or the initiation of osteoblast differentiation, but potentiated BMP-induced alkaline phosphatase activity (ALP). In long-term cultures, Dkk-1si completely inhibited formation of mineralized bone nodules, suggesting involvement in the transition of an ALP-positive in a mineralizing osteoblast. The effects of Dkk-1si could be rescued by addition of recombinant Dkk-1 protein. In contrast, Dkk-2si had a modest inhibitory effect on cell proliferation and blocked the initiation of osteoblast differentiation as well as mineralized nodule formation, which could not be rescued by recombinant Dkk-1. Our data suggest that Wnt-signaling in maturing osteoblasts needs to be down-regulated to enable the formation of a mineralized bone matrix. This is established, at least in part, by induction of Dkk-1 and Dkk-2. Furthermore, they point to a crucial role for Wnt-antagonists in fine-tuning of Wnt-signaling during successive stages of osteoblast differentiation. Finally, they suggest that Dkk-1 and Dkk-2 may have distinct functions in osteoblast differentiation: Dkk-1 may preferentially antagonize Wnt(s) inducing ALP activity and blocking the transition of an ALP-positive in a mineralizing osteoblast, while Dkk-2 may specifically antagonize Wnt(s) involved in cell proliferation and the initiation of osteoblastic differentiation.

OC014

In Vivo Overexpression of Circulating Dlk1/Pref-1 Protein Leads to Increased Bone Turnover and Decreased Bone Mass

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Dlk1/Pref-1 (delta like1/preadipocte factor-1) is an imprinted gene encodes for transmembrane protein belongs to EGF-like repeats protein family. We have recently identified Dlk1/Pref-1 as negative regulator for late stage differentiation of human mesenchymal stem cells (hMSC) into osteoblast and adipocyte [Abdallah BM, et. al., JBMR, May, 19(5):841–852, 2004]. To further investigate the in vivo effect of Dlk1/Pref-1 on bone remodeling, we generate mice expressing high serum level of FA1 (biological soluble form of Dlk1) using the hydrodynamic-based gene transfer procedure. Full length of mPref-1 cDNA was subcloned under human ubiquitin promoter and rapidly injected via tail vein into BALB/cA male mice (16 weeks old, $n = 15$) every 2 weeks over a period of 2 months. DNA, quantitative mRNA analysis, immunohistology and ELISA measurements of FA1 were assayed to identify the efficient expression of the transgene. Using this method, we succeeded to elevate the FA1 serum level of Dlk-injected mice (Dlk1+ mice) by more than 15 folds vs. control (saline injected), while DNA immunohistochemistry analysis could only localized the plasmid in liver as expected. After 2 months, the Dlk1+ mice displayed lower total body mass and reduced total fat mass. Interestingly, Dlk1+ mice displayed (16.6%, $P < 0.005$) lower total BMD than control group and BMD was negatively correlated with the circulating level of FA1. This bone loss phenotype was associated with marked increases in serum biochemical bone turnover markers CTX-I (+15.7%; $P < 0.04$) and osteocalcein (+36.2%; $P < 0.001$). Micro-CT analysis revealed significantly lower microarchitectural parameters in the distal femur and proximal tibia of the Dlk1+ mice compared to the control group (see table). We conclude that naked DNA delivery by hydrodynamic injection proved to be simple and safe approach for evaluating the effect of Dlk1/Pref-1 on bone phenotype in vivo. The current data together with our previous in vitro data suggest that Dlk1/Pref-1 is a novel inhibitor of bone mass.

Table

Micro-CT parameters Groups ($n = 8$), Measurements	BV/TV	TbTh (μm)	TbSp (μm)	TbN (1/mm)	CD ($1/mm^3$)
Distal femur	0.34 ± 0.11	0.07 ± 0.01	0.19 ± 0.03	5.80 ± 1.10	155.1 ± 37.4
Control Dlk+ mice	0.19 ± 0.06	0.06 ± 0.00	0.24 ± 0.04	4.60 ± 0.84	113.3 ± 37.8
Tibia	0.20 ± 0.07	0.07 ± 0.01	0.24 ± 0.04	4.60 ± 0.57	98.6 ± 21.27
Control Dlk+ mice	0.14 ± 0.03	0.06 ± 0.01	0.25 ± 0.02	4.26 ± 0.42	75.5 ± 25.7

OC015

Adult Mice Heterozygous for the Transcription Factor Sox4 Exhibit Reduced Bone Mineral Density and Suppressed Osteoblast Activity in Culture

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The PTH-regulated transcription factor Sox4 is vital for normal fetal development, as *Sox4*$^{-/-}$ mice die in utero from circulatory failure. We have previously demonstrated that *Sox4* mRNA is expressed in the embryonic growth plate and regulated via the parathyroid hormone/parathyroid hormone-related peptide receptor (PTH/PTH-rP) in osteoblast-like cells [Reppe S et al. (2000) JBMR 15: 2402-12]. While adult *Sox4*$^{+/-}$ animals appear phenotypically normal, our data, supported by others, suggest a potential role for Sox4 in osteoblast differentiation. Here, we evaluated time-dependent changes in bone mineral density (BMD) of *Sox4*$^{+/-}$ and wildtype (wt) mice. We also characterized the bone morphologically, and studied the ability of primary calvarial osteoblasts from *Sox4*$^{+/-}$ and wt mice to differentiate and form bone nodules in vitro.

Methods: BMD was measured by DEXA every 6 weeks (from 2 to 11 months, males and females, *Sox4*$^{+/-}$ and wt). Epoxy-embedded tibiae were analyzed by light and electron microscopy. Osteoblast primary cultures were derived from 8- to 10-day-old female mice and grown to 70–90% confluence before use. Osteoblast phenotype was evaluated by alkaline phosphatase (ALP) histochemistry, [^{3}H]-thymidine incorporation and von Kossa staining of mineralized nodules in osteoblasts cultured for 3 weeks. mRNAs characteristically expressed by osteoblasts were analysed by real-time PCR.

Results: By 6 months, BMD was reduced 5.1% (males, $P < 0.05$) and 3.1% (females, n.s.) in *Sox4*$^{+/-}$ mice compared to sex- and age-matched controls. At 10 and 11 months, the BMD of female *Sox4*$^{+/-}$ mice was reduced further to 5.3 and 5.0% ($P < 0.01$), respectively. Morphological analyses showed reduced number and thickness of trabeculae in *Sox4*$^{+/-}$ mice. In *Sox4*$^{+/-}$ osteoblast cultures, the number of ALP-expressing cells and mineralized bone nodules appeared decreased by ~50% and *Sox4*$^{+/-}$ cells incorporated less (~30%, $P < 0.05$) [^{3}H]-thymidine. Preliminary data suggested reduced expression of osterix mRNA in the *Sox4*$^{+/-}$ osteoblasts compared to wt.

Conclusions: This study reports that *Sox4*$^{+/-}$ mice display reduced BMD compared to wt, with differences starting to appear in young adults and being maintained during aging. Our data showing impaired differentiation and function of osteoblasts derived from *Sox4*$^{+/-}$ mice provide a possible explanation to these observations. Supported by: Osteogene.

OC016

The Record Trial: An Evaluation of Calcium and/or Vitamin D in the Secondary Prevention of Osteoporotic Fractures

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Objectives: To determine the efficacy of oral supplementation with calcium or vitamin D or both in the secondary prevention of low-trauma fractures in men and women aged over 70 years who had already experienced a low trauma fracture.

Materials and methods: 5292 participants, recruited from 21 centres in the UK, were randomized to daily calcium (1 g as carbonate), vitamin D3 (800 IU/20 μg), both, or placebo. Subjects and investigators were blind to allocation. The principal outcome was new low-trauma fracture. The study had 80% power ($2P < 0.05$) to detect an absolute reduction in fractures from 15% to 12%. Other outcomes were health status, all cause mortality, hospital admissions, change of residence, falls and adverse events. Planned sub-group analyses examined the effect of age, gender, recruitment fracture type, time since fracture, calcium intake, vitamin D status, body weight, and compliance. Recruitment started in 1999 and follow-up ended in 2004. Recruitment fractures included proximal femur (17%) and distal forearm (35%). At recruitment, most participants were within 3 months of a fracture and were able to walk out of doors. The main reasons for trial ineligibility were cognitive impairment (43%) and current treatment for osteoporosis (34%). The study was approved by the Multi-centre Research Ethics Committee for Scotland.

Results: 698 (13.2%) had a further low-trauma fracture, including 183 hip fractures. There were no statistically significant differences between those allocated calcium and those not [331 (12.6%) vs. 367 (13.7%); HR 0.94, 95% CI 0.81 to 1.09]; those allocated vitamin D and those not [353 (13.3%) vs. 345 (13.1%); HR 1.02, 95% CI 0.88 to 1.19]; and those allocated both calcium and vitamin D versus placebo [165 (12.6%) vs. 179 (13.4%)]. No differences were detected in all reported fractures, X-ray confirmed fractures, hip fractures, death, falls, quality of life, or in sub-group analyses. Compliance with calcium was significantly poorer, due to gastrointestinal symptoms. Potentially serious adverse events were rare and did not differ between groups.
Conclusions: The findings do not support the use of routine supplementation with calcium and/or vitamin D for the prevention of further fractures in people who have a recent low-trauma fracture.
Funding: The RECORD Trial was funded by a project grant from the Medical Research Council (UK). Shire Pharmaceuticals and Nycomed provided active and placebo treatments free of charge.

OC017

LRP5 and LRP6 Variants Determine Fracture Risk in Elderly Men

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The low-density lipoprotein receptor-related proteins 5 and 6 (LRP5 and 6) are co-receptors for Wnt-ligands. Loss-of-function mutations of the LRP5 gene in both human and mice lead to decreased BMD. Mice heterozygous for mutations in the LRP5 and LRP6 gene showed that LRP5 and LRP6 genetically interact in limb development. We previously described association of the Val-allele of the A1330V LRP5 polymorphism with low BMD, reduced frame size and higher fracture risk in men. We now studied association of an amino-acid variant of the LRP6 gene (I1062V) with bone parameters and fracture risk, and analyzed interaction between LRP5 and LRP6 variants in 6373 participants of a large prospective population-based cohort of elderly subjects. In men, carriership of the Val-allele (f = 20%) of the I1062V polymorphism in LRP6 was associated with increased height (P = 0.01) and increased vertebral body size (P = 0.04). Male carriers of the Val-allele of the LRP6 polymorphism had a 70% increased risk (P = 0.01) for fragility fractures. In addition, we found a non-significant trend (P = 0.13) towards a higher risk for vertebral fractures. We subsequently stratified the subjects according to carriership of the LRP5 1330-V and LRP6 1062-V alleles into 4 groups. While carriers of a single risk allele for either LRP5 or LRP6 had a fracture risk of 1.5, carriers of both risk alleles had a 2.2 times increased risk (P = 0.01) for fragility fractures compared to non-carriers of both risk alleles. In addition, the double risk allele carriers had a 90% higher (radiological defined) vertebral fracture risk compared to the reference (P = 0.02). All the fracture risks were independent of age, height, weight, or BMD. Although similar trends were seen in women, all of these associations were weaker compared to men. In terms of functional effects of the LRP6 variant, the SIFT software program predicted the 1062 Val-allele to be deleterious, based on good conservation of the 1062-Ile allele. Further experiments are needed to elucidate the effects of this variant on function of the LRP6-protien.
In conclusion, in men, the V-1062 variant of the LRP6-gene is associated with frame size and fracture risk. An additive effect was seen for fracture risk between the LRP5 and 6 variants.

OC018

Integrin Beta 3 Leu33Pro Polymorphism and Risk of Hip Fracture: 25 years Follow-up of 9233 Adults from the General Population

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Integrin $\alpha_v\beta_3$ is essential for mature osteoclast function. In β_3 knockout mice, osteoclasts show deranged microstructure with an abnormal actin cytoskeleton, impairing the formation of the ruffled membrane. The defective bone resorption leads to an increased skeletal mass. Antagonists of $\alpha_v\beta_3$ are able to prevent bone loss in ovariectomized rats. The Leu33Pro polymorphism changes the conformational structure of the β_3 subunit. In platelets, this results in increased cell adhesion and actin polymerization. Given similar effects in osteoclasts, we would expect increased fracture risk in 33Pro carriers. We tested the hypothesis that the Leu33Pro polymorphism in the integrin β_3 subunit associates with risk of hip fracture. We performed a prospective study of participants in the Copenhagen City Heart Study with 25 years follow-up comprising 9233 Caucasian men and women selected at random to represent the Danish general population. Main outcome measure was first-ever hip fracture (n = 267). Additionally, bone mineral density (BMD) was measured cross-sectionally by digital X-ray radiogrammetry (DXR) in a subgroup of 1981 postmenopausal women attending a planned follow-up visit. Genotyping rendered 69.9% non-carriers, 27.3% heterozygotes, and 2.7% homozygotes.

Incidence of hip fracture was 2.8 and 1.5 per 1000 person-years in homozygotes and non-carriers (log-rank: $P = 0.02$). Multifactorial adjusted Cox regression revealed a hazard ratio of 2.0 (95% CI: 1.1–3.5) for hip fracture in homozygotes versus non-carriers. After stratification by gender, equivalent hazard ratios were 2.0 (1.0–4.1) in women and 2.0 (0.8–5.0) in men. In 2193 postmenopausal women, hazard ratio for hip fracture in homozygotes versus non-carriers after additional adjustment for age at menopause and use of hormone replacement therapy was 2.6 (1.2–5.3). Hazard ratio for hip fracture in heterozygotes versus non-carriers did not differ from 1.0. DXR-BMD measured cross-sectionally did not differ between genotypes (ANCOVA $P = 0.14$). The study design did not allow conclusions about whether the association with fracture was mediated partly through an effect on BMD. In conclusion, individuals homozygous for the integrin \beta$_3$ Leu33Pro polymorphism have a 2-fold risk of hip fracture. The increased risk is even more pronounced in postmenopausal women. Given our results are confirmed, integrin \beta$_3$ Leu33Pro homozygosity could prove a useful marker for risk of future hip fracture.

OC019

Population-Based Geographic Variations and Determinants of BMD Change in Elderly Men and Women Across Europe. Results from the Network for Male Osteoporosis (NEMO) Study

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While much is known about determinants of BMD change in women, there have been few longitudinal studies in men. As part of the Network for Male Osteoporosis (NEMO) study, data was analyzed from 1337 men and 1490 women aged 50–86 years (mean = 67 years) from 12 centers across Europe to assess determinants of BMD change and between-gender contrasts. BMD was measured at the femoral neck, trochanter, and/or L2–L4 spine on 2 occasions 0.8–8 years apart (mean = 3.5 years) using DXA densitometers manufactured by Hologic ($n = 6$), Lunar ($n = 4$), and Norland ($n = 2$). Densitometers were cross-calibrated using European Spine Phantom data and annual rate of BMD change (g/cm^2/year) was calculated from the standardized paired BMD values. The EVOS risk factor questionnaire was administered at baseline.

In men, the mean annual BMD changes in g/cm^2/year (95% CI) were: femoral neck −0.003 (−0.004, −0.002); trochanter −0.001 (−0.001, −0.000); and spine 0.003 (0.002, 0.005). In women, the respective estimates were: −0.005 (−0.005, −0.004); −0.003 (−0.004, −0.003); and −0.002 (−0.004, −0.001). In multivariate linear regression models, there were highly significant between center differences in the mean rates of BMD change in all 3 sites for both genders ($P < 0.0001$). Higher baseline BMD value was associated with subsequent greater decline in BMD ($P < 0.008$) and weight gain preserved BMD ($P < 0.039$) in all 3 sites for both genders, except male spine. Higher baseline body weight preserved BMD in all 3 sites in men ($P < 0.012$) but not in women. Increasing age was associated with faster BMD decline at the trochanter in both genders ($P < 0.026$) and with slower decline at the female spine ($P = 0.008$).

Effects of lifestyle, physical activity, medications, and reproductive factors were not consistent across sites or between genders. At the male femoral neck, calcium, alcohol, and yoghurt consumption preserved BMD (all $P < 0.034$). Higher activities of daily living score marginally preserved trochanter BMD ($P = 0.092$). In women, receiving hormone replacement ($P = 0.009$) and greater frequency of milk consumption ($P = 0.005$) preserved BMD at the femoral neck. Moderate alcohol consumption and high self-rated health status preserved trochanter BMD ($P < 0.016$).

The results show major variations in rates of BMD change in men and women over 50 years of age across diverse European populations and shows that body weight and weight gain are key determinants of BMD change in men.

OC020

Population-Based Assessment of Bone Strength Indices and Traumatic Loads: Relationship to Patterns of Fragility Fractures at Wrist and Hip

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Measurements of bone mineral density (BMD) fail to capture major components of bone strength or satisfactorily explain patterns of fragility fractures, and population-based assessments of bone strength indices and traumatic loads have not been made. By quantitative computed tomography at ultra-distal radius (UDR) and femoral neck (FN) in an age-stratified sample of 375 females (W) and 325 males (M), ages 21–97 years, from Rochester, MN, we assessed

volumetric BMD (vBMD, g/cm^3) and strength indices that estimate bone's resistance to compressive (EA, Newtons [N]) and bending forces (EI, N * mm^2). We also estimated the force (*F*, in N) that would be applied to the wrist and hip during forward and sideways falls, respectively, and computed the ratio of fall force to bone strength.

Conclusions: (1) Bone strength decreases less than vBMD with aging because of compensatory changes in bone structure; (2) *F*/strength ratios increase with age and increase 2- to 3-fold more in W vs. M; (3) lower bone strength in young adult W, due to smaller bone size, persists over life and explains much of their 6-fold greater sex incidence of wrist fractures; (4) lower initial bone strength, greater subsequent bone loss, and greater increases in *F*/strength ratios with aging all contribute to the 2-fold greater incidence of hip fractures in W vs. M; (5) the moderate decreases in bone strength with aging are insufficient to explain the 4-fold increase in hip fracture incidence after age 75, suggesting that increased frequency of falls and impaired protective reflexes in the elderly play major roles.

Variable	W		M		(M−W)/W, % (20–29 years)
	Mean ± SD (20–29 years)	Δ % over life	Mean ± SD (20–29 years)	Δ % over life	
UDR					
vBMD	593 ± 61	−33**	651 ± 99	−28**	10[a]
EA	3.31 ± 0.39	−27**	5.16 ± 0.79	−17**	56[b]
EI	9.32 ± 1.93	−19**	20.3 ± 5.8	0	117[b]
F/EA	0.80 ± 0.09	43**	0.56 ± 0.08	23**	−32[b]
F/EI	0.30 ± 0.06	28**	0.15 ± 0.03	4**	−50[b]
FN					
vBMD	428 ± 55	−46**	367 ± 48	−34**	−14[b]
EA	5.73 ± 0.65	−38**	7.28 ± 1.06	−30**	27[b]
EI	4.59 ± 0.75	−29	8.97 ± 2.45	−24**	95[b]
F/EA	109 ± 0.17	62**	1.21 ± 0.14	34**	11[a]
F/EI	1.39 ± 0.29	40**	1.02 ± 0.20	22**	−26[b]

For age changes: **<0.005; for sex differences: [a]<0.05, [b]<0.005.

OC021

Transient Receptor Potential Channel TRPV5 and 1a-Hydroxylase Double Knockout Mice Demonstrate Significance of Renal Reabsorption and 1,25(OH)$_2$D$_3$ for Calcium Homeostasis

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We recently demonstrated that transient receptor potential channel V5 (TRPV5) deficiency (TRPV5$^{-/-}$) in mice causes disturbances in control of Ca^{2+} homeostasis. Despite normocalcemia and normal PTH levels, a strong increase was observed in the 1,25(OH)$_2$D$_3$ levels compared to wildtype littermates. Moreover, trabecular and cortical bone thickness was reduced in these mice. In an attempt to explain why the TRPV5$^{-/-}$ mice do not have hypocalcemia and have high 1,25(OH)$_2$D$_3$ levels, we generated TRPV5 and 1\alpha-hydroxylase double knockout mice (TRPV5$^{-/-}$1\alpha-OHase$^{-/-}$).

We measured a number of serum [Ca^{2+}, 1,25(OH)$_2$D$_3$ and PTH] parameters in 10-week-old wildtype, TRPV5$^{-/-}$, 1\alpha-hydroxylase$^{-/-}$ (1\alpha-OHase$^{-/-}$) and TRPV5$^{-/-}$ 1\alpha-OHase$^{-/-}$ mice (n = 7–9) and renal and intestinal TRPV5 and 6, calbindin-D$_{9k}$ and -D$_{28k}$ and NCX1 gene expression was assessed. Femurs of these mice were analyzed, using X-ray imaging and \muCT.

TRPV5$^{-/-}$ mice are normocalcemic and have normal PTH levels but strongly increased 1,25(OH)$_2$D$_3$ levels. In 1\alpha-OHase$^{-/-}$ mice serum Ca^{2+} was low and 1,25(OH)$_2$D$_3$ was undetectable. Despite intact renal reabsorption and increased PTH levels, Ca^{2+} homeostasis was disturbed in these mice. In mice lacking both TRPV5 and 1\alpha-OHase, the phenotype was even more severe than in the 1\alpha-OHase$^{-/-}$ mice. Despite the 50-fold increased PTH levels, lack of renal reabsorption and 1,25-(OH)$_2$D$_3$, these mice suffered from severe hypocalcemia. In all mice models, gene expression of the transcellular Ca^{2+} transport proteins in intestine (TRPV6 and calbindin-D$_{9k}$), but not in kidney (calbindin-D$_{9k}$, -D$_{28k}$ and NCX1), is positively correlated with 1,25-(OH)$_2$D$_3$ levels. Detailed analyses of the femurs showed that TRPV5$^{-/-}$ mice had reduced cortical and trabecular thickness and the 1\alpha-OHase$^{-/-}$ mice show a rickets-like phenotype (hypomineralization and epiphyseal growth plate widening). However, the double knockout mice display the most dramatic phenotype with severely reduced femur length and bone mineralization as well as extensive epiphyseal growth plate widening, precluding to analyze in detail a direct role for TRPV5 or 1\alpha-OHase in bone metabolism. In conclusion, we demonstrate that elevated serum 1,25-(OH)$_2$D$_3$ prevents hypocalcemia in renal reabsorption deficient TPRV5 knockout mice.

OC022

BMP-6 Restores Lost Bone in Ovariectomized Rats and Mediates the Effect of Estradiol on Trabecular Bone in BMP-6 Knock-out Mice

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Bone morphogenetic proteins (BMPs) induce new bone formation when administered locally. However, there is still no evidence that a human recombinant BMP, given systematically, can restore bone in an osteoporotic rat model. Rats were ovariectomized at 6 months of age and therapy started 6 months later. Total body, lumbar spine, and hind limbs BMD was measured at 6- and 12-week period. Within 3 months,

BMD in BMP-6 treated rats reached the values of sham treated animals at hind limbs, while spine BMD was slightly lower. BMP-6 was most effective at g/kg 3 times weekly. In vivo measurements were μ the lowest dose of 1 subsequently confirmed by ex vivo BMD values, by pQCT, microCT and histomorphometry of distal femurs, and by biomechanical testing of long bones. In similar experiments, BMP-7 was not effective, while estradiol was less effective, and did not have a synergistic effect with BMP-6. To further explore whether estradiol exerts its bone activity via BMP-6, Bmp-6 mutant and wild type mice were ovariectomized (OVX) and 3 weeks later treated for the next 6 weeks as follows: (1) sham, (2) OVX, (3) OVX + BMP-6 (10 μg/kg i.v. 3 × week), and (4) OVX + 17β-estradiol (E2) (50 μg/kg i.v. 3 × week). As revealed by μCT Bmp-6 −/− sham animals had decreased BV/TV and trabecular number as compared to wild type sham mice. Unlike in wild type mice, both OVX and estradiol therapy did not influence the BMD values in Bmp-6 −/− mice. On the contrary, BMP-6 therapy increased BV/TV, trabecular number and trabecular thickness in Bmp-6 −/− OVX mice to higher values than in sham animals. The results suggest that systemically administered BMP-6 has a specific anabolic effect on bone volume in aged osteoporotic rats, while estradiol mediates its anabolic activity on trabecular bone in mice at least partially via BMP-6.

OC023

Arrestins Regulate Osteoprotegerin (OPG) and RANKL Expression by Intermittent and Continuous PTH in Primary Cultures of Mice Osteoblasts

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We previously reported that the G-protein-coupled receptor regulatory molecule β-arrestins inhibit PTH-stimulated intracellular signaling and PTH activity in bone. In particular, arrestins moderate PTH-activated bone resorption, but the molecular mechanisms by which arrestins exert these effects remain to be elucidated. Since osteoprotegerin (OPG) and RANKL are important mediators of PTH-stimulated bone turnover, we used quantitative real-time PCR to investigate OPG and RANKL mRNA expression in primary cultures of osteoblasts (POB) from β-arrestin2 KO and WT mice. Sub-confluent cell cultures (n = 2 to 4 in each experiment) were exposed to bPTH (1–34) (10–100 nM) or vehicle intermittently (i.e., for 6 h every 2 days) or continuously (i.e., for 24–48 h) and this repeatedly for 2 days up to 2 weeks. The relative expression level of OPG and RANKL mRNA was reported to the β-2microglobulin housekeeping gene.

Overall, OPG levels were lower in KO compared to WT POB (P = 0.027, n = 44), whereas RANKL mRNA levels did not significantly differ between these cells, with thus a higher OPG/RANKL ratio in WT cells (P = 0.048). PTH significantly decreased OPG (P = 0.0001), respectively increased RANKL (P = 0.009), depending on the time of exposure (P interaction = 0.013 and 0.006 for OPG and RANKL, respectively, by 3F-ANOVA) and the presence/absence of β-arrestin2 (P interaction = 0.0008 for OPG, ns for RANKL). Indeed, intermittent PTH inhibited OPG expression after 6 h in both WT (−36%) and KO (−42%) POB, but a sustained inhibition was observed 48 h after intermittent PTH exposure and after continuous PTH treatment only in KO cells (P = 0.0012). In contrast, PTH-stimulated RANKL mRNA expression was detectable after 6 h, peaked at 24 h, and was still detectable, albeit at lower levels, after longer exposure to PTH in both WT (P = 0.01) and KO (P = 0.007) cells. Nevertheless, after more than 6 days of continuous exposure to PTH, RANKL mRNA levels eventually declined in WT cells, whereas they remained increased about 3-fold above vehicle levels in KO POB.

These findings indicate that absence of β-arrestins prolongs inhibition of OPG and stimulation of RANKL expression by PTH in osteoblasts. Thus, by inhibiting intracellular signaling and prompting receptor internalization and recycling, β-arrestin2 may allow desensitization of OPG and RANKL response to PTH. In turn, these molecular mechanisms can partly explain how arrestins modulate the effects of PTH on bone turnover.

OC024

Risk of Fracture in Patients Using High-Dose Intermittent Oral Glucocorticoids

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The aim was to estimate the fracture risk in users of oral glucocorticoids (GC) for respiratory disease (aged 40+). The study included 92,000 GC users (from the UK General Practice Research Database). The period of follow-up was divided into time periods of current and no exposure, with daily dose (DD) and cumulative dose (CD) determined for each period. Odds ratios (ORs) were estimated using Cox proportional hazards models, adjusted for age, gender, body mass index, smoking and disease and drug history. Fractures included were those of radius/ulna, humerus, rib, femur/hip, pelvis, or vertebrae.

Intermittent high-dose GC use (>30 DD) was associated with only a small increased risk of osteoporotic fracture, but without any statistical difference between first-time users and repeat users (>3 months after end of prior use). Hip fracture risk was not increased in these patients.

Table
Risk of Fracture by Daily Dose and Cumulative Dose

	Any fracture	Hip fracture
DD < 2.5 mg		
CD < 1 g	0.88 (0.37–2.12)	0.58 (0.08–4.12)
CD > 1 g	1.78 (1.25–2.53)	1.34 (0.64–2.83)
DD 2.5–5 mg		
CD < 1 g	1.50 (0.99–2.29)	1.04 (0.43–2.51)
CD > 1 g	1.67 (1.34–2.08)	1.37 (0.89–2.11)
DD 5–7.5 mg		
CD < 1 g	1.20 (1.00–1.44)	1.08 (0.75–1.56)
CD > 1 g	1.88 (1.67–2.10)	1.46 (1.16–1.82)
DD 7.5–15 mg		
CD < 1 g	2.10 (1.63–2.71)	1.70 (1.02–2.83)
CD > 1 g	2.18 (1.89–2.52)	1.74 (1.30–2.32)
DD 15–30 mg		
CD < 1 g	1.55 (1.27–1.90)	1.63 (1.11–2.39)
CD > 1 g	2.64 (2.09–3.32)	1.85 (1.10–3.09)

OC025

Glucocorticoid Blocks AP-1 Activity Leading to Transcriptional Repression of an Osteogenic Cytokine Interleukin-11: A Mechanism of Impaired Bone Formation

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Glucocorticoid (GC) suppresses bone formation through impaired differentiation and enhanced apoptosis of osteoblasts. We have reported that GC suppresses expression of an osteogenic cytokine, interleukin (IL)-11, both in vitro and in vivo. We have also shown that IL-11 expression by bone marrow stromal cells is dependent on AP-1 and is decreased in aged mice due to decreased activity of AP-1, especially Jun D, and that IL-11 is a target of bone anabolic stimuli such as PTH and mechanical stress. The role of IL-11 in bone formation in vivo has been established by our observation that transgenic mice over-expressing IL-11 exhibits enhanced bone formation and thereby increased bone mass. Taken together, these results suggest that decreased IL-11 expression may be involved in the pathogenesis of GC-induced osteoporosis (GIO). In the present study, we aimed to clarify the mechanism by which GC inhibits IL-11 gene expression and to further establish the role of IL-11 in GIO, particularly in GC-induced osteoblast apoptosis.

Deletion analysis with the mouse IL-11 promoter revealed that PTH stimulated IL-11 gene transcription, which was strongly inhibited by dexamethasone (DEX) in an AP-1-dependent manner. DEX had no effects on induction of the fos family genes such as deltafosB or upstream signals including ERK activation. Inhibitory effects of DEX on IL-11 gene transcription appeared steroid-specific, because no inhibition was observed with other steroid hormones such as 1,25-dihydroxyvitaminD3, 17beta-estradiol and dihydrotestosterone. DNA precipitation experiments demonstrated that Jun D and deltafosB, a fos family member that has been shown to stimulate bone formation in vivo, co-precipitated with the AP-1 binding sequences on the mouse IL-11 promoter. We found that the GC receptor (GR) was also co-precipitated with AP-1 in a ligand-dependent manner. Interestingly, Jun D binding to the AP-1 site was diminished by GC, suggesting a direct interference of GR with Jun D binding. Relevance to the pathogenesis of GIO was underscored by our observation that both PTH and IL-11 inhibited GC-induced osteoblast apoptosis. Experiments with neutralizing antibodies and siRNA to IL-11 suggested that PTH inhibition of GC-induced apoptosis was partly dependent on IL-11. We therefore conclude that GC inhibits IL-11 gene transcription in part through inhibition of AP-1 binding, thereby enhancing osteoblast apoptosis and ultimately suppressing bone formation.

OC026

ADAMTS 10 Mutations in the Autosomal Recessive Weill-Marchesani Syndrome

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Weill–Marchesani syndrome (WMS, [MIM 277600]) is characterized by the association of short stature, brachydactyly, joint stiffness, and eye anomalies including microspherophakia, ectopia of the lenses, severe myopia and glaucoma and occasionally, heart defects. Despite clinical homogeneity, autosomal recessive (AR) and autosomal dominant (AD) modes of inheritance have been reported and we have recently identified an in frame deletion of the fibrillin-1 gene in an AD WMS family. Using a homozygosity mapping strategy in two consanguineous families from Lebanon and Saudi Arabia, we have reported linkage of the AR WMS gene to chromosome 19p13.3–p13.2 in a 12.4-cM interval. Several candidate genes involved in the extracellular matrix structure were considered including a member of the extracellular matrix proteases, ADAMTS 10 (a disintegrin and metalloprotease with thrombospondin motifs). Here, we present null mutations in ADAMTS 10 in two consanguineous families and in one sporadic WMS case. A total of three distinct mutations was identified including one stop mutation (R237X) and two splices mutations (1190 + 1G > A, 810 + 1G > A). Expression studies of ADAMTS 10 using RT-PCR, Northern blot, and dot blot analyses showed that ADAMTS 10 is expressed in skin, fetal chondrocytes and fetal and adult heart. Moreover, electron microscopy and immunological studies of the skin fibroblasts of affected patients confirmed the impairment of the extracellular matrix. We conclude therefore that

ADAMTS 10 plays a major role in skin, growth, lens and heart development in human.

OC027

Osteoprotegerin (OPG) Mutations that Cause Hyperphosphatasia Impair OPG Protein Secretion and Biological Activity

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Familial Idiopathic Hyperphosphatasia (FIH) is a rare genetic bone disease characterized by increased bone turnover. There is a considerable phenotypic variation from presentation in infancy with severe progressive deformity, through to presentation in late childhood with minimal deformity. Recently, genetic studies have established that mutations in OPG, a key regulator of bone remodeling, can cause FIH. The aim of the study was to investigate genotype-phenotype correlation between specific mutations, the function of the mutant proteins and the severity of disease in five families.

The patients were grouped into mild, intermediate, and severe phenotypes using clinical, biochemical, and radiographic data. We produced constructs corresponding to five different mutations: two from patients with intermediate disease (\DeltaD182 and F117L), two from patients with severe disease (C65R and C87Y), and a mild C-terminal mutation (CtFS). When expressed in the human osteoblastic cell line $SaOS_2$, the constructs did not effect cell proliferation and measurement of OPG mRNA by real-time PCR demonstrated that all constructs were transcribed with comparable efficiency. However, different levels of OPG protein were secreted into the media: F117L had similar levels to wtOPG; while C65R, C87Y, and CtFS all had greatly reduced yields; \DeltaD182 had an intermediate secretion level. Functional studies using surface plasmon resonance technology (BIAcore) showed significant variability in the ability of the different mutant proteins to bind the OPG ligand, RANKL. Measuring the activity of the intermediate mutants in an osteoclastogenesis assay showed they were significantly less potent than wtOPG in inhibiting osteoclast formation.

The various OPG mutations identified in FIH families and the phenotypic variation of the disease offered a unique opportunity for a structure–function study of OPG. Our investigations suggest that the FIH phenotype results from a combination of impaired intracellular processing and reduced activity of the OPG mutants.

OC028

Identifying Targets Against Multiple Myeloma by Characterizing the Plasma Cell Specific Unfolded Protein Response

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Multiple Myeloma (MM) is a frequent, severe, and still incurable hematological malignancy that originates from the clonal expansion of plasma cells. Recently, a new class of drugs, proteasome inhibitors (PI), proved effective in MM therapy, and are currently in phase 3 clinical trial. The anti-tumor effect of these drugs owes to their ability to prime apoptosis selectively in MM cells, via undetermined mechanisms. The normal counterpart of MM, plasma cells, the factories of soluble Ig, are subject to intense ER stress and rely on the adaptive unfolded protein response (UPR), for their differentiation and activity. PI induce a robust UPR, providing a framework to understand their peculiar efficacy on MM cells.

To explore the molecular bases of such therapeutic effect, we first asked whether plasma cell differentiation confers apoptotic sensitivity to PI on different models (in vitro, from the murine inducible B lymphoma I.29 μ+, and ex vivo, from primary mouse B cells). Our findings reveal that plasma cell differentiation generates apoptotic sensitivity to PI at late stages of differentiation, in correlation with UPR activation. The selective toxicity of PI on plasma cells was then confirmed in vivo in LPS-injected mice, indicating that proteasomes participate in regulating plasma cell lifespan and the duration of the humoral immune response.

The UPR is a key inducer of apoptosis in many diseases. To gain insights on the mechanisms that trigger death in plasma cells, we investigated the ordered unfolding of the B cell-specific UPR over time by assessing histone post-translational modifications and cofactor recruitment to UPR target genes by chromatin immunoprecipitation. Our data reveal that: (1) the plasma cell-specific UPR, unlike that induced by pharmacological ER stressors, is accompanied by profound chromatin remodelling at UPR genes (like XBP-1 and CHOP); (2) plasma cell differentiation activates a potentially protective heat shock response (HSR); (3) histone acetylation at target genes correlates with recruitment to gene promoters of the key UPR transcriptional coactivator XBP-1. Our findings link the molecular bases of sensitivity of MM to PI to the Ig-secreting phenotype, and reveal that chromatin remodelling is involved in the plasma cell UPR. Altogether, our data, by characterizing plasma cell differentiation, may help identify potential targets and design more specific therapeutic tools against MM.

OC029

Homozygosity for a Dominant-Negative Type I Collagen Mutation Attenuates the Type IV OI Phenotype of the Heterozygous Brtl Mouse: Insight into Disease Mechanism

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The Brtl mouse is a dominant-negative model for type IV osteogenesis imperfecta, caused by a glycine substitution (G349C) knocked into one col1a1 allele. Brtl/+ pups have 30% perinatal lethality and surviving Brtl mice are smaller in size with weaker and more brittle bones than Wt. In murine models for other dominant genetic disorders, homozygous animals have a more severe or lethal phenotype than do the heterozygous mice. We present here the novel genetic situation in which homozygosity for a dominant mutation (Brtl/Brtl) attenuates the phenotype of the heterozygous mice. Perinatal survival of Brtl/Brtl is normal. Their weight is intermediate to Wt and Brtl/+ and they lack the rib fractures, flared thorax, osteoporotic calvarium and vertebrae seen in Brtl/+. Brtl/Brtl femurs have normal areal and vBMD, but intermediate cross-sectional area, trabecular thickness and BV/TV. They withstand normal loading to fracture and are less brittle than Brtl/+. Cell numbers, MAR, and BFR/BS were unchanged in all genotypes at 2 months. Matrix insufficiency and collagen chain composition may contribute to the difference in homozygous and heterozygous phenotype. Brtl/Brtl fibroblasts synthesize only mutant alpha1(I) mRNA and virtually all alpha1 chains form disulfide-linked homodimers. Brtl/Brtl procollagen is efficiently secreted and incorporated into matrix in culture and in tissue. In Brtl/+, type I collagen containing one mutant chain is selectively retained by the cells and is deficient in the lung and skin tissue. Approximately 1/3 of collagen with one mutant alpha1 chain and 2/3 of collagen with two mutant alpha1 chains are secreted from the Brtl/+ fibroblasts. This results in about 40% matrix insufficiency of type I collagen in Brtl/+ mice vs. only 15% in Brtl/Brtl. In addition, the collagen with one mutant chain present in Brtl/+ has a reactive SH group which might contribute to illegitimate collagen cross-links, while collagen of Brtl/Brtl contains only disulfide linked alpha1 dimers. Interestingly, dermal fibril diameter is significantly larger in Brtl/Brtl than in either Brtl/+ or Wt mice, supporting a direct effect of different collagen composition on matrix regulation. The relative contributions of matrix insufficiency, illegitimate collagen crosslinking and mutant collagen composition to the attenuation of phenotype in the homozygotes are under investigation.

OC030

Effect of depot medroxyprogesterone acetate on attainment of peak bone mass

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The injectable contraceptive depot medroxyprogesterone acetate (DMPA, Depo-Provera) is used by over nine million women worldwide. It suppresses estrogen secretion and induces amenorrhea, raising concern about skeletal effects. Previous studies have produced differing results; a bone mineral deficit has been demonstrated in studies of the lumbar spine and hip, but not in studies of the forearm. The effect may be greatest in young users, but DMPA users are likely to be smokers, pregnant at a young age, and not educated beyond high school level, which could all affect bone density.

This study aims to determine if the effects of DMPA on the skeleton are age-specific, and to eliminate the effect of social and lifestyle factors by the use of individually matched controls.

We recruited 100 pairs of women ages 18–25, and 35–45 from Sheffield general practices and family planning clinics. DMPA use was of at least 12 months duration (mean 37 months) and commenced before the age of 20 or after the age of 34. Controls were matched for source of recruitment, postcode, age, height, body mass index and smoking habit. We included users of the combined oral contraceptive in the control group as there is good evidence it has no effect on bone mineral density, and is the most common contraceptive choice in this population.

Bone mineral density was assessed by DXA at the spine, hip and forearm.

Paired *t* test showed a deficit in young DMPA users of 5.6% at the spine (95% CI: −9.3 to −1.9%) and 5.2% at the hip (95% CI: −9.4 to −0.92%), but no difference at the forearm. Estimation of bone volume and volumetric density in the young DMPA users found a greater difference in bone volume at the femoral neck, but a greater difference in volumetric density at the lumbar spine.

The older age group showed no significant differences between DMPA users and controls.

We conclude that DMPA may influence bone acquisition in the immature skeleton, but has no detrimental effect after attainment of peak bone mass. The site-specific effect may be due to differing maturity of the peripheral and central skeleton at the time of exposure, differing sensitivity to estrogen deficiency or a protective effect of DMPA at the forearm. The age specificity of our results suggests that estrogen deficiency is unlikely to be the sole mechanism through which DMPA acts on bone.

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OC031

Use of Beta-Blockers is Associated with BMD and Fracture Risk

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There is accumulating evidence that the sympathetic nervous system is involved in the regulation of bone metabolism. Sympathetic innervation of bone and adrener-

gic receptors on both osteoblasts and osteoclasts were recently demonstrated. Furthermore, propranolol increased bone formation in mice. We examined the association between dose and duration of beta-blocker use and bone mineral density (BMD), bone loss and (non)vertebral fracture incidence. We performed our study in the cohort of the Rotterdam Study, a population-based cohort study in men and women of 55 years and older. BMD was measured by DXA. Nonvertebral fractures were reported by general practitioners and vertebral fractures were assessed from spinal X-rays. Medication use was available on a day-to-day basis from complete medication histories of pharmacies. For 3009 participants, follow-up femoral neck BMD measurements were available and for 7892 and 3469 participants follow-up data were present on nonvertebral fractures and vertebral fractures, respectively. After adjustments for age, gender, baseline BMD, body mass index, cardiovascular disease risk, hypertension and use of thiazides and statins, mean BMD at end of follow-up (0.858 g/cm^2; 95% CI 0.851–0.864) of long-term beta-blocker users (>4 years) was significantly higher than BMD of non-users (0.841; 0.839–0.844). Long-term users had a significantly lower rate of loss of BMD per year; 0.38% (0.27%–0.47%) than non-users (0.63%; 0.59%–0.67%). Risk for all nonvertebral fractures was not decreased, but there was a significant association between long-term beta-blocker use and frailty fracture risk (upper arm, hip and pelvis) (HR 0.67; 0.46–0.97) Vertebral fracture risk was lower for long-term users, but not significantly decreased (OR 0.83; 0.49–1.41). In conclusion, we report a significant and duration-dependent association between use of beta-blockers, BMD, rates of bone loss and risk of frailty fracture.

OC032

Use of Beta-Blockers and Risk of Hip and Vertebral Fractures: Associations with Daily and Cumulative Dose

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Background: Following findings that propranolol increases bone formation in mice, a recent study conducted in the UK General Practice Research Database (GPRD) has found that use of beta-blockers is associated with a decreased risk of hip and vertebral fractures. However, the authors did not report whether daily dose or cumulative dose of beta-blocker use were associated with fracture risk.
Objective: To evaluate the association between daily and cumulative dose of betablockers use and risk of hip and vertebral fractures.
Methods: A large case-control study (n = 23,100 cases) was conducted among adults using data from the GPRD. Cases were defined as patients with a first record for a clinical symptomatic fracture of the hip or vertebrae. For each case, one control patient without a history of a fracture was matched by age, gender, and practice and index date. Use of betablockers, thiazides, and other antihypertensives 3 months before the index date were compared to never use. We calculated atenolol-equivalents of the last prescribed daily dose before the index date, and the cumulative exposure before the index date. Conditional logistic regression analysis was used to calculate odds ratios (OR) and 95% confidence intervals (CI). We adjusted our analyses for concomitant antihypertensive use and 20 general risk factors associated with fractures.
Results: Patients who were current users of beta-blockers [adjusted (adj.) OR 0.79, 95% CI 0.70–0.89], or thiazides (adj. OR 0.91, 95% CI 0.84–1.00) had a decreased risk of hip fracture. Risk of vertebral fracture was reduced among patients who were current users of betablockers (adj. OR 0.66 95% CI 0.59–0.78), but not thiazides (adj. OR 0.99 95% CI 0.87–1.14). Among current users, a cumulative dose–response relationship between beta-blocker use and fracture risk was observed, yielding the strongest effect among patients in the highest dose category (adj. ORs 0.75, 95% CI 0.63–0.88 and 0.54,95% CI 0.43–0.68 for hip fracture and vertebral fracture, respectively). Furthermore, the strength of last daily dose was inversely associated with risk of hip and vertebral fracture respectively, yielding adj. ORs of 0.74 (95% CI 0.60–0.91) and 0.64 (95% CI 0.49–0.85) among patients in the highest dose tertile. Results were similar for exposure to selective and non-selective beta-blockers.
Conclusions: Daily and cumulative dose of beta-blockers are associated with a decreased risk of hip and vertebral fracture.

OC033

Mice Null for β1β2-Adrenergic Receptors Have Low Bone Mass and Architecture and are Resistant to Isoproterenol-Induced Inhibition of Bone Growth

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It is postulated that β-adrenergic stimulation reduces bone mass by decreasing bone formation. We previously reported that administration of isoproterenol, a β-adrenergic receptor agonist, significantly decreases vertebral trabecular and femoral mid-shaft cortical bone mass in mice. To further evaluate the role of the adrenergic system in the regulation of bone mass and architecture, we treated 6-week-old wild-

type (WT) and β1β2 adrenergic receptor knock-out (AR KO) mice with isoproterenol (ISO 10 mg/kg/day) or vehicle (VEH) for 8 weeks (n = 7–12/group). The skeletal response was evaluated by pDXA, μCT, and biochemical markers. At baseline, AR KO mice had lower total body bone mineral density (TB BMD) (−15%, $P < 0.0001$), lower serum osteocalcin (−14.1% $P = 0.01$) and higher serum TRACP5B (+24.5%, $P = 0.07$) than WT. In WT, ISO treatment diminished the gain in TBBMD (+21.2% vs. +28.7%, $P = 0.045$ for ISO vs. VEH) and decreased total body % fat (2% in ISO vs. 28% in VEH, $P < 0.0001$). In contrast, ISO did not affect TB BMD gain (+26.6% in ISO vs. +27.8% in VEH) or % fat (36% in ISO and VEH) in AR KO mice. In WT, ISO decreased cortical thickness (−8.1%, $P = 0.009$ vs. VEH), vertebral trabecular (Tb) BV/TV (−14.1%, $P = 0.022$) and Tb number (−14.0%, $P = 0.018$), whereas, bone micro-architecture was unaffected by ISO in AR KO. ISO had no effect on osteocalcin in either WT or AR KO mice, but significantly increased TRACP5B levels in both WT (+103.9%, $P = 0.003$ vs. VEH) and to a lesser extent in AR KO mice (+23.2%, $P = 0.005$; $P = 0.03$ for the difference between WT and KO). Altogether, these data demonstrate that β-adrenergic stimulation inhibits acquisition of bone mass and modeling of vertebral and cortical architecture during growth through β1 and/or β2 AR, mostly by promoting bone resorption. In addition, in absence of treatment, bone mass and architecture were lower in AR KO mice at baseline, suggesting that deficit of adrenergic signaling causes an imbalance in bone remodeling that may negatively influence the skeleton. Hence, pharmacological modulation of the adrenergic system for treatment of osteoporosis needs to be thoroughly evaluated.

OC034

Residual Lifetime Risk of Fractures in Elderly Men and Women

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The assessment of osteoporotic fracture risk is shifting from a relative risk-based to an absolute risk-based approach. The present study was undertaken to estimate the remaining lifetime risk of fracture in elderly men and women by age and bone mineral density (BMD) level.
Eight hundred and fifty-eight men and 1358 women aged 60+ years as at 1989 of Caucasian background who have participated in the Dubbo Osteoporosis Epidemiology Study had been followed for 15 years (1989–2004). During the follow-up period, fracture incurred by the individuals was recorded and confirmed by X-ray and personal interview. Traumatic and pathological fractures and fractures of skull, cervical spinal, or digits were excluded from the analysis. During the follow-up period, all-cause mortality was also recorded. Bone mineral density at the femoral neck was measured by dual energy X-ray absorptiometry (GE-LUNAR) at baseline. Residual lifetime risk of fracture from the age of 50 was estimated by the survival analysis with left truncation by using Cox's proportional hazards model, taking into account the competing risk of death.
From the age of 50, 180 men and 496 women had sustained at least one fracture, and 374 men and 465 women had died. The lifetime risk of any fracture for men and women from age 50 was 32% (95% CI: 23–36) and 54% (95% CI: 48–59), respectively. The lifetime risk of hip fracture was 17% (95% CI: 11–21%) for women and 6% (95% CI: 2–36%) for men; vertebral fracture: 25% (95% CI: 5–29%) for women and 11% (95% CI: 7–14%) for men. In men with osteoporosis, the short-term (10-year) risk and lifetime risks of fracture were 30% and 47%, respectively. The corresponding estimates for women were 31% and 70%. In men with BMD in the range of −2.0 to −2.4, the short-term risk was as high as 20%, and 24% in women.
These results suggest that the lifetime risk of any fracture from age 50 is one in three men and one in two women. These estimates provide a means to communicate the risk of fracture to an individual patient, and can be used to promote identification of high-risk individuals and target for treatment in the population.
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OC035

Incidence of Hip Fracture Over a 10-Year Period (1991–2000): Reversal of a Secular Trend

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Hip fractures in elderly are the main burden of osteoporosis in terms of mortality, disability, impairment of quality of life and costs. There is a large regional variation in hip fracture incidence. With the aging of the population, an increase of the number of fractures is expected. Furthermore, there is an increase of age-adjusted hip fracture incidence (secular trend) in many studies. Long-term data on secular changes in women and men within a defined community are still scarce. This study specifically examined over 10 years from 1991 to 2000 the age distribution of patients with hip fracture and secular changes in the incidence rates of hip fracture in women and men 50 years of age and older in a well defined community. All patients discharged with a diagnosis of a hip fracture (ICD-10 code: S72.0 and S72.1) were retrospectively identified from the computer records of the main hospital in Geneva, which is receiving 95% of hip fracture occurring in a well defined area. From 1991 to 2000, 4115 hip fractures were recorded in 2981 women and

822 men aged 50 years and over. Mean age (±SD) of patients with hip fracture was 83.1 ± 8.9 years in women and 78.3 ± 11.6 in men. The overall hip fracture incidence rate was 455 (95% CI: 439–471) per 100,00 person-years in women aged 50 years and over and 153 (95% CI: 143–163) in men. Female:male hip fracture incidence ratio was 2.99 (95% CI: 2.80–3.18, $P < 0.001$). Over the 1991–2000 period, the mean age of patients with hip fracture increased each year by 0.13 year in women ($P = 0.019$) and by 0.04 year in men (NS). Over this 10-year period, the age-adjusted incidence rate of hip fractures, standardized to the 2000 Geneva population, decreased significantly by 1.4% per year in women ($P = 0.021$) and remained unchanged in men (0.5% per year, $P = 0.66$). The female:male hip fracture incidence ratio significantly decreased by 0.07 per year ($P = 0.024$). In conclusion, despite an increase in the population at risk and an increase in the mean age of hip fractured women, there was a significant decrease in age-adjusted incidence in women but not in men. These results may suggest a reversal of the previously observed secular trend.

OC036

Cannabinoid Receptor 1 Knockout Mice Have Increased Bone Mass and are Protected from Ovariectomy-Induced Bone Loss

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We have recently shown that cannabinoid receptors are expressed in bone cells and that cannabinoid receptor antagonists inhibit osteoclast formation and bone resorption in vitro by promoting osteoclast apoptosis. Here, we investigated the physiological role of the endocannabinoid system in bone metabolism by conducting skeletal phenotyping of mice with targeted inactivation of type I cannabinoid receptors (CB1 KO). We found that adult CB1 KO mice had significantly higher BMD than wild type littermates at several skeletal sites (11% at the lumbar spine, 22% at the femur, and 26% at the proximal tibia; $P < 0.01$ to $P < 0.001$ between groups). As expected, ovariectomy of wild type mice resulted in a loss of up to 40% of trabecular bone at the proximal tibia, whereas CB1 KO mice were completely protected against ovariectomy-induced bone loss ($P < 0.01$ between genotype groups). Further in vivo studies showed that the AM251 – a pharmacological antagonist of CB1 receptors – prevented ovariectomy induced bone loss in mice by inhibiting osteoclastic bone resorption. We found that AM251 also inhibited osteoclast formation in vitro in a concentration dependent manner with 50% inhibition at 750 nM. In contrast, the endogenous cannabinoid receptor agonist anandamide reversed these effects and stimulated osteoclast formation in a concentration-dependent manner. Osteoclasts derived from CB1 KO mice were resistant to osteoclast inhibition mediated by CB1 receptor antagonists indicating a CB1-mediated effect. Furthermore, treatment with AM251 resulted in osteoclast apoptosis and prevented RANKL induced activation of several key osteoclast survival factors including c-Jun, c-Fos, and NFATc1. Our results show that the endocannabinoid pathway and the CB1 receptor in particular, plays a hitherto unrecognised role in the regulation of bone mass and identifies the CB1 receptor as key mediator of ovariectomy induced bone loss and novel molecular target for osteoclast inhibition. Therefore, CB1 receptor antagonists may represent a promising new class of anti-resorptive drugs for the treatment of osteoporosis and other bone diseases associated with increased osteoclast activity.

OC037

DKK Proteins Control Osteoblast Function Both In Vitro and In Vivo

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Wnt/β-catenin signaling plays an essential role in bone biology. Reduced Wnt signaling, as consequence of loss of function mutations in the Wnt co-receptor Lrp5, causes severe osteoporosis, while gain of function mutation in this same gene results in a high bone mass phenotype in both humans and mice. Wnt activity is tightly regulated by a number of secreted proteins including Dickkopfs (Dkks). By bridging LRP5/6 and Kremen, Dkks induce the internalization of the complex and therefore antagonize Wnt/β-catenin signaling. We have investigated the activity of Dkk1 and Dkk2 on osteoblast differentiation and bone formation both in vitro and in vivo. First, overexpression of Dkk proteins in rat primary osteoblasts results in complete inhibition of mineralized nodule formation. Interestingly, in these cells Dkk overexpression induced adipocyte differentiation as observed by red-oil staining. These data were further confirmed by using purified recombinant murine

Dkk proteins in M3CT3-E1 osteoblastic cells. In these cells, rmDkk proteins inhibit spontaneous cell mineralization as well as mineralization induced by morphogenic proteins including Sonic Hedgehog and BMP-2. In addition, rmDkk proteins reduce alkaline phosphate expression in MC3T3-E1 cells. Our data clearly demonstrate that Dkk proteins are able to antagonize osteoblast differentiation and matrix mineralization in vitro. To analyze the role of Dkk1 in vivo, we generated transgenic mice displaying a dkk1 insufficiency. While homozygous dkk1-deficient mice are embryonically lethal, heterozygous dkk1 deficient mice develop normally. However, dkk1+/− mice display an increased bone mineral density as well as an increased trabecular number as consequence of enhanced osteoblast activity. It is concluded that Dkk proteins regulate bone formation in vivo by antagonizing the LRP5 and Wnt-dependent activation of the β-catenin pathway in osteoblast differentiation.

OC038

Lithium—A Potential Bone Anabolic Treatment

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Background: Lithium has been shown to inhibit bone resorption and to interact with Wnt signaling, potentially pointing at bone-anabolic properties. We therefore studied the effects of lithium on fracture risk.
Design: Case-control study.
Subjects: Cases were all subjects with any fracture sustained during the year 2000 (n = 124,655). For each case, three controls (n = 373,962) matched on age and gender was randomly drawn from the background population. Adjustments were made for use of other psychotropic drugs (neuroleptics, antidepressants, anxiolytics/sedatives), psychiatric disease (manic depressive states, schizophrenia, other psychoses), and other confounders (alcoholism, previous fracture, etc.).
Results: In the crude analysis, there was a decreasing risk of any fracture with increasing dose of lithium. After adjustment for psychotropic drug use, the risk of any fracture was decreased (OR = 0.74, 95% CI 0.60–0.92 for 250–849 DDD, and 0.67, 95% CI 0.55–0.81 for ≥850 DDD of lithium). For Colles' fractures and spine fractures a significant decrease was seen with ≥850 DDD (OR = 0.57, 95% CI: 0.35–0.94 for Colles' fracture and 0.32, 95% CI: 0.11–0.95 for spine fractures). For hip fractures, a non-significant trend towards a decrease was seen, however, without a dose–response relationship. Adjustment for further confounders did not change the results.
Conclusions: Lithium was associated with a decreased risk of fractures potentially pointing at bone anabolic properties.

OC039

Allogeneic In Utero BMT Rescues the Mutant Phenotype in a Murine Model of Osteopetrosis

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Infantile autosomal recessive osteopetrosis (ARO) is a severe bone disease, due to osteoclast malfunction, that causes many severe abnormalities as macrocephaly, deafness, blindness, hepatosplenomegaly, and severe anemia, beginning in early infancy or in fetal life. Deafness and blindness are thought to be the effects of bone pressure on nerves. In more than 50% of cases, the defect is in the TCIRG1 gene, coding for the a3 subunit of the acidifying proton pump of the osteoclast. The only available treatment is bone marrow transplantation (BMT), which needs an HLA-matched donor and a conditioning regimen in order to avoid graft versus host disease (GVHD). However, postnatal BMT does not usually cure all the stigmata of the disease and even when engraftment is achieved, neither the growth impairment nor the cranial nerve defects are rescued. These considerations make ARO the best candidate to verify whether the in utero BMT approach could benefit patients who need to be treated early during fetal life. We exploited a spontaneous mutant mouse strain (*oc/oc*) whose underlying genetic defect and phenotype are identical to TCIRG1-dependent human ARO patients. Affected mice die early in life at 3 weeks of age with severe anemia, high bone density and complete absence of tooth eruption. We used this mouse model as recipient of in utero bone marrow transplantation (IUT). BM cells from a GPF positive outbred mouse strain (CD-1) were used as cell donors, simulating what occurs in patients who do not have HLA-matched donors. From thirty-eight females treated by in utero injection at 14.5 p.c (5×10^6 BM cells/per fetus), 14 *oc/oc* mice were born, five of these were alive after 4 weeks of life, showing a drastic improvement of the phenotype. Dramatic rescue of the phenotype is obtained by both permanent and transient engraftment, suggesting that differentiation of donor hematological progenitors along the osteoclast lineage in the critical perinatal period is sufficient to prevent most of the skeletal changes which are the basis of the severity of ARO. Moreover, all the mice examined in our study did not show any sign of GVHD, which represents the major complication in postnatal BMT. The results described here suggest that in utero BMT in ARO patients could greatly

improve and even normalize their clinical picture and could also of benefit in other diseases in which severe symptoms develop in fetal life.

OC040

Identification of a Quantitative Trait Locus for Bone Mineral Density on Human Chromosome Xp22 by Interspecies Synteny Mapping

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Bone mineral density (BMD) is a highly heritable trait, and is an important clinical predictor of osteoporotic fracture risk. Despite intensive efforts, most of the genes responsible for BMD regulation remain to be identified. Here, we report the use of a cross-species strategy to identify such genes, proceeding from quantitative trait locus (QTL) mapping in inbred mice to association mapping of the syntenic region in the human genome. We identified a QTL on mouse chromosome X for post-maturity change in BMD by linkage analysis in an experimental cross of SAMP6 and AKR/J mice (LODmax = 4.0, $P < 0.001$). We conducted association mapping of the corresponding syntenic region on human chromosome Xp22 by DNA pooling using a case-control design. We analysed 76 single nucleotide polymorphisms (SNP) from the candidate region in DNA pools prepared from 200 individuals with lumbar spine BMD values in the bottom decile of a population-based study of 3224 British women and compared the allele frequencies in these subjects with those in DNA pools prepared from 200 individuals with lumbar spine BMD values in the top decile. SNPs associated with BMD in the first set of pools were examined in a replication set of pools from the same population. A region ~15 cM from the "p" telomere of the human X chromosome showed significant association in both pool sets, based on differing allele frequencies in the low and high BMD pools, for two SNPs within the PIRIN gene (rs234494, pool-set-1 $P = 0.037$ and pool-set-2 $P = 0.013$; rs234495, pool-set-1 $P = 0.043$ and pool-set-2 $P = 0.024$). PIRIN encodes a highly conserved nuclear protein, which interacts with the transcription factor NF1A, but its function is largely unknown. Association of these two PIRIN SNPs with lumbar spine BMD was also confirmed in a subset of 1046 women randomly drawn from the main study cohort (rs234494, $P = 0.003$; rs234495, $P = 0.005$). For this sample, each T allele at rs234494 conferred an average decrease in lumbar spine BMD of 0.27 Z-score units after correcting for confounding factors such as age, weight, menopausal status, and HRT use. Our study illustrates the utility of using interspecies synteny to identify and refine QTLs, and implicates PIRIN as a novel candidate gene for the regulation of BMD in women.

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OC041

C-SRC Inhibition Decreases Breast Cancer-Induced Lethality and Incidence of Experimental Bone Metastases in Balb nu/nu Mice

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Breast carcinomas are prone to metastasize to bone. We investigated whether modulation of the proto-oncogene c-Src could affect survival and bone metastasis incidence in vivo. Balb-c nu/nu mice were subjected to intracardiac injection of the human breast cancer parental cell line MDA-MB231, or of MDA-MB231 cells stably transfected with c-Src wild type (MDA-SrcWT) or c-Src kinase-dead and dominant negative (MDA-SrcDN) constructs. Similar progression and incidence of cachexia was observed in animals injected with parental or MDA-SrcWT or parental cells. In contrast, no cachexia was noticed in mice injected with MDA-SrcDN cells. Lethality appeared earlier and was higher in mice injected with parental or MDA-SrcWT cells, while all animals inoculated with MDA-SrcDN cells survived for the time frame of the experiments (38 days). X-ray analysis showed 57% and 71% incidence of bone metastases in parental- and in MDA-SrcWT cell-injected mice, respectively, while in MDA-SrcDN cell-injected mice, the onset of bone metastases was delayed, reaching only 25% incidence. To assess any therapeutic relevance of our findings, we treated parental cell-injected mice with 100 mg/kg/day of the c-Src inhibitor CGP76030, and observed an analogous delayed and reduced cachexia and lethality compared to control animals. In untreated mice, osteolytic lesions appeared earlier and progressively increased up to 57% incidence, while in c-Src inhibitor-treated mice, the incidence was again not higher than 25%. In vitro, c-Src inhibition caused a concentration- and time-dependent reduction of MDA-MB231 cell proliferation, adhesion, spreading, and migration. Furthermore, it also significantly inhibited bone marrow osteoclast formation and bone resorption. However, when conditioned media (CM) from parental, MDA-SrcWT, and MDA-SrcDN cells were added to bone marrow cultures, no differences in stimulation of osteoclast formation was observed, suggest-

ing that modulation of c-Src in cancer cells did not affect synthesis and/or release of osteoclastogenesis factors. We also noted that MDA-MB231 CM increased endothelial cell activity, with again no changes occurred upon c-Src modulation. In conclusion, we demonstrated a role for c-Src in the development of in vivo bone metastases and in vitro breast cancer cells, and identified c-Src as a pharmacological target for the treatment of experimental bone metastases, which could also reduce cachexia and lethality.

OC042

The Effect of AZD0530, a Highly Selective Src Inhibitor, on Bone Turnover in Healthy Males

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AZD0530 is a highly selective, dual-specific, orally available small molecule inhibitor of Src kinase and Bcr-Abl. Src kinase plays an essential role in RANK-mediated osteoclast activation, and may also inhibit osteoblast activity. To examine the effect of AZD0530 on osteoclast and osteoblast activity, we have investigated the changes in markers of bone turnover in response to AZD0530 treatment in a multiple ascending dose study in healthy male volunteers (ages 18–55 years).The study comprised placebo and 4 dose levels given to cohorts each of 12 volunteers. Volunteers in each cohort received a single dose of 60–250 mg AZD0530 (n = 9) or placebo (n = 3) repeated, 7 or 10 days later, as multiple daily doses for 10 or 14 days. Serum and second morning urine were collected after overnight fast prior to and 24 and 48 h after the single dose and the final dose. Further samples were collected at the follow-up visit 10–14 days after the last dose in the cohorts receiving the two highest doses. Resorption markers measured were serum cross-linked C telopeptide of type I collagen (sCTX), urinary cross-linked N telopeptide (NTX), corrected for creatinine, and serum tartrate resistant acid phosphatase 5b (TRAP 5b). Formation markers measured were procollagen serum type I N terminal propeptide (PINP) and bone-specific alkaline phosphatase (Bone ALP). Provisional PK-PD modeling suggests the relationship between AZD0530 plasma concentrations and CTX suppression is well described by an inhibitory sigmoid E-max model. Levels of sCTX and uNTX/Cr but not TRAP 5b appear to rise back rapidly towards baseline following cessation of dosing with the two highest doses. PINP tended to decrease after cessation of treatment, possibly reflecting the temporal difference in resorption and formation during bone remodeling. We conclude that suppression of Src kinase activity inhibits osteoclast-mediated bone resorption. The potential effect of Src inhibition on markers of bone formation warrants further investigation. AZD0530 may have therapeutic benefit in treating osteoclast-driven metastatic bone disease and osteoporosis.

Table
Mean (95% CI) percentage 24 h after final dose

Dose of AZD0530	sCTX	uNTX/Cr	TRAP 5b	PINP	Bone ALP
Placebo	17 (−7, +49)	+5 (−23, +44)	+9 (0, +17)	+17 (−1, +38)	+3 (−5, +11)
60 mg	−24 (−41, −1)	−3 (−32, +37)	+2 (−7, +11)	+3 (−14, +24)	+5 (−4, +15)
125 mg	−55 (−65, −43)	−39 (−56, −15)	−13 (−19, −5)	+23 (+3, +46)	+10 (−1, +19)
185 mg	−71 (−77, −63)	−69 (−78, −56)	−14 (−21, −6)	+33 (+11, +58)	−3 (−11, +5)
250 mg	−88 (−91, −84)	−67 (−77, −53)	−11 (−18, −3)	+13 (−6, +35)	+9 (0, +19)

P001-Su

Gender Differences in Trabecular and Cortical Microstructure of the Distal Radius Shown in an Elderly Population as Assessed by High-Resolution 3D-pQCT

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Introduction: Fractures in the region of the distal radius are amongst the most common in humans and their incidence is increasing. Due to the differences of osteoporosis in women and men, it is of importance to better understand gender differences in bone microstructures. Structural parameters such as trabecular thickness and trabecular number often predict better the mechanical properties of cancellous bone than bone mineral density alone. Therefore, 3D high-resolution peripheral quantitative computed tomography (3D-pQCT) was employed to quantify structural parameters non-destructively.
Methods: 166 human cadaveric forearms (81 women and 85 men) were measured with high-resolution pQCT. From these measurements, two elderly population groups of 42 women and 42 age-matched men (83 ± 7 years) were selected. PQCT was performed using a new generation in vivo 3D-pQCT scanner (Scanco Medical, Switzerland) providing an isotropic nominal resolution of 93 μm. The measurements were performed at a site that corresponded to 20% of the forearm length. Five different regions of interest were chosen, each representing 4% of the analyzed site. Subsequently, direct 3D morphometry was used to compute bone volume (BV), bone volume density (BV/TV), trabecular thickness (Tb.Th), number (Tb.N), and separation (Tb.Sp) in these five regions.
Results: Gender differences of the two groups in trabecular and cortical microstructures were investigated. Men had a

factor of two higher trabecular BV/TV than women in the most distal region decreasing by a factor of two in proximal direction whereas BV/TV in the women group stayed almost constant over these regions. Tb.N decreased and Tb.Sp increased in both groups in proximal direction. Where males demonstrated constant Tb.Th throughout all regions, women showed a 35% increase in thickness from region 1 (distal) to 5 (proximal). Cortical properties showed similarly interesting differences between men and women; with values for females always being lower.
Conclusion: From the differences found between female and male bone microstructures, we expect different gender-related mechanisms in distal radius fractures. We hypothesize that where the critical structure in men seems to be the cortical bone, strong trabecular bone appears to be necessary in all regions to maintain adequate bone strength in women.
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P002-Mo
Fracture Risk Among Women with the Lowest Bone Turnover During 10 Years of Alendronate Treatment
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Alendronate (ALN) reduces the rate of bone turnover to within the premenopausal range, increases BMD, and reduces the risk of both vertebral and nonvertebral fractures. We previously reported that fracture incidence among ALN-treated women with the lowest on-therapy bone turnover was similar to, and perhaps lower than, that of women with higher levels of turnover during up to 4.5 years of follow-up in the Fracture Intervention Trial (FIT) (1). The current analysis extends those observations for a total of up to 10 years follow-up among women who continued to receive ALN (N = 399) in the FIT Long-term EXtension (FLEX), a 5-year extension of FIT. We used a per-protocol analysis of two turnover markers, urine N-telopeptides (NTX) and serum bone specific alkaline phosphatase (bone ALP), obtained at the 12-month visit in FLEX, because the full effect of ALN on turnover markers occurs within a few months and is subsequently maintained. The number of women with new nonspine and morphometric vertebral fractures during the final 5 years of ALN treatment (during the FLEX study) was calculated. The sample size in the low turnover group is small; hence, statistical comparisons were not performed. The fracture incidence in the 20% of ALN-women with the lowest NTX levels at the 12-month FLEX visit was similar to or lower than that observed in women with higher levels of bone turnover. Among the 87 women with the lowest 20% of NTX values (NTX < 12 pmol BCE/μmol Cr), 13 women (14.9%) had nonspine fractures compared to 53 women with nonspine fracture (16.9%) among the 313 women with NTX > 12 pmol/μmol Cr. Three women (3.5%) with the lowest 20% of NTX values had new vertebral fractures compared to 20 women (6.4%) among those with higher NTX levels. Results were similar with bone ALP. Cox models using turnover levels as a continuous predictor variable did not detect any significant relationship between turnover levels at the 12-month visit and fracture risk during FLEX. Thus, there is no evidence of an increase in risk among ALN-treated women with the lowest NTX levels during up to 10 years of ALN treatment.

[1] Bauer DC, et al. JBMR 2004;19(8):1250–8.

P003-Tu
Fracture Index Validation in a Population-Based Sample of Swiss Elderly Women
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Background: Due to the magnitude of osteoporosis and its related morbidity and mortality, the identification of high-risk women for fracture is essential. The Fracture Index (6 questions), an assessment tool with or without DEXA measurement, was shown to be predictive of hip fracture, as well as vertebral and nonvertebral fractures in post-menopausal women [D. Black et al. Osteoporosis Int. 2001]. The Swiss Evaluation of the Methods of Measurement of Osteoporotic Fracture Risk (SEMOF) study is a prospective multicenter study which compares 3 QUS devices for the assessment of fracture risk in a population-based sample of Swiss elderly women. The aim of the study was to validate the Fracture Index in the SEMOF study.
Method: Among the 7062 women age 70 years or older (75.2 ± 3.1), 607 clinical fractures were reported. According to the Fracture Index scoring, women were divided in quartiles. A linear increase of fracture risk was assumed during the follow-up (conservative approach). The follow-up duration varied from 976 to 1111 days, according to the quartile group and the type of fracture.
Results: 1021, 2088, 1983, and 1970 women were included, respectively, in quartile one to four. The total number of clinical fractures was 56, 146, 174, and 231, respectively. The annualized incidence of clinical fracture per 1000 women increased from 18.5, 24.7, 32.0, to 43.9 with quartile one to four. The total number of hip fracture was 2, 11, 25, and 42 corresponding to an annualized incidence per 1000 women of 0.6, 1.8, 4.4, and 7.6. The corresponding annualized incidence of clinical nonvertebral fracture was 16.8, 21.6, 27.6, and 36.9.
Conclusion: The Fracture Index is a very simple tool that identified women at high risk for fracture, and may defined

a threshold for treatment. The risk of fracture observed in our Swiss cohort was lower than in the SOF or EPIDOS studies. It may be in part explained by the shorter follow-up period or by the younger age of women. Another explanation may be the difference of fracture risk related to different population or country.

P004-Su

Ten-Year Absolute Risk of Osteoporotic Fractures According to BMD *T* Score at Menopause. The Danish Osteoporosis Prevention Study

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Background: International recommendations highlight the importance of absolute fracture risk in interpreting BMD results and establishing intervention thresholds. The best estimates of long-term risk have been derived by combining relative risks from meta-analyses with US normative BMD-data and Swedish fracture incidence records. We have compared the Kanis risk algorithm with the numbers of fractures observed in the first 10 years of the DOPS Study.
Study population and methods: We analyzed DXA of the spine and hip from 862 women, who participated in the non-HRT arms of the study and had not received HRT, bisphosphonates, or rlx. We collected verified reports of incident fractures at each visit. We focused on fractures of the hip, forearm, shoulder, and the spine to provide risk estimates comparable with those in the Kanis algorithm. Asymptomatic radiographic vertebral fractures were not included. Risk calculated using logistic regression analysis. Age at inclusion: 50.7 ± 2.9 years. Participants were not selected by risk factors.
Results: 79 women (9.1%) sustained relevant fractures. The risk depended on baseline *T* score of the total hip [Exp(B) = 0.756 for each unit increase in *T* score, $P < 0.05$], femoral neck [Exp(B) = 0.740, $P < 0.05$] and spine [Exp(B) = 0.769, $P = 0.01$]. Fracture risk was higher than expected from the Kanis algorithm at all *T*-score levels. The difference was greatest for participants in the higher range of *T* scores, where fracture risk was more than twice that estimated by the algorithm.
Conclusion: In this cohort of healthy women, examined in the first year or two after menopause, 10-year fracture risk was higher at each level of BMD *T* score than expected from the model derived by Kanis et al. Inclusion of HRT users in the cohorts used may have led to higher BMD values and lower fracture risk in the model. The present longitudinal single-cohort data can be used directly in estimating absolute fracture risk in untreated women from BMD at menopause.

Table
Ten-year fracture risk (forearm, hip, shoulder, spine)

Total hip *T* score	Obs. risk (DOPS)	Expec. risk (Kanis)
+1	6.4%	2.4%
+0.5	7.4%	3.0%
0	8.4%	3.8%
−0.5	9.5%	4.7%
−1	10.8%	5.9%
−1.5	12.1%	7.4%
−2	13.7%	9.2%
−2.5	15.4%	11.3%

DOPS: Women aged 50.7 ± 2.9 years, no HRT.

P005-Mo

Intravenous Ibandronate Injections are at Least as Effective and Similarly Well Tolerated as Daily Oral Ibandronate in Postmenopausal Osteoporosis: 1-Year Results from DIVA

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Objectives: Oral bisphosphonates may be unsuitable for some patient groups in postmenopausal osteoporosis (PMO). A proven intravenous (i.v.) bisphosphonate could be of significant utility for such patients. Ibandronate, a potent, nitrogen-containing bisphosphonate with significant antifracture efficacy(1), can be effectively and safely administered as a rapid (15–30 s) i.v. injection with an extended between-dose interval(2). The ongoing DIVA study is establishing the efficacy and safety of novel intermittent i.v. ibandronate regimens in PMO.
Methods: DIVA is a 2-year, randomized, double-blind, double-dummy, phase III, non-inferiority study involving 1395 postmenopausal women (aged 55–80 years; ≥5 years since menopause) with osteoporosis (lumbar spine [L2–L4] BMD *T* score <−2.5 and ≥−5). Participants are receiving daily calcium (500 mg) and vitamin D (400 IU), plus either 2 mg once every 2 months (q2mo) or 3 mg once every 3 months (q3mo) i.v. ibandronate injections or an established active comparator [2.5 mg daily oral ibandronate; 3-year vertebral fracture risk reduction: 62%(1)].
Results: At 1 year, increases in lumbar spine BMD (primary study endpoint) of 5.1%, 4.8%, and 3.8% were observed in the 2 mg q2mo ($n = 355$), 3 mg q3mo ($n = 368$), and daily ($n = 381$) groups, respectively. Non-inferiority and superiority ($P < 0.001$) versus the oral regimen were demonstrated for both i.v. regimens. Greater increases in total hip, femoral neck and hip trochanter BMD were also observed in the i.v.

arms versus the oral arm. Pronounced and similar reductions in the biochemical marker of bone resorption serum CTX were obtained in all treatment arms at 1 year (−64.6% in the 2 mg q2mo group vs. −58.6% in the 3 mg q3mo group vs. −62.6% in the daily group). A similar overall incidence of adverse events (AEs; 76–81%), related AEs (33–44%) and related AEs leading to withdrawal (4.5–6.6%) was observed in the i.v. and oral ibandronate groups. A low and similar incidence of related serious AEs was also reported (<1% all arms; $n = 7$), with most events considered unrelated to treatment.

Conclusions: I.v. ibandronate injections (2 mg q2mo, 3 mg q3mo) are at least as effective and similarly well tolerated as an established daily oral ibandronate regimen in PMO. I.v. ibandronate injections are likely to be of significant utility in patients for whom oral bisphosphonate are contraindicated or unsuitable.

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[2] Adami S, et al. Bone 2004;34:881–9.

P006-Tu

Strontium Ranelate Improves Bone Microarchitecture and Intrinsic Bone Quality

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Strontium ranelate reduces the risk of vertebral and hip fracture in postmenopausal osteoporotic women. We previously reported that strontium ranelate treatment increases ultimate strength without affecting stiffness in adult female rats. We investigated the specific role of various bone strength determinants in specimens of rats treated over 26 months with strontium ranelate at a daily dose of 0 (control) or 900 mg/kg ($n = 12$ per group). The load deflection curve obtained by axial compression of vertebral body provided ultimate strength, stiffness, total and plastic energies values. Three-dimensional micro computed tomographic analysis investigated microarchitecture providing trabecular bone volume, trabecular number, thickness and spacing, and cortical thickness values. Intrinsic bone tissue quality (elastic modulus, hardness and dissipated energy) was evaluated by nanoindentation test performed at the trabecular nodes level under either hydrated and dry conditions. Results are means ± SEM, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ vs. control by Student's t test. Strontium ranelate significantly increased vs. control group ultimate strength (+23%, $P < 0.05$), total energy (+71%, $P < 0.05$), plastic energy (+143%, $P < 0.01$) and microarchitecture parameters. Moreover, at the tissue level, strontium ranelate significantly increased elastic modulus, hardness and dissipated energy vs. control in hydrated conditions. The increased energy to failure achieved with strontium ranelate was essentially due to the increment of plastic energy suggesting that bone formed under strontium ranelate treatment can withstand greater deformation before fracture with similar elastic properties as untreated normal bone. The positive effect of strontium ranelate on bone strength could thus be related to improvements of trabecular and cortical microarchitecture and of intrinsic bone tissue quality. By a step-way regression analysis, intrinsic bone tissue quality is predominant in predicting bone strength. These results show for the first time a direct action of strontium ranelate on bone tissue quality, which could explain part of the reduction in fracture risk in postmenopausal osteoporotic treated-patients.

	BV/TV (%)	Cortical thickness (mm^{-1})	Elastic modulus (GPa)	Hardness (GPa)	Dissipated energy (mN*nm)
Control	52.5 ± 3.6	3.63 ± 0.04	12.4 ± 0.3	0.46 ± 0.02	4142 ± 146
strontium ranelate	73.3 ± 1.5**	4.46 ± 0.07**	14.2 ± 0.4**	0.51 ± 0.02*	4677 ± 160*

P007-Su

An Individualized Model of Cost-Effectiveness of Bisphosphonates in Elderly Women

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Clinical treatment guidelines often include cost-effectiveness data based on group averages, restricting its generalizibility to those with below- or above-average risk. An individual patient-based pharmaco-economic model on the cost-effectiveness of bisphosphonates was developed.

Data were obtained from the UK The Health Improvement Network research database of general practitioners, comprising a general population of women >50 years ($N = 350{,}000$). Mortality and hip, vertebral, and other osteoporotic fracture risks for each individual were estimated by age, sex, body mass index, smoking, and fracture history and other clinical risk factors. The vertebral risks were standardized to those of the EPOS study (1/3 of the vertebral fractures were considered to be clinically symptomatic). UK costs on medication (£284 per year) and direct costs of fracture were obtained from a UK national report (NICE) and discounted annually by 6%. Using the individual mortality and fracture risks, outcomes were simulated over a 10-year period, comparing presence and absence of bone protection. The assumption was that bisphosphonates were given for 5 years and reduced (hip) fractures in all women by 30–40% (with linear efficacy offset over remaining 5

years). Data on the distribution of bone mineral density in the UK population were obtained from Holt et al.
The median 5-year hip fracture risk among the women was 0.3% at age 60 and 2.8% at age 80. Using an acceptability cost-utility ratio of £30k per QALY (as used by NICE), it was found that bisphosphonates were cost-effective in women with a fracture history who had a 5-year hip fracture risk of 1.2% or higher (3.8% in those without fracture history). At age 60, this risk was achieved in women with fracture history who had risk factors with a relative rate of 3.5 compared to women with the median risk (relative rate of 11.2 in those without fracture history). Including bone mineral density in the risk assessment, women at age 60 with a fracture history and a *T* score of −2.5 had a fracture risk higher than required for cost-effectiveness (cost per QALY gained of £29k; at age 80, £5k).
An individual-based pharmaco-economic model can help to identify individual patients, rather than broad populations.

P008-Mo
In Vivo Monitoring of Bone Formation by Near-Infrared Fluorescence Mediated Tomography
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Detection of bone formation rates by histomorphometric evaluation of fluorochrome markers in bone is slow. We demonstrated recently that we can monitor local bone forming activity rapidly in vivo by near-infrared fluorescence (NIRF) imaging [1], using a NIRF bisphosphonate derivative homing to bone and binding to hydroxyapatite, as suggested previously [2]. In the present studies, we validated this method further using a novel tomographic detection method. We delivered sc. twice daily 100 nM hPTH or vehicle onto the calvaria of 4-, 5-, and 6-month-old OF1/IC mice (n = 6/group) for 5 days followed by an i.v. administration of 0.1 mg/kg Cy5.5 labeled pamidronate. NIRF images of the calvaria of the anesthetized mice were recorded 4 h after administration of the NIRF bisphosphonate derivative. A time-domain small animal optical imager (GE eXplore Optix) was used allowing for calibration of Cy5.5 concentration. Depth measurements confirmed that the NIRF signal came from within the calvarial bone. An age-related decline of NIRF signal intensity was observed in vehicle and PTH treated mice consistent with decreasing bone formation with increasing age. PTH induced significant increases in signal intensity. The absolute level of NIRF signal was highest in 4-month-old PTH -treated animals, while fold-induction above vehicle treated controls was highest in 6-month-old mice. Comparison with classical histomorphometric evaluation of fluorochrome marker based bone formation rates demonstrated excellent agreement between the two methods (1.6-fold PTH-stimulated bone formation rates by histomorphometric readout correspond to 1.7 fold increases in NIRF signal in 4-month-old mice). We proceeded to testing the effect of a resorption inducer on NIRF signal intensity, since it has been claimed that bisphosphonates home to bone matrix under osteoclasts. To this end, we applied in the same experimental set up daily s.c. the retinoid Ro 13 6298 or vehicle for 4 days to induce severe bone resorption as described recently [3]. A positive control group receiving PTH showed increased NIRF signal intensity, while animals treated with retinoid presented even with a slight decrease compared to vehicle control. Taken together these results provide further evidence that bone formation responses can be quantified rapidly and selectively in vivo by the use of this technology. [1] Gremlich et al. 2004 JBMR 19 1069; [2] Zaheer et al. Nat Biotech 19 2001; [3] Kneissel et al 2005 Bone in press.

P009-Tu
Serum Cathepsin K Levels Reflect Osteoclastic Activity in Women with Postmenopausal Osteoporosis and Patients with Paget's Disease
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Cathepsin K, a cysteine protease, plays an essential role in osteoclast-mediated degradation in cleaving both helical and telopeptide regions of collagen type I. Recently an immunoassay to quantify cathepsin K in serum has been developed. No data are available assessing cathepsin K as a marker of bone resorption in patients with metabolic bone diseases. The aim of this study was to assess the performance of serum cathepsin K in comparison to known biochemical markers of bone turnover. The study cohort consisted of 40 healthy controls (13 premenopausal women [age, 30.6 ± 6.6 years (mean ± SD)]; 11 postmenopausal women [64.1 ± 8.3 years]; 16 men [41.6 ± 12.4 years]), 21 women with postmenopausal osteoporosis (66.1 ± 7.9 years) and 10 patients with Paget's disease of bone (67.1 ± 11.6 years). All patients were started on bisphosphonate treatment (oral or IV) and were followed prospectively over 6 months with serum collection at baseline, after 1 and 6 months. Circulating cathepsin K levels were determined using an enzyme immunoassay (Biomedica, Austria). Serum carboxyterminal cross-linked telopeptide of type I collagen (bCTX) and bone-specific alkaline phosphatase (BAP) were assessed as a markers of bone resorption and bone formation. Serum cathepsin K levels did not differ between the three groups of healthy controls (mean ± SD, 3.1 ± 1.7 pmol/L). In contrast, women with postmenopausal osteoporosis (11.3 ± 13.1 pmol/L, P = 0.01) and patients with

Paget's disease (6.2 ± 4.4 pmol/L, $P = 0.01$) had significantly higher cathepsin K levels at baseline as compared to controls. In postmenopausal osteoporotic women, bisphosphonate treatment resulted in a significant decrease in serum cathepsin K ($P < 0.005$). Cathepsin K levels reached its nadir after 1 month ($P = 0.01$ vs. baseline) with no further change thereafter. Mean % changes in serum levels after 1 and 6 months were −26.7% and −37.8% for cathepsin K, −34.4% and −59.8% for bCTX, and −6% and −31.6% for BAP, respectively. In patients with Paget's disease (baseline BAP, 73.3 ± 50.4 U/L), cathepsin K tended to decrease during bisphosphonate therapy (mean % change after 1 month: −21.9%). In conclusion, circulating cathepsin K levels seem to reflect osteoclastic activity in patients with postmenopausal osteoporosis and Paget's disease. Its changes during bisphosphonate treatment to a similar extent as serum markers of bone resorption may indicate, that cathepsin K levels could potentially be used for clinical assessment of metabolic bone diseases.

P010-Su

Adiponectin Increases Bone Mass in Mice by Suppressing Osteoclastogenesis and Activating Osteoblastogenesis

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Adiponectin is an adipose-derived hormone discovered by our group from human fat cDNA. It exhibits various biological functions, such as increasing insulin sensitivity, protecting hypertension and suppression of atherosclerosis, liver fibrosis, and tumor growth. Here, we report the role of adiponectin on bone metabolism. C57BL/6J mice were treated with adenovirus expressing lacZ or adiponectin, and their bones were analyzed by three-dimensional micro computed tomography. Adiponectin-adenovirus treatment increased bone mass especially in trabecular bone, accompanied by decreased number of osteoclasts and decreased levels of plasma NTx, a marker of bone resorption. In vitro studies showed that adiponectin also inhibited M-CSF- and RANKL-induced differentiation of mouse bone marrow macrophages and human CD14-positive mononuclear cells into osteoclasts. Adiponectin also suppressed the bone-resorption activity of osteoclasts derived from human CD14-positive mononuclear cells. Furthermore, adiponectin enhanced mRNA expression of alkaline phosphatase and mineralization activity of mouse MC3T3-E1 osteoblasts. Our results indicate that a fat-derived hormone, adiponectin, exerts an activity to increase bone mass by suppressing osteoclastogenesis and activating osteoblastogenesis, suggesting that adiponectin manipulation could be therapeutically beneficial for patients with osteopenia.

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P011-Mo

PTHR1 Polymorphisms and their Role in Variation in Bone Mineral Density

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Background: The chromosome 3p21 region, in which PTHR1 is encoded, has been linked with bone mineral density (BMD) variation. Mutation in the PTHR1 gene is known to cause Jansen-type metaphyseal dysplasia and Blomstrand chondrodysplasia. We sought to investigate association of PTHR1 polymorphisms with BMD in families in which we have previously demonstrated linkage of chromosome 3p21 with BMD (1), and to confirm that finding in a larger data set.

Methods: The PTHR1 gene including its 14 exons, their exon–intron boundaries, and 1500 bp of its promoter region were screened for polymorphisms by dHPLC and sequencing in 36 osteoporotic cases. 11 SNPs, one tetranucleotide repeat and one tetranucleotide deletion were identified. These were genotyped in our initial chromosome 3p21-linked cohort of 168 families ascertained with probands with extreme low BMD ($z < -2.0$, $t < -2.5$). A further cohort of 472 families ascertained with low but less extreme BMD ($z < -1.5$) was genotyped for 5 informative SNPs (minor allele frequency >5%) and one tetranucleotide repeat. The findings were analyzed as two separate groups and combined. The total cohort consisted of 1281 men (39%) and 1978 women (61%). Total association between PTHR1 polymorphisms and LS and FN raw BMD measures corrected for age, gender, and height was tested using the program QTDT.

Results: In our initial cohort, strong association was noted between FN BMD and alleles of a known functional tetranucleotide repeat in the PTHR1 U4 promoter region (5 repeat allele $P = 0.0019$, 6 repeats $P = 0.0037$). In the follow-up cohort and in the total data set, we confirmed association with variation in BMD, although at the LS (5 repeat allele, follow-up cohort = 0.05, total data set $P = 0.04$). No association was observed with height in either data set.

Conclusions: The strong positive association findings in our initial data set support a role for the U4 tetranucleotide repeat of PTHR1 in BMD variation in population variation in BMD, which is partially supported in the follow-up screen. A significant role for this polymorphism in osteoporosis risk is further supported by previous data demonstrating that the same alleles of this polymorphism affect height, bone resorption, and PTHR1 transcription (2).

References:

[1] Duncan et al. J Bone Miner Res. 1999;14:1993–1999.
[2] Minagawa et al. J Clin Endocrinol Metab 2002;87: 1791–1796.

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P012-Tu

PTHrP Expression in Cartilage Cells is Regulated by the Hedgehog Signaling Molecule, GLI2

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Parathyroid hormone-related peptide (PTHrP) plays an important physiological role in the regulation of chondrocyte proliferation in the growth plate. Its expression is controlled by Indian hedgehog (Ihh), and both Ihh and PTHrP null mutant mice have overlapping growth plate abnormalities, with premature differentiation and decreased proliferation of growth plate chondrocytes. Although these data suggest that Ihh controls PTHrP expression in cartilage cells, the mediators responsible are unknown. Hedgehog signaling is mediated by the Gli family of transcriptional regulators and Gli2 null embryos exhibit increased hypertrophic chondrocytes and decreased PTHrP expression (Miao et al., 2004), suggesting that Gli2 has an important and non-redundant role in chondrocyte regulation. We therefore reasoned that Gli2 may regulate PTHrP expression in chondrocyte cell lines. To examine this we co-transfected the growth plate chondrocyte cell line TMC-23 with various Gli expression constructs and a 1.1-kb PTHrP promoter construct containing the P2 and P3 promoters (Cataisson et al., 2004) linked to a luciferase reporter. We found that Gli2 specifically increased PTHrP promoter activity approximately 4-fold in these cells, while other Gli family members had no effect on PTHrP promoter activity. A Gli2 construct, which acts as a dominant negative by blocking Gli2 DNA-binding, decreased basal PTHrP promoter activity. Furthermore, Gli2 siRNA duplexes that specifically decrease Gli2 expression by >90% dramatically blocked the Gli2-stimulated PTHrP promoter activity, suggesting that Gli2 is a physiologic regulator of PTHrP expression in cartilage cells. We next analyzed the PTHrP promoters for consensus Gli binding sites. The site with the closest homology was a 6/9 match, which by EMSA did not bind Gli2, indicating that the effects of Gli2 on the PTHrP promoter are likely indirect. Furthermore, we used a full-length (4 kb) PTHrP promoter luciferase construct that contains the P1 promoter region in addition to the P2 and P3 promoters. There was no additional effect of Gli2 on the 4-kb promoter over the response observed with the 1.1-kb promoter. The effect of Gli2 on the PTHrP promoter mapped to a region overlapping the 3′ region of P2 and the 5′ region of P3 using PTHrP promoter deletion constructs. Taken together, our data suggest that Gli2 is an important regulator of PTHrP transcription in growth plate chondrocytes.

P013-Su

Vitamin D Status and the Threshold for Secondary Hyperparathyroidism in the Longitudinal Aging Study Amsterdam (LASA)

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Vitamin D deficiency is common in the elderly. It causes secondary hyperparathyroidism and increased bone resorption, and contributes to osteoporosis and fractures. Controversy exists regarding the required serum 25-hydroxyvitamin D [25(OH)D] concentration. Traditionally, a serum 25(OH)D <25 nmol/l has been associated with vitamin D deficiency. More recently, serum 25(OH)D concentrations between 25 and 50 to 75 nmol/l have been considered mild vitamin D deficiency or vitamin D insufficiency. The latter has been estimated by the threshold serum 25(OH)D, i.e., the point where the serum concentration of parathyroid hormone (PTH) starts to rise. The aim of this study was to assess vitamin D status and the threshold serum 25(OH)D in the healthy elderly Dutch population. The study was done in the framework of the Longitudinal Aging Study Amsterdam (LASA), a follow-up study in a representative sample of the older Dutch population. Blood samples were obtained in 1320 community-dwelling men and women, aged 65 years and older on January 1, 1996. Serum 25(OH)D and serum PTH were measured by competitive protein binding assay and IRMA, respectively.

Serum 25(OH)D (mean ± SD) was 53.2 ± 24.1 nmol/l. It was <12.5 nmol/l in 1.6% of the participants, 12.5–25 nmol/l in 9.9%, 25–50 nmol/l in 36.9%, 50–75 nmol/l in

34.0%, and >75 nmol/l in 17.6%. Mean serum PTH decreased gradually from 4.7 pmol/l when serum 25(OH)D <25 nmol/l to 2.9 pmol/l when serum 25(OH)D >75 nmol/l. Serum PTH was at its lowest point when serum 25(OH)D >75 nmol/l, a threshold higher than expected.

This study shows that vitamin D deficiency and insufficiency are very common in the elderly. The high dietary calcium intake, as is usual in the Netherlands, does not protect against secondary hyperparathyroidism. General public health measures such as fortification of milk and milk products with vitamin D3 should be considered to improve vitamin D status in the elderly.

P014-Mo

Y2 Receptors and the Central Regulation of Cortical Bone: Reciprocal Activity to Leptin

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The hypothalamus is known to modulate potent bone anabolic signals. Lack of Y2 receptor [Y2R KO] and leptin [ob/ob] signaling in this brain region markedly increase cancellous bone volume and osteoblast activity. However, their effects on cortical bone are less clear. We examined the effect of Y2R deletion on cortical bone mass and osteoblast activity and compared these findings to leptin deficiency.

Male, 16-week-old wild type [wt], Y2R KO, ob/ob and Y2R/ob knockout mice were examined in groups of 5–8 animals. In addition, the hypothalamic nature and adult-inducibility of Y2 activity was assessed in Y2R floxed mice injected into the hypothalamus with adeno-virus expressing either CRE or GFP at 11 weeks of age. Cortical bone was examined by DXA [BMC mg, BMD mg/cm^3] in isolated whole femora, in midshaft and in distal thirds. Osteoblast activity was measured by mineral apposition rate [μm/day] in sagittal sections following dual tetracycline labeling.

Whole femoral BMC was significantly greater in Y2R KO compared to ob/ob and Y2R/ob, with differences of up to 40%. However, BMD was not different, consistent with greater bone size in Y2R KO and was the opposite of that seen in ob/ob mice. Similar changes were evident in the femoral shaft, with greater BMC in Y2R KO compared to ob/ob and Y2/ob with no change in BMD. In contrast, in the distal femur BMC in Y2/ob mice was increased to Y2R KO levels, with both greater than ob/ob, and again no difference in BMD. The bone mass changes in the distal region were consistent with endocortical osteoblast activity, which was greater in Y2R KO and Y2/ob than wt and ob/ob, with differences of over 30%. This stimulation of cortical osteoblast activity was also evident in the adult-induced hypothalamic Y2R KO compared to the GFP control (0.23 ± 0.01 vs. 0.17 ± 0.02, $P < 0.05$) a difference of over 35%.

In summary, these results reveal an adult-inducible regulation of cortical bone mediated by hypothalamic Y2 receptors, stimulating osteoblast activity. Y2 deficiency increased BMC and osteoblast activity in Y2/ob mice, whereas leptin deficiency was associated with reduced cortical bone mass. The Y2R and leptin pathways have opposing effects on cortical bone mass.

Table

		wt	Y2R KO	ob/ob	Y2R/ob
Total BMC	(mg)	30 ± 3	34 ± 2	24 ± 02$^{\#}$	26 ± 1$^{\#}$
Total BMD	(mg/cm^3)	72 ± 4	68 ± 3	67 ± 3	66 ± 3
Distal BMC	(mg)	10 ± 1	12 ± 1	9 ± 1$^{\#}$	11 ± 1
Distal BMD	(mg/cm^3)	72 ± 5	70 ± 4	71 ± 3	68 ± 3
EndoCo MAR	(μm/day)	0.21 ± 0.03$^{\#}$	0.31 ± 0.03	0.24 ± 0.01$^{\#}$	0.32 ± 0.01

$^{\#}P < 0.05$ vs. Y2R KO.

P015-Tu

Beta-Catenin is a Powerful Enhancer of BMP-2 Gene Expression in Osteoblasts In Vitro and In Vivo

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Although BMP-2 is an important growth factor for postnatal bone formation, and a recent human genetic study has indicated that BMP-2 is an osteoporosis-associated gene, the molecular mechanisms that control BMP2 gene expression in health and aging are not fully understood. We found that the 5′ flanking region of BMP-2 gene contains putative binding sites for TCF, a co-activator of the transcription factor beta-catenin. Beta-catenin is a pivotal signaling molecule that transduces Wnt signal to target genes. The Wnt pathway is known to play a vital role in skeletogenesis, and disruption of Wnt signaling inhibits bone formation in mice and humans. To determine the effect of beta-catenin on the BMP-2 promoter, we performed promoter reporter assays, and found that (1) both wild type and stable mutant beta-catenin dose-dependently increased BMP-2 promoter activity; (2) TCF4 strongly enhanced this stimulation; (3) known antagonists of Wnt signaling DKK1, ICAT, sFRP1, and mutant TCF4 significantly reduced BMP-2 promoter activity and attenuated beta-catenin-mediated enhancement on the promoter; (4) the E3 ubiquitin ligase beta-TrCP, which mediates the proteolytic processing of beta-catenin,

abolished the effects of beta-catenin on the BMP-2 promoter. BMP-2 promoter deletion studies indicated that beta-catenin may recruit transcription factors other than TCF for the transactivation, such as CREB and Sox17. In further studies to determine the significance of beta-catenin effects, we determined that beta-catenin is expressed in a series of human and rodent cells of the osteogenic lineage, namely, C3H10T1/2, ROS17/2.8, MG63, C2C12, 2T3 cells. To confirm if beta-catenin also modulates BMP-2 transcription in vivo, we examined BMP-2 expression in beta-catenin transgenic mice that we generated using the bone-specific 2.3-kb Col1a1 promoter. BMP-2 mRNA expression assessed by qPCR was significantly elevated in the bones of Col1a1-beta-catenin mice compared with wt littermates. We also found that overexpression of beta-catenin stimulated osteoblast differentiation and bone formation in these mice. These results were confirmed by the marked enhancement of BMP-2 mRNA transcription and osteoblast differentiation following transient or stable overexpression of beta-catenin in C2C12 cells. These data strongly suggest that beta-catenin plays an important role in the regulation BMP-2 gene expression, which in turn is essential for normal osteoblast differentiation and bone formation.

P016-Su

Secreted Frizzled-Related Protein 1 (SFRP1) Regulates Mesenchymal Stem Cell Differentiation Toward Osteogenic or Adipogenic Lineage

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Mesenchymal stem cells have the potential to differentiate into osteogenic, adipogenic, and chondrogenic lineages. Secreted frizzled-related proteins 1 (SFRP1), a Wnt protein antagonist has been found to regulate skeletogenesis. We characterized the contribution of SFRP1 to the commitment of mesenchymal stem cells. Increased soluble SFRP1 and nuclear beta-catenin expression were coincided with osteogenic differentiation of murine mesenchymal stem cells cultured in osteogenic medium (10 nM dexamethasone, 10 mM beta-glycerophosphate and 50 μg/ml L-ascorbic acid). Interrupting SFRP1 signaling by SFPR1 antibody neutralization or SFRP1 RNA interference unexpectedly promoted alkaline phosphatase activity and bone nodule formation. Recombinant SFRP1 treatment markedly reduced osteogenesis of cell cultures, indicating that SFRP1 acted as a negative regulator for osteoblastogenesis. The inhibition of osteogenesis resulted to increased adipogenic gene expression (peroxisome proliferator-activated receptor-gamma2 and aP2) and adipocyte formation. SFRP1 modulation of osteogenesis and adipogenesis appeared to be regulated by beta-catenin-dependent signaling, because recombinant SFRP1 reduction of osteogenic differentiation was associated with decreased nuclear beta-catenin expression. Sustained stable beta-catenin by transfection of beta-catenin (beta-catS33Y) mutant gene completely abrogated the adipogenesis-stimulatory effect of recombinant SFRP1 on cell cultres. These observations suggest that SFRP1 is a crucial molecule in modulating beta-catenin regulation of osteogenesis and adipogenesis of mesenchymal stem cells. Control of SFRP1/beta-catenin signaling can be used for understanding the imbalance between osteoblast and adipocyte populations in bone marrow of osteoeporosis and an alternative strategy for skeletal tissue regeneration.

P017-Mo

p53 Represses the Expression of Transcription Factor Osterix and Functions as a Negative Regulator of Osteoblastogenesis and Bone Formation

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p53 is a critical player in safeguarding against cancer development. More than 50% of primary human tumors have p53 mutations. p53 executes its functions mainly by activating or repressing target genes involved in cell cycle arrest, apoptosis and cell senescence. Here, we report an additional role for p53 in osteoblast differentiation and bone remodeling. We showed that p53 functions as a negative regulator of osteoblast differentiation and postnatal bone development as p53−/− mice showed osteosclerotic phenotypes, manifested by an increase in bone mineral density and trabecular bone volume, and a 50% increase in bone formation rate. Compared to wild type cells, bone marrow osteoprogenitors and calvarial osteoblasts isolated from p53−/− mice displayed accelerated differentiation and enhanced proliferation, two cellular events that are believed to be mutually exclusive. p53−/− mice also showed an increase in bone resorption and in the number of osteoclasts. However, differentiation and resorption activity of p53−/− osteoclast appeared normal, suggesting that the increase in bone resorption is probably caused by enhanced osteoblast activities in p53−/− mice. Accelerated differentiation displayed in p53−/− osteoblasts was found to be mediated by elevated expression of osterix, an osteoblast specific transcription factor essential and sufficient for differentiation, while expression of Runx2, another lineage-specific transcription factor that acts upstream of osterix, was not affected. Knocking down the levels of

osterix by antisense oligos in p53−/− osteoblasts inhibited their differentiation. Further studies using luciferase assays suggest that p53 represses osterix transcription by the minimal promoter in a DNA-binding independent manner. Moreover, deficiency of p53 rescued the differentiation defects of osteoblasts deficient for c-Abl, which is a physical and functional interacting partner of p53 in DNA damage response. As p53 expression is regulated during osteoblast differentiation in addition to genotoxic and other stresses, we postulate that p53 may serve as a differentiation checkpoint for osteoblasts.

P018-Tu

Tu Role of Fibroblast Growth Factor Receptor-2 in the Altered Osteoblast Phenotype Induced by Twist Haploinsufficiency in the Saethre–Chotzen Syndrome

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Genetic mutations of Twist, a bHLH transcription factor, induce premature fusion of cranial sutures (craniosynostosis) in the Saethre–Chotzen syndrome (SCS). We previously showed that craniosynostosis induced by Twist haploinsufficiency in SCS induces alterations in osteoblast differentiation. In this study, we investigated the role of fibroblast growth factor-2 (Fgfr2) in the abnormal osteoblast differentiation in SCS. Cranial osteoblasts from an SCS patient with a Y103X mutation inducing deletion of the bHLH domain in Twist and reduced Twist dosage showed decreased Fgfr2 mRNA levels associated with decreased expression of Runx2, bone sialoprotein and osteocalcin, markers of differentiated osteoblasts, compared to wild type osteoblasts. Chromatin immunoprecipitation (ChiP) analysis indicated that Twist present in osteoblast nuclear extracts binds to a specific region containing a consensus E-box in the Fgfr2 promoter. Additionaly, nuclear extracts from Twist mutant osteoblasts showed reduced Runx2 binding to a target OSE2 site in the Fgfr2 promoter, suggesting that reduction in both Twist and Runx2 may contribute to Fgfr2 mRNA downregulation in Twist mutant cells. Accordingly, transfection with Twist or Runx2 expression vectors restored Fgfr2 as well as Runx2, bone sialoprotein and osteocalcin expression in Twist mutant osteoblasts. A dominant-negative Fgfr2 construct further decreased Runx2, bone sialoprotein and osteocalcin expression in Twist mutant osteoblasts. Consistently, forced expression of Fgfr2 restored Runx2 and osteoblast marker genes despite Twist haploinsufficiency, indicating that alteration of Fgfr2 mRNA results in downregulation of osteoblast genes in Twist mutant osteoblasts. We conclude that bHLH deletion in Twist reduces Fgfr2 mRNA expression, resulting in reduced Runx2 and osteoblast-specific gene expression in human calvarial osteoblasts. This provides novel genetic and biochemical evidence for a role of Fgfr2 in the altered osteoblast phenotype induced by Twist haploinsufficiency in the Saethre–Chotzen syndrome.

P019-Su

Apo2L/TRAIL is a Potent Anti-Cancer Agent that Prevents Breast Cancer-Induced Bone Destruction in a Mouse Model

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Breast cancer is the most common carcinoma that metastasizes to bone. Bone metastases in breast cancer patients are associated with extensive bone destruction, leading to bone pain, hypercalcemia, pathological fractures, spinal cord compressions, and eventually death. To examine the in vivo anti-tumor effects of Apo2L/TRAIL, we established a mouse model in which MDA-MB-231 human breast cancer cells are transplanted orthotopically into the tibiae of athymic mice. These tumor cells grow reproducibly and produce osteolytic lesions in the area of injection. Untreated animals transplanted with MDA-MB-231 breast cancer cells all developed large lesions that invaded the marrow cavity and eroded the cortical bone, as assessed by radiography, micro computed tomography (mCT) and histology. In contrast, animals treated i.p. with 30 mg/kg/dose of Apo2L/TRAIL for five consecutive days followed by once weekly for 4 weeks, showed dramatic conservation of the tibiae. In addition, the tumor burden was reduced by 10-fold with Apo2L/TRAIL treatment, although tumor cells persisted in the marrow cavity. The tumors were significantly smaller and were confined to the site of transplantation. The presence of tumor cells in the Apo2L/TRAIL-treated animals may be an indication that the therapy and dosing is insufficient or that Apo2L/TRAIL treatment results in the selection of TRAIL-resistant clones. We have tested this hypothesis by isolating tumor cells from the bones of Apo2L/TRAIL-treated animals to assess their resistance to Apo2L/TRAIL in vitro. Indeed, cancer cells explanted from Apo2L/TRAIL-treated animals were significantly more resistant to the effects of Apo2L/TRAIL compared to cells explanted from the untreated animals. However, this resistance was readily reversed when Apo2L/TRAIL was used in combination with other agents, including chemotherapy or the histone deacetylase inhibitor, SAHA. This is an important finding because for the first time we have demonstrated the acquisition of Apo2L/TRAIL resistance in vivo and our data suggests that, while Apo2L/TRAIL monotherapy may not be an effective treatment for bone cancer, a combinatorial approach with other therapeutics may be effective in eradication of cancer cells from the bone microenvironment.

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P020-Mo

Sclerostin is a Delayed Secreted Product of Osteocytes that Regulates BMU Width and Cortical Canal Infilling

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Sclerostin, the SOST gene product, is an important regulator of bone formation. We have investigated the precise location of sclerostin in fresh frozen undecalcified sections of adult human iliac crest bone using histochemistry. 14 biopsies from patients participating in a study of bone loss in hemiplegia were taken within 3 months of an acute stroke. The spatial relationships between sclerostin-positive osteocytes and forming/mineralizing surfaces were evaluated using serial sections stained for alkaline phosphatase (ALP) and unstained sections with demeclocycline labeling. Overall, 86% of 6231 cortical osteocytes were positive for sclerostin, whereas 74% of 1018 cancellous osteocytes were positive. Osteoblasts and lining cells were consistently negative for staining in all biopsies. Sclerostin-negative osteocytes were located significantly closer to haversian canals and endosteal/periosteal surfaces (Median Distance from Surface, MDS 53.5 μm, IQR 31.5–84.9) than sclerostin positive cells (MDS 88.6 μm, IQR 60.8–116.8; $P < 0.0001$, paired t test). A detailed analysis of individual osteons/cortical BMU's (280 osteons from 5 patients) indicated that the distribution of sclerostin-positive and -negative osteocytes was strongly related to the forming status of the osteon. ALP-positive (forming) osteons were significantly more likely to contain osteocytes negative for sclerostin (chi-square 33.5, $P < 0.0001$ *Table*). Qualitative assessment indicated that recently embedded osteocytes (defined by their proximity to ALP-positive surfaces and demeclocycline labels on serial sections) were negative for sclerostin. Osteocytes in mineralized bone were positive for sclerostin with diffuse staining in canaliculi (*Image*). Sclerostin secretion by new osteocytes is therefore a delayed event. These findings are consistent with the concept that newly embedded osteocytes secrete sclerostin after the onset of mineralization to inhibit cortical bon formation and BMU infilling by cells of the osteoblast lineage.

Table
Sclerostin status of osteocytes within an osteon

	Osteocytes all +ve	Osteocytes mixed	Osteocytes all −ve
ALP −ve Osteons (n = 235)	66%	32.3%	1.7%
ALP +ve Osteons (n = 45)	23.4%	66%	10.6%

P021-Tu

Low Bone Turnover and Functional Hypoparathyroidism Reduce Mortality in Vitamin D Deficient Elderly

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Background: Vitamin D deficiency is common in the elderly and normally leads to secondary hyperparathyroidism. Elderly subjects who fail to respond in this way may have altered bone turnover and fracture risk. We prospectively assessed the associations of vitamin D and PTH status with bone turnover, fracture risk and mortality in a cohort with a high prevalence of vitamin D deficiency.

Methods: We measured serum levels of the aminoterminal propeptide of type I collagen (PINP), a marker of bone formation, and serum concentrations of the carboxyterminal telopeptide of type I collagen (CTX-I), a marker of bone resorption, as well as serum intact parathyroid hormone (PTH) and serum 25 hydroxyvitamin D (25OHD) in 1167 men and women living in residential care.

Results: The mean age of subjects was 85.8 (±6.86 SD) years and 83.0% had hypovitaminosis D (defined as a serum 25OHD level <39 nmol/L). Subjects in the highest tertile of serum CTX and PINP had higher mortality rates (hazard ratio 1.53 (95% CI 1.27 to 1.84) and 1.46 (95% CI 1.21–1.75), respectively ($P < 0.0001$ for both), than those in the middle and lower tertiles. Subjects with low serum 25OHD and high serum PTH levels (group 1, n = 433) were older and had significantly lower serum calcium, albumin and 25OHD levels and higher serum creatinine values than individuals with low serum 25OHD and low or normal serum PTH levels (group 2, n = 536), or subjects with normal serum 25OHD concentrations (group 3, n = 198). After adjustment for age, sex, creatinine, and bone turnover (as assessed as the logarithm of serum PINP and CTX levels), mortality was significantly higher in group 1 than groups 2 or 3. In multivariate analyses that adjusted

for age, sex, and creatinine, both PTH group and elevated CTX or PINP were significantly associated with increased mortality.

Interpretation: In the elderly with vitamin D deficiency, high bone turnover and secondary hyperparathyroidism were independently associated with increased mortality. In contrast, functional hypoparathyroidism in the face of vitamin D deficiency was associated with lower bone turnover and lower mortality. The mechanism of the protective effect of normal PTH in the presence of hypovitaminosis requires further investigation.

P022-Su

BMD Maintained After Withdrawal of Milk Supplement: A Follow-up Study in Postmenopausal Chinese Women in Malaysia

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Background: A randomized controlled trial in 173 postmenopausal Chinese women in Kuala Lumpur showed that milk supplementation was effective to reduce bone loss at the total body, lumbar spine, femoral neck and total hip compared to the control group on a usual diet.

Objective: The objective was to determine whether the results were sustained after the conclusion of the study.

Design: A total of 139 participants was followed up 21 months after the study ended. Bone mineral density (BMD) was measured at the total body, lumbar spine, femoral neck and total hip by dual energy X-ray absorptiometry, and anthropometric measurements as well as changes in dietary habits were measured. Differences between and within treatment groups were assessed using repeated measures analysis of variance (SPSS version 11.0, Chicago, USA).

Results: At the follow-up, the milk supplement group did not show significant bone loss from baseline at most sites (mean differences ± SE) (total body 0.42 ± 0.25%, femoral neck 0.44 ± 0.58%, total hip −0.06 ± 0.46%), unlike the control group (total body −1.07 ± 0.28% $P < 0.005$, femoral neck −1.49 ± 0.56% $P < 0.05$, total hip −0.89 ± 0.57% $P < 0.05$). The calcium intake of the milk group remained significantly higher than the control group (milk 710 mg/day, control 466 mg/day, $P < 0.005$) despite discontinuation of the milk supplement.

Conclusions: The results showed that some of the beneficial effects of a milk supplement were still evident at follow-up and it was possible to motivate subjects to adopt a positive change in dietary calcium intake after intervention.

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P023-Mo

High Bone Turnover is an Independent Predictor of Mortality in the Frail Elderly: A Prospective Cohort Study

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Background: Osteoporotic fractures are associated with accelerated bone turnover and excess mortality. In a prospective cohort study of elderly subjects, we assessed whether the rate of bone turnover is a direct predictor of mortality.

Methods: Baseline data from 1112 men and women (21% male; mean age: 86 ± 6.9 years) living in residential care facilities were analyzed. Parameters included: age, gender, comorbidity, incident hip fractures, serum amino-terminal propeptide of type I collagen (PINP), carboxyterminal telopeptide of type I collagen (CTX-I), intact parathyroid hormone (PTH), serum 25 hydroxyvitamin D (25OHD). Serum calcium, phosphate, and creatinine were measured in a randomly selected subgroup of 448 subjects (40%).

Results: Over a mean follow-up of 782 ± 414 (range: 4–1883) days, a total of 517 (46.5%) subjects died. In univariate analyses, time to death was significantly ($P < 0.05$) associated with age (HR 1.64, per 10 years), gender (HR 1.32, male vs. female), comorbidity (HR 0.31, mild symptoms vs. severe illness), hip fracture (HR 2.36, yes vs. no), serum LN creatinine (HR 1.81), LN PTH (HR 1.26), LN CTX (HR 1.44), and LN PINP (HR 1.33). These associations remained essentially unchanged when adjusted for serum creatinine levels. Death rates/person/year continuously increased with increasing serum CTX-I or PINP concentrations (highest quintile vs. lowest quintile, CTX: 31.4 vs. 17.6%, PINP: 30.1 vs. 19.5%). Similarly, the rate of hip fractures increased with increasing serum CTX-I, but not with PINP levels. In multivariate analyses adjusting for age, gender, comorbidity, 25OHD, PTH, and hip fracture status, both bone turnover markers remained significantly associated with time to death: LN CTX-I, HR 1.21 (95% CI 1.05–1.40, $P = 0.01$), LN PINP, HR 1.21 (95% CI 1.05–1.40, $P = 0.007$). We conclude that in the frail elderly, high bone

turnover predicts death independent of age, gender, comorbidity, renal function, serum PTH levels, and hip fracture status.

P024-Tu

Bone Mineral Density in Premenopausal Women with Rheumatoid Arthritis

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Aims: To ascertain changes in central bone mineral density (BMD) in premenopausal women with rheumatoid arthritis (RA), we conducted a study of axial BMD in a cohort of 79 RA patients.

Materials and methods: Premenopausal, regularly cycling women with RA (n = 79) attending our hospital were randomly recruited for a 2-year follow-up study and were divided into two groups: women with RA (n = 42), and women with RA and treatment with corticosteroids (cts), (n = 37). The control group (n = 50) had age-matched healthy controls. BMD measurements of the lumbar spine and the proximal femur were made at the introduction of the follow-up by dual X-ray absorptiometry.

Results: The mean age in women with RA was 38 (SD 6), in women with RA and cts 36 (SD 5), in the controls 37 (SD 6) years, respectively. The mean weight in women with RA was 64 (SD 11), in women with RA and cts 59 (SD 8), and in controls 59 (SD 8) kg (P = 0.024), respectively. Women with RA were assessed mainly (74%) into Steinbrocker's functional grades I and II, as 63% of the women with RA and cts were assessed into functional grades III and IV. Median Health Assessment Questionnaire-index was higher in RA patients with corticosteroids, 0.87 and 0.37 (P value 0.016), respectively. The median daily dose of cts was 5 mg. The mean duration of cts use was 3.3 (SD 3.4) years, range 0.1 to 12 years. Women with RA and cts had lower mean BMD at the lumbar spine and femoral neck, 1.09 (SD 0.13) [95% CI: 1.04 to 1.13], 0.85 (SD 0.11) [95% CI: 0.81 to 0.88] than women with RA and controls 1.20 (SD 0.12) [95% CI: 1.16 to 1.23], 0.95 (SD 0.13) [95% CI: 0.91 to 0.99] and 1.20 (SD 0.15) [95% CI: 1.16 to 1.25], 0.95 (SD 0.12) [95% CI: 0.92 to 0.99] g/cm^2, respectively. Women with RA and controls did not differ from each other according to measured BMD values. Women with RA and cts had probably more severe disease affecting their functional grade, which may have effect on BMD. There was no statistical difference in disease duration between the two RA groups.

Conclusions: Premenopausal women with RA mainly seem to preserve their axial BMD, although there are premenopausal women with RA having reduced axial bone mass. RA itself does not explain reduced BMD values. Further studies are needed to evince possible related factors to this phenomenon.

P025-Su

Delay in Bone Density Gain Compared with Height Growth may Cause Transient Increase in Fracture in Growing Japanese Children

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Incidence rate of fracture in Japanese children has increased progressively in these decades. The highest fracture rate across the school ages is observed at 7th grade (13 or 14 years) of compulsory school in boys and 5th grade (11 or 12) in girls, even though areal bone mineral density (aBMD) increases linearly over these ages. We examined possible causes of this transient increase in bone fragility in growing children by estimating volumetric BMD (vBMD) in a longitudinal study.

We examined 579 healthy children (283 boys) from 4th (10 or 11 years) to 9th (15 or 16) grade of a compulsory school (G4 to G9, respectively) for whom height, weight, and bone indices including bone mineral content (Bc), bone projection area (Ba), and aBMD at the spine (LS) and total hip (TH) and length of range of interest (Lr) at LS were measured (QDR4500A, Hologic). The subjects were invited for a follow-up survey conducted 3 years after the baseline which comprised the same bone measurements. 432 subjects (203 boys) (74.4%) completed the follow-up survey. vBMD was estimated at LS under an assumption of the spine being a cylinder. The mean diameter of the spine was estimated by Ba divided by Lr. vBMD was determined using the equation, $vBMD = Bc/(pi(Ba/(2Lr))^2Lr)$. Annual rates of changes in height and bone indices were determined.

Height and aBMD at LS and TH at baseline increased linearly with advancing in age in both genders. A similar increase was observed in vBMD in girls but not in boys, who showed the lowest vBMD in G6 (12 or 13 years) and the highest one in G9. At follow-up, both aBMD and vBMD increased linearly with age in boys but plateaued at high school ages in girls. The greatest annual rate of change in height was observed during G6 to G7 in boys and G4 to G5 in girls, and a similar pattern of change was found for aBMD. However, the annual rate of change in vBMD was the greatest during G8 to G9 in boys and during G7 to G8 in girls.

The lowest vBMD was observed at the age of peak velocity of height growth in both genders and these ages are around the age when the highest fracture rate across the adolescence appears. Delay in gaining bone density compared with height growth may cause low bone density, which may result in the transient increase in fractures in growing children.

P026-Mo

Italian Pediatric Reference Curves for Quantitative Ultrasound Bone Measurements at Multiple Skeletal Sites

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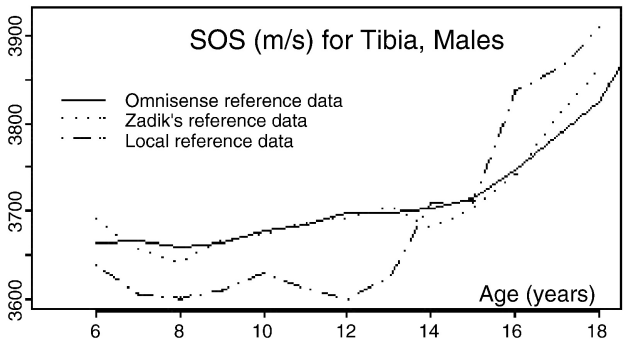
Bone health survey in childhood and adolescence is very important to attent an ideal peak bone mass. Differently from DXA and QCT, QUS devices are easy to use, cheap and safe: they represent a simple technique to assess bone quality. However, results of QUS measurements need an accurate interpretation in the clinical practice. The aim of this work is to demonstrate that local reference curves are necessary, in order to give a sensible diagnostic interpretation to speed of sound (SOS) measures obtained by Omnisense™ device (Sunlight Technologies). This multisite device is released with reference curves derived from Israelian Caucasian subjects aged 0–20, reporting SOS mean ± 1 SD as a function of age, gender, and site. Recently, Zadik et al. published reference curves derived from 1141 Caucasian Israelian children studied with Omnisense™: values were very similar to the reference data provided by the instrument, except for higher values found for tibia in females after puberal phase. We measured SOS in 662 Italian Caucasian healthy subjects, aged 6–18, 346 males and 316 females. Differently from Zadik's results, a common feature of our comparisons was that Omnisense™ reference values are significantly higher for ages 6–12, while for ages 13–18, tibial measures are lower, and radius ones are similar. As an example, the figure compares Zadik's and our results with Ominsense™ reference data. In these subjects, for some age categories, the difference is as high as a standard deviation, and this should imply an incorrect diagnostic classification. Our conclusion is that each country should develop its own reference curves. Further studies are necessary to understand reasons of such differences (diet, physical activity, etc.) and to consequently assess the size of geographical areas to consider for developing reference curves.



P027-Tu

The Retrospective Analysis of Mineral Content in Bone Samples from Donors Aged 0–99 Years Living in the Southern Urals Over the Second Half of the XX Century

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This study is based on the results of a long-term monitoring of the radionuclides content in human bones sampled in 1959–1988 from deceased residents of the Southern Urals (Russia). These data were compiled in an Autopsy Registry established at the Urals Research Center for Radiation Medicine (Chelyabinsk, Russia). The Registry is a part of the Database containing medical follow-up data on Urals residents. The bone mineral content (in terms of ash content, g per kg of bone) in samples of rib (n = 5685), cranium (n = 400), vertebra (n = 469), sternum (n = 270), and long bones of the lower extremities (n = 636) were analyzed. Age and gender features as well as secular trends in bone mineral content were studied. The donor ages were 0–99 years; years of birth ranged from 1872 to 1984; and the total number of bone samples analyzed was 4717 from men and 2184 from women. The average values based on combined data for the general population were: Mineralization rates for children and adolescents were found to vary from 1.3–1.5% year^{-1} (rib) to 0.5–0.9% year^{-1} (cranium and fibula). For some types of bone (vertebrae, sternum, cranium, fibula), a plateau or the period of minor changes in bone mineral content was observed. The average rates of bone loss in adulthood depended on gender and the type of bone, and varied from 0.8% year^{-1} (fibula, women after 50 years of age) to 0.2–0.3% year^{-1} for men and women in different sites of the skeleton. Thus, the rate for rib was equal to 0.4–0.5% year^{-1}, for trabecular bones 0.16–0.3% year^{-1}, and for long bones of the lower extremities 0.3–0.8% year^{-1}. However, as was found by the example of rib, the rates of bone mineral loss in the same bones can differ 2 or more times depending on the period of investigations (decades of the XX century). In the second half of the XX century (from the 1960s to the 1980s), a decrease in rib mineralization occurred in all age groups. As a result, the minimal level of rib mineralization for women (about 250 g/kg) was reached for persons born in 1931–1941 by the age of 50, for women born in 1911–1920 by the age of 70, and for women born before 1900 by the age of 90. Similar, but less pronounced trends, were observed in men. These data indicate the inapplicability of using cross-sectional studies to estimate actual age dependences of bone mineral loss, which can only be obtained in longitudinal studies. This work has been funded by the Russian Foundation for Fundamental Investigations (Projects 01-04-96490 and 04-04-96085).

P028-Su

Assessments of the Cortical Bone Resorption Rates for Men and Women Aged 30–80 Years in a Longitudinal (25 Years) Study Using Strontium-90 as a Radioactive Tracer

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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 population over a wide age range (30–80 years old). Measured levels of ^{90}Sr-body burden (5–150 kBq) did not influence the rate of cortical resorption. Longitudinal studies were conducted for 108 men (1769 measurements) and 81 women (1337 measurements). The study did not show statistically significant age dependence in individual rates of cortical resorption over individual period of observation; however, some women were of appropriate ages and had enough data to detect an increase in resorption rate after the menopause. 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. The analysis of average-grouped resorption rates has shown gender and age dependencies: The rate of cortical resorption sharply increases in men after the age of 55 years from 2.8% year^{-1} to 3.3% year^{-1} and after the age of 45 years in women from 3.2% year^{-1} to 4.5% year^{-1}. Further, slower increases with increasing age in both genders is seen reaching 3.5% year^{-1} in men and 6.0% year^{-1} in women by the age of 70–80 years. It was found that women manifested statistically higher cortical resorption rates compared to men over the entire age period (30–80 years). Averaging according to individual menopausal age resulted in a sharper increase in the cortical resorption rate compared to averaging according to calendar age. It was found in the study that there was no relationship between the number of previous pregnancies/labors and the cortical resorption rate. The analysis of the influence of diseases affecting bone turnover shows 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 US Department of Energy and Russian Foundation for Fundamental Investigations (Project 04-04-96085).

P029-Mo

Gene Expression Analysis of Major Lineage Defining Factors of Human Bone Marrow Cells Related with Aging and Age Related Disorders in Bone

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Adult bone marrow cells (BMCs) include two populations of stem cells: mesenchymal stem cells (MSCs), which can differentiate into bone, cartilage, and fat; hematopoietic stem cells (HSCs), which give rise to all mature lineages of blood. How the BMCs have initiated differentiation into a defined lineage, and how the cells with defined lineage transdifferentiate into another lineage are still unknown. Aging and age-related disorders might be potent factors to regulate the lineage of BMCs. In order to evaluate whether lineages differentiation is modified by aging and age-related disorders, we analyzed, by quantitative RT-PCR, mRNA expression of the major factors defining lineage of BMCs, such as cbfa1 for osteoblast, ppar-gamma for adipocyte, sox-9 for chondrocytes, rankl for osteoclast, in bone marrows of healthy subjects and patients with two age-related disorders, osteoarthritis (OA) and rheumatoid arthritis (RA). The ages of a total of sixty individuals vary from 14 to 72. Moreover, two apoptosis-related genes, bcl-2 and DRAK1, were also studied. We found that RANKL and PPAR-Gamma levels exhibited a clear positive correlation with age in female patients, but not visible in male. There was obviously an age-related decline in CBFa1 transcripts. SOX9 levels were age-independent until 60 years old, but then showed inverse correlation with age. DRAK1 expression showed age associated ascending trend in female. There were significantly greater transcripts of RANKL and DRAK1 in female than in male ($P < 0.01$). No age-related correlation for BCL-2 was detectable. Compared with age-matched controls, patients with RA exhibited the increased RANKL, PPAR-Gamma and DRAK1 mRNA level than normal individuals ($P < 0.05$), and OA showed the higher RANKL and PPAR-Gamma transcripts ($P < 0.05$). Furthermore, the expression of SOX9 and DRAK1 in RA group were higher than in OA group ($P < 0.05$). Therefore, our data indicate that aging and age-related disorders affect differently on gene expression, suggesting that in aging, the lineage of bone marrow cells modified, with prominent changes in decreased bone marrow osteoblastogenesis, increased adipogenesis, and osteoclastogenesis, while in age-related disorders, marrow adipogenesis and activity or number of osteoclast may play an important role in the

pathogenesis of arthritic bone loss. Furthermore, gender-dependent differences in RANKL, PPAR-Gamma, and DRAK1 expression suggest that estrogen deficiency may also play the role in bone loss process.

P030-Tu

Fall Mechanisms in a Healthy Older Population with Minimal Trauma Fractures

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Falls frequently relate to neurological, musculoskeletal, cardiorespiratory, or cognitive impairment in older people. In the context of fracture prevention falls mechanisms are of interest. In a study of an otherwise healthy elderly population aged 60–79 years, the mechanisms for falls resulting in a minimal trauma fracture were identified.

Methods: 120 clients (12.6% male; 87.4% female) were interviewed to qualitatively determine fall mechanisms. Excluded were those with any disorder related to inactivity or osteoporosis. Interviews were conducted in the fracture clinic or ward. Injuring mechanisms of the current and any former fractures were qualitatively assessed.

Results and discussion: Fracture prevention had not been discussed with any client. Fracture mechanisms were varied, and in descending order of incidence, these were:

Tripping (31%; 37/120) usually over an uneven surface or step (81%; 29/37) but causes included foot catching—in a seatbelt when exiting from a car, or the bedclothes, tripping over shoe laces, when chasing animals in the garden.

Slipping (24%; 29/120); 9 on ice, 16 on wet surfaces (leaves/moss/ floor), and 4 off steps.

Loss of balance (18%; 22/120) when turning, pulling or pushing objects (5 occurred when gardening).

No apparent reason (9%, 11/120); Falling down a step (9%, 10/120; Joint instability-leg/ankle 'giving way' (7%; 8/120); Knocked over by a dog (2.5%, 3/120); Blown over by the wind (2%; 2/120); 'Other' causes: e.g., loss of balance on crutches, taking weight on fractured metatarsal; small weight landing on the wrist (4%; 5/120); No fall (stress fracture) (2.5%; 3/120).

Tripping and slipping caused falls in 55% of cases. Interestingly, only 22 (18%) falls occurred inside the home, half being falls with no obvious cause (e.g., fainting). Seven falls occurred while gardening; 3 at swimming pools and a further 4 in shopping precincts. The remainder occurred in various public places including pavements, parks, and car parks. Slippery surfaces caused a quarter of falls, and research is needed into suitable footwear for different situations. In this healthy older population, 91% of falls resulted from causes related to activity—most of which were preventable. In the context of fracture prevention, rehabilitation management following minimal trauma fractures should include client education in falls prevention when undertaking activity both within and outside the home.

P031-Su

Targeted Disruption of the Src Substrate, Sam68, Protects Against Age-Related Bone Loss

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Src was first implicated in bone biology over a decade ago when mice homozygous for targeted disruption of the c-src gene died in the peri-natal period with an osteopetrotic phenotype. The predominant pathogenetic mechanism in the Src−/− mice was impaired activity of mature osteoclasts involved in bone resorption. However, evidence also exists for altered osteoblast function in these mice. Around the same time, Sam68 was named as an Src-associated protein during mitosis of 68 kDa, and has subsequently been identified as a pro-apoptotic agent in neuronal cells. Despite extensive biochemical characterization of the RNA-binding properties of Sam68 over the past decade, there has been little progress in defining its physiological function. Homologous recombination in embryonic stem cells was therefore used to generate mice lacking Sam68 function. Although many of the Sam68−/− mice died in the peri-natal period of yet-to-be identified causes, a significant number survived to old age. Examination of the skeletal phenotype of surviving mice revealed no differences between the Sam68−/− and Sam68+/+ littermates at 4 months of age. In contrast, the age-related loss of bone seen in the femur and vertebra of 12-month-old wild type mice was not apparent in Sam68−/− mice, whose bones resembled those of the 4-month-old mice. Quantitative micro-CT and histomorphometric analyses confirmed the relatively high bone mass in Sam68−/− mice. In situ staining for tartrate-resistant acid phosphatase and alkaline phosphatase indicated a relative decrease in osteoclast and osteoblast activity in 12-month-old Sam68+/+ mice, which was not apparent in the age-matched Sam68−/− mice. Preliminary evidence from bone marrow stromal cell cultures suggests that the underlying mechanism involved in the Sam68−/− bone phenotype is altered osteoblast function, rather than altered osteoclast function, as was seen in the Src−/− mice. These studies show that we have generated a new model in which bone mass is preserved during aging and have identified the Src substrate Sam68 as a potential target for the prevention and treatment of osteoporosis. This research was supported in part by the Canadian Institutes of Health Research.

P032-Mo

Age Changes in the Mandibular Condyle and Tibia in Rats

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In the present study, the age changes in the cartilages of both mandibular condyle and proximal tibial growth plate in rats ranging from 1 month to 1 year and 10 months of age were investigated histologically, histometrically, and immunohistochemically. These cartilages are the sites of endochondral bone formation. Histologically, at 1 month, the mandibular condyle showed distinctly the layers of anticular surface, proliferation, differentiation, and hypertrophy. At 3 months, the 4 layers of the mandibular condyle showed decreased cellularity. Both at 6 months and at 1 year and 1 month, the 4 layers exhibited further decreased cellularity and became indistinct from each other. At 1 year and 10 months, the mandibular condyle lost the layers of anticular surface and proliferation and displayed only the layer of cartilage (layers of differentiation and hypertrophy together) which was exposed to the joint space. As for the tibia, at 1 month, the proximal growth plate showed distinctly the zones of resting, proliferation, and hypertrophy. At 3 months, the 3 zones showed decreased cellularity, and at 6 months they exhibited further decreased cellularity. Both at 1 year and 1 month and at 1 year and 10 months, the proximal tibial growth plate was seen only as the scattered zone of cartilage (zones of proliferation and hypertrophy together) within the bone. Histometrically, from 1 to 6 months, both the 4 layers of the mandibular condyle and the 3 zones of the proximal tibial growth plate showed a decrease in thickness. Both at 1 year and 1 month and at 1 year and 10 months, the histological layers of the mandibular condyle and also the histological zones of the proximal tibial growth plate could not be measured histometrically because of the indistictiveness of the layers and of the scattering of the zones. Immunohistochemically, at all ages, staining of type II collagen was observed in the mandibular condyle. From 1 to 6 months, staining of type II collagen was also found in the proximal tibial growth plate, but both at 1 year and 1 month and at 1 year and 10 months, it was observed sporadically. The present results indicated that the cartilage of the mandibular condyle showed age changes in a different pattern from that of the proximal tibial growth plate.

P033-Tu

Effect of Mechanical Components of Physical Activity on Bone Mineral Density in Japanese Children: Cross-Sectional Analyses

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It is well recognized that physical activity during childhood plays an important role in determining final bone mass. However, how the ground reaction forces, the intensity, and the duration of physical activity affect bone growth is not well known. In this study, we examined these effects on bone mineral density (BMD) in a cross-section of Japanese children. We studied 579 healthy Japanese children aged 10–16 years (283 boys, 296 girls) from a town in northern Japan. We recorded their heights and weights. BMDs at the spine and hip were measured using dual-energy X-ray absorptiometry (QDR4500A, Hologic Inc., Bedford, MA, USA). A history of each child's physical activity from the first compulsory grade of school (at age 6 years) was estimated, using a questionnaire administered by interviewers who were trained public health nurses in the town. Physical activity was scored in both the metabolic components of physical activities (METPA) and the mechanical components of physical activities (MECHPA). Each child's METPA was calculated as a total score of the different activities, multiplied by the duration of the intensity categorized by metabolic rate for each activity. Each child's MECHPA was calculated as the sum of the scores of the different activities categorized by their estimated ground reaction forces. Multivariate regression analyses were conducted among four groups stratified by sex and pre- or post-pubertal status. MECHPA data, or METPA divided into quartiles, were included in the model as dummy categorical variables. When adjusted for age and for years after the onset of puberty and for height and weight, this showed that METPA during elementary school (ages 6–13 years) was significantly and positively related with hip BMD among post-pubertal boys, and with hip and lumbar BMD among pre-pubertal boys. Similar analysis showed that MECHPA during elementary school was significantly and positively related with hip BMD among post-pubertal boys and girls, with lumbar BMD among post-pubertal girls, and with hip and lumbar BMD among pre-pubertal boys. When we included METPA in the analysis, a MECHPA score of 3 or more was still significantly and positively related with lumbar BMD among pre-pubertal boys, and with hip and lumbar BMD among post-pubertal girls. Thus, the intensity of mechanical strain may be even more important for child bone growth than the metabolic intensity of exercise.

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P034-Su

Repeated Measurements Analysis of Bone Fragility Risk Factors: First Results

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The contribution of genetic factors to interindividual differences in main components of bone strength, such as bone mineral density (BMD), bone size (BS) and bone geometry (BG) are presently well established. However, very little is known about the genetic effects on the rate and pattern of age related changes in these variables. The objective of the present study was to examine the contribution of putative genetic influences and lifestyle factors (alcohol consumption, smoking and obesity) on the rate of change in BMD, BS, and BG in an ethnically homogeneous sample of Chuvasha families (Caucasians). The study was conducted on some 800 healthy individuals, of which 290 were measured twice after an interval of eight years. Computerized digital images of hand bones radiographs were examined for BMD, BS, and BG in all participating individuals. The studied phenotypes were not modified by HRT or any other continuous medical treatment. The family data were subjected to model fitting statistical–genetic analysis in order to find the most parsimonious and best fitting description of the above traits variation. The results showed that alcohol consumption and smoking did not significantly affect any of the studied bone traits. Age, sex, and obesity significantly affected variation of the baseline levels of each of the traits, but did not contribute to the rate of their change after the effect on the baseline level was taken into account. Genetic effects were highly significant and prominent for baseline levels of all bone variables (but not their change) as expected. The rate of change in BS and BG depended very little on genetic factors (3–7% of the variation, $P < 0.06$). However, the latter were highly significant ($P < 0.001$) and strong, explaining 44% to 51% of the variation for changes of all examined BMD characteristics. The present analysis suggests that baseline variations in BMD and the rate of age-related changes are likely influenced by different genetic sources.

P035-Mo

Biochemical Markers of Bone Metabolism and Serum Leptin in Obese Postmenopausal Women

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Introduction: Leptin, a circulating peptide of adipocyte origin, is a potential mediator in relation between fat mass and bone tissue. Although obesity is considered one of the protective factors in epidemiology of osteoporosis, Leptin in most studies showed a negative correlation with bone mineral density. The relation of serum Leptin with biochemical markers of bone metabolism is uncertain.

Materials and methods: Seventy-one postmenopausal obese women and ten postmenopausal women with normal body weight (control group) participated in the study. Women were recruited in Outpatient Osteoporosis Dept. in Institute of Agricultural Medicine in Lublin (Poland). Biochemical markers of bone metabolism: Osteocalcin and C-terminal telopetyde of collagen type (CTX) were measured using ELISA method. Serum Leptin was determined using RIA method.

Results and conclusions: There were no statistically significant differences in serum Osteocalcin between control group and subgroups with BMI 25–29.9, BMI 30–39.9 and BMI > 40 (P values: 0.77, 0.58, 0.89, respectively). There were no statistically significant differences in serum CTX between control group and subgroups with BMI 25–29.9, BMI 30–39.9 and BMI > 40 (P values: 0.46, 0.82, 014, respectively). Mean values of (3.7) (BMI ± serum Leptin study group were: 9.61 < 25)—control group, (2.3) ± (1.8) (BMI 30–39.9), 11.94 ± (1.6) (BMI 25–29.9), 11.90 ± 12.04 (BMI > 40), and the differences with control group were statistically significant ($P = 0.01$). In all study subgroups serum Leptin was positively correlated with Osteocalcin and negatively correlated with CTX, but the relations were statistically non-significant (all P values > 0.05).

P036-Tu

Quantitative Multi-Slice CT Study on Lumbar Vertebrae of Osteoporotic Elderly Women: Comparison of Volumetric BMD with DXA

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Purpose: To determine the identification of vertebral fracture by several volumetric BMD values of osteoporotic elderly women with vQCT technique on multi-slice CT (MSCT), and to compare the bony structure of lumbar vertebrae in osteoporotic elderly women with and without fracture.

Materials and methods: Spinal MSCT (GE LightSpeed16) scanning of L1 and L2 were acquired in a cohort of 56 DXA (GE-Lunar DPX) diagnosed osteoporotic women, of whom 26 (mean age 67.8 ± 5.1 years) had radiographically confirmed atraumatic vertebral fractures (Group One) and 30 (mean age 65.9 ± 5.4 years) were nonfractured subjects (Group Two). All MSCT data of L1 and L2 were retro-reconstructed into 1.25 mm slice thickness and transferred to Workstation to measure traditional BMD (2D-TRAB, 2D-INTGL) and volumetric BMD (3D-INTGL, 3D-CORT, 3D-TRAB) in volume rendering (VR) images. BMD indexes in DXA were AP-SPINE and bone mineral apparent density (BMAD). Reconstructed L1 images of the Normal Group were used to reformat 3D-VR images to analyse the bony structure

of trabecula and calculate the ratio of bone volume to total volume (BV/TV) in the central volume as 2.0 × 2.0 × 2.0 cm and to compare the indexes of ten patients randomly selected in 56 women in Group One and Group Two. ANOVAs were used when comparing the differences of indexes between the Group One and Group Two. ROC curve was used to identify the different ability of 3D-INTGL and 3D-TRAB. The difference of values in BV/TV of Normal Group and 10 OP patients was compared by Student's t test.

Results: AP-SPINE and BMAD measurements showed no statistically significant differences, and all volumetric BMD measurements showed statistically differences (the decrements 18%~23%) between Groups One and Two. The value of BV/TV of L1 was 8.12% ± 1.96% in OP patients, significantly lower than 39.13% ± 2.15% of normal elderly women ($P < 0.01$).

Conclusion: vQCT technique of lumbar vertebra in MSCT is superior than DXA in identifying the vertebral fractured elderly women. 3D reformatted image by MSCT would detect the changes of vertebral structure in OP patients and thus improve the osteoporosis evaluation.

P037-Su

The Backbone of Trabecular Network—A New Approach

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It is well-established observation that trabecular bone mass is reduced in osteoporosis. There are, however, subjects sustaining osteoporotic fractures in spite of apparently adequate bone mass. Different measures of trabecular architecture have been postulated to contribute to the overall trabecular bone strength. In particular, it has been shown that the number of free end trabeculae in thick sections of trabecular bone is significantly higher for fracture subjects than for non-fracture ones. These results suggest that there is some amount of the trabecular bone mass (the mass of free end trabeculae), which is irrelevant for the strength of the analyzed structure, but contributing to the net trabecular mass, measured clinically by either DXA or QCT. Basing on the methods of statistical physics, an algorithm is developed to detect irrelevant trabeculae, i.e., the trabeculae, which could be removed from the network, without reducing the stiffness of the structure. The algorithm was used for the analysis of the microCT 3D images of distal radius specimens. Bone volume fraction of the analyzed specimens ranges from 4% to 16%. The part of the initial structure, which actively participates in the transfer of the loads, is referred to as trabecular backbone (backbone = initial structure-irrelevant trabeculae). It is shown that the mass of irrelevant trabeculae ranges from 1% to 17% and is especially high for low-density subjects. There were also considerable differences between the mass of the backbone of subjects matched for bone volume fraction. Because the difference between backbone mass and the mass of the whole trabecular structure is the simplest factor, which should be incorporated into the diagnosis of fracture risk, the results implies that there is a need for the study of the backbone mass, especially under in vivo image acquisition circumstances.

P038-Mo

Differential Diagnosis of Connective Tissue Disorders, Marfan Syndrome

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608 patients with inherited connective tissue diseases were examined by means of a diagnostic system based on revised criteria for Marfan and Ehlers–Danlos syndromes as well as for osteogenesis imperfecta, benign joint hyperelasticity, and juvenile osteoporosis of the youth. There were 336 adult persons (135 males, 201 females) and 262 children (114 girls and 148 boys up to 18 years). In 415 patients/68.3% of the total examined population/all data necessary for differential diagnostics were obtained: 143 patients met the criteria for Marfan syndrome (MFS), 82 patients for Ehlers Danlos syndrome (EDS) and 62 persons for osteogenesis imperfecta (OI). 154 patients had benign joint hyperelasticity (BJH) and the remaining patients demonstrated other diagnoses. Examined biochemical parameters osteocalcin (OC) was significantly increased in patients with type IV of OI when compared with type I. Procollagen I C peptid/PICP/in patients with OI achieved significantly lower level when compared to other diagnostic groups. Crosslinks were significantly higher in patients with OI as well as in patients with Marfan syndrome up to 13 years, but in older children no difference was found. The highly specific markers for Marfan syndrome were bird chest and thumb test. On the other hand, in osteogenesis imperfecta, drum chest and larger head circumference were typical. Decreased vital pulmonary capacity was found in all severe chest deformities and in scolioses greater than 25° Cobb angle. Patients with Marfan syndrome were tall and had longer extremities to trunk, the highest incidence of hernias and in addition to that, they had also longer anteroposterior bulbus length measured by ultrasonography. Acetabulum protrusion or spondylolisthesis also occur only in MFS patients. Recurrent luxations, varicose veins, and chronic pains were observed only in Ehlers–Danlos syndrome.

Conclusion: The presented diagnostic system including clinical, biochemical, densitometric, radiological, and ultrasonic parameters seems to be adequate for differential

diagnosis of connective tissue diseases. Molecular genetic examination was indicated only in few unclear/15/cases.

P039-Tu

Two Years' Experience of Raloxifene Therapy

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Aim of study: Compare different effect of raloxifene and calcium and vitamin D on bone mass during 2 years.
Methods: In a case-control study, we have 154 women on therapy with 60 mg raloxifene hydrochloride i 100 women on therapy with 1000 mg calcium and 800 i.j. vitamin D only. Bone mass has been measured on hip, spine, and forearm on the beginning and after 2 years. Median age in both groups was 57.
Results: Increasing bone mineral density (BMD) on spine was 3.7%, Odds ratio (OR) 5.85 with confidence interval (CI) 95% (1.535–53.967), χ^2 test 24.67 ($P < 0.05$); on hip 1.2% OR 0.015, 95% CI (0.016–0.896), χ^2 test 43.14 ($P < 0.05$); on forearm decrease 1.2% with OR 0.122, 95% CI (0.0021–0.0568), χ^2 test 27.86 ($P < 0.05$).
Conclusion: Raloxifen statistically significantly increase BMD on spine. There is no statistically significant effects on hip and forearm.

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P040-Su

The Existing Bone Phenotype of Mice Lacking Transient Receptor Potential Channel 5 (TRPV5) Persists During Aging

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We recently demonstrated that transient receptor potential channel V5 (TRPV5) deficiency in mice causes disturbances in Ca^{2+} homeostasis, including hypercalciuria and $1,25(OH)_2D_3$-induced intestinal hyperabsorption in 8-week-old mice. In the bones of these TRPV5 knockout ($TRPV5^{-/-}$) mice, trabecular and cortical bone thickness are reduced compared to wildtype littermates ($TRPV5^{+/+}$). In addition, recent in vitro data indicate that osteoclastic bone resorption is severely reduced in murine bone marrow cultures. In this study, we assessed whether the bone phenotype of $TRPV5^{-/-}$ mice persists with aging.
We measured a number of serum parameters in 8-, 30-, and 52-week-old wildtype and $TRPV5^{-/-}$ mice ($n = 7–9$) including Ca^{2+}, $1,25(OH)_2D_3$ and tartrate-resistant acid phosphatase (TRAP). Furthermore, the femurs were analyzed for several structural parameters, including trabecular bone thickness (Tb.th) in the femoral head as well as cortical bone thickness (Ct.th) and polar moment of inertia (MOI) in the diaphysis, using muCT. Finally, in femoral bone sections, we analyzed calcein labels and calculated mineral apposition rate (MAR) and bone formation rate (BFR).
In the $TRPV5^{-/-}$ mice, $1,25(OH)_2D_3$ levels were very high, which persisted during aging. At 8 weeks of age, both Tb.th and Ct.th are reduced in $TRPV5^{-/-}$ mice. During aging, these differences in Tb.th and Ct.th between the genotypes progressively increased. MOI, which is a proxy for bone quality, was reduced in all $TRPV5^{-/-}$ age groups in comparison with the $TRPV5^{+/+}$ mice. TRAP was reduced in all $TRPV5^{-/-}$ age groups compared to $TRPV5^{+/+}$ mice, confirming our previous in vitro studies that resorption is reduced in $TRPV5^{-/-}$ mice. In bone sections from the young $TRPV5^{-/-}$ age group, both MAR and BFR were reduced compared to $TRPV5^{+/+}$ littermates and this difference between the genotypes was similar in the 52-week-old animals.
In conclusion, the bone phenotype of $TRPV5^{-/-}$ mice persists with aging and the differences with wildtype mice even become greater. The observed reduced bone thickness and quality as a consequence of reduced bone resorption and mineralization may be indicative of a low bone turnover state in mice lacking TRPV5.

P041-Mo

The Age of Attainment of Peak Bone Mass is Site Specific in Swedish Men—The Good Study

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Introduction: Peak bone mass (PBM) is an important determinant for the risk of contracting osteoporosis. In men, the age of attainment of PBM has been under some controversy. The objective of the present study was to determine if peak bone mass had been attained, and whether it is site specific or not, in 18- to 20-year-old Swedish men

included in the Gothenburg Osteoporosis and Obesity Determinants (GOOD) study.

Subjects and methods: 1068 18- to 20-year-old Swedish males from the Gothenburg area (Sweden) were included in the present population-based study. Bone mineral density was measured using both dual-energy X-ray absorptiometry (DXA) and peripheral quantitative computerized tomography (pQCT). Environmental factors, such as dietary intake and physical activity, were assessed through questionnaires. The independent predictors of bone mineral density were assessed through multiple linear regression, including age, height, weight, calcium intake, smoking, and physical activity.

Results and conclusions: We here demonstrate, in a large well-characterized cohort that age was not an independent predictor of bone mineral density of the lumbar spine, femoral neck, or total body, indicating that peak bone density has been achieved in these skeletal sites, while it was an independent predictor of BMD of the radius, suggesting that peak bone density is not yet attained in the long bones. pQCT analyses revealed that age was associated with cortical volumetric BMD and endosteal contraction of the radius and tibia. These results demonstrate that the age of attainment of PBM is site specific.

P042-Tu

Periosteal Apposition Rate with Age is Identically Low in Both Sexes: Cross-Sectional Measurements of Metacarpal Bones by Digital X-ray Radiogrammetry

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The increasing focus on cortical bone in the pathogenesis of osteoporosis has brought new information on sex- and age-related differences. The net loss in cortical bone is believed to be larger in women since the endosteal resorption is larger and the periosteal gain is smaller in women than in men.

In the present study, the cortical dimensions of metacarpal 2–4 were determined in 2090 postmenopausal women and 233 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/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, a very small periosteal apposition is seen in both sexes, but it is not known whether this is only a characteristic of the metacarpal bones or a general phenomenon. Measurements of the inner diameter revealed an annual loss of 1.42% in females, but only the half in males, see table below. The outer diameter is 15% larger in males, but there was found no sex difference in the (small) increase with age in the periosteal diameter.

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.

Table
Annual change in percent

	Females	Males
Cortical thickness	−1.00%	−0.56%
Periosteal diameter	+0.12%	+0.08%
Endosteal diameter	+1.42%	+0.72%
Bone area	−0.67%	−0.34%

P043-Su

Young Women's Bone Mineral Density Changes and their Lifestyle Behaviours; A Grounded Theory Analysis

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Background: Limited information is available on healthy young women's own perspective of lifestyle behaviours. By lifestyle behaviours, e.g., smoking and physical activity, individuals have the possibility to influence bone mineral density (BMD). The aim of this study was to generate a theoretical model of lifestyle behaviours among young women with different BMD changes.

Methods: Data were collected through interviews with 11 women, and the material was analysed by means of the grounded theory method.

Findings: Two core categories were generated (1) the respondents outlook on life and (2) their life situation. The respondents' outlook on life consisted of two categories, either a rigid outlook on life or a relaxed outlook on life. Respondents who had a rigid outlook on life were adjusting to others and had a decreased BMD while respondents with a relaxed outlook on life were doing things for fun and had an increased BMD change. Life situation also consisted of two categories: stagnation and development. Respondents in a static life situation did not pursue any active actions while in a developing life situation the respondents were actively striving towards a goal. Four dimensions emerged which characterised the respondents' outlook on life in relation to their life situation: subordinating and enduring with a decreased BMD change or

compromising and discerning with an increased BMD change.

Conclusion: It seems as if outlook on life has a greater influence than the acted lifestyle behaviour for bone development. Further research is needed to be able to generalise the findings of this study and to explore the importance of outlook on life among women all ages.

P044-Mo

The Gender Difference in Bone Strength Continues to Increase into Old Age

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Osteoporotic fractures are more common among elderly women than men. This sexual dimorphism may elucidate important underlying mechanisms in bone fragility. We studied the interplay of bone density, bone size and geometry on bone strength estimates in the Age Gene Environment Susceptibility-Reykjavik Study (AGERS), an ongoing population-based study in Iceland. After excluding 588 individuals using drugs known to affect BMD or with evidence of fractures in L1 or L2, we compared quantitative computed tomography (QCT) measures of BMD, bone geometry and estimated bone strength in 807 men and 905 women, age 67–93. In lumbar spine (L_1 and L_2) we measured trabecular and integral BMD, cross-sectional area (CSA) and estimated compressive strength ($g^2/cm^4 = BMD^2 \times SA$). In the femoral neck, we measured trabecular and integral BMD, minimum cross-sectional area, estimated compressive strength, an estimate of bending/torsional strength (section modulus, polar cross-sectional moment of inertia divided by the neck width) and buckling ratio (half bone diameter/cortical thickness reflecting bone instability). In the trochanter, we measured trabecular and integral BMD, maximal cross-sectional area and estimated compressive strength. In the mid-femur, we quantified total cross-sectional area, medullary area and the buckling ratio. We used regression models to assess the effects of age and gender adjusting for current weight and measured height in mid-life for cohort effects in standing height. Results, see Table.

This cross-sectional study indicates that there is a gender difference in the rate of loss of bone strength in old age. This difference, which may be an important factor in the gender difference in osteoporotic fracture incidence, appears to be strongly related to the greater decline in BMD and cortical thickness in women. Age-related increases in bone size, however, were similar in both genders. Bone strength estimates seem to differentiate more clearly between sexes in this age group than conventional BMD measurements.

Supported by a grant by the National Institute on Aging and the Icelandic Government.

Measure		Age Δ (%) men/10 years	Age Δ (%) women/10 years
$L_1 + L_2$	Compressive strength	−6.5	−20.6**
Femoral neck	Compressive strength	−3.5	−14.5***
	Section modulus	−0.8	−4.2**
	Buckling ratio	+2.7	+7.8**
	Cortical thickness	−1.7	−6.2***
Trochanter	Compressive strength	−2.5	−16.5***
Mid-femur	Buckling ratio	+1.1	+5.5***
	Medullary CSA	+5.8	+16.7***

*Statistical significance of gender difference in age-related change, *<0.05. **<0.01, ***<0.001.

P045-Tu

High Dose of Bisphosphonate Enhances the Effect of PTH on Trabecular Bone in Ovariectomized Rats

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Osteoporosis can be therapeutically approached in two different ways: by use of anti-resorptive agents or by use of anabolic agents. Nowadays, anti-resorptive agents like bisphosphonates, estrogens, and SERMs are widely used to prevent menopause-related bone loss. However, these treatments are only limited in preventing bone loss and do not restore the lost bone. Daily administration of parathyroid hormone (PTH) has been found to possess this anabolic quality and is therefore a promising agent to treat osteoporosis. However, PTH withdrawal results in a rapid loss of newly gained bone. Preclinical studies have been described in literature to address the hypothesis that there may be an advantage to using PTH in combination with anti-resorptive agents. The results published so far are inconclusive, partially due to the large variety of different experimental set-ups used. In the present study, we evaluated the effect of PTH (16.5 μg/kg/day sc), ethinyl estradiol (EE, 2 μg/kg/day sc) and bisphosphonate (BP, 200 μg/kg/day sc) on the bone mass and bone strength of ovariectomized female rats (OVX). Sixty-four 3-month-old female Wistar rats were ovariectomized and randomized in 8 groups ($n = 8$). The animals were left untreated for 2 weeks to await development of moderate osteopenia. The sham-operated and the control OVX animals were treated with vehicle starting two weeks after surgery, while other groups (all OVX) received EE, a high dose BP, PTH, or a combination of PTH plus either EE or a high dose BP. All animals were sacrificed after 6 weeks of treatment. Both femurs were collected for bone mineral density measurement by pQCT and biomechanical testing by use of an indentation test, three-point bending test and cantilever bending test. In addition, the fourth lumbar vertebral body (L4) was dissected for a compression test. Our study demonstrated that treatments with either BP or PTH alone,

or combined treatment of PTH with EE or BP significantly restored the ovariectomy-induced bone mass and bone strength loss in rats. Combined treatment of PTH with BP strongly augmented the effect of PTH alone. In conclusion, these data suggest that a bisphosphonate and PTH may act synergistically on bone mass bone strength in rats. This is in contrast to a recent clinical study that failed to demonstrate any additive effect of the combined treatment of both agents in women. Further animal studies to explain this discrepancy are ongoing.

P046-Su

Absolute Measurement of Bone Mass from Whole Bones of Women and Men, 20 to 90 Years of Age

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Our approach is to improve methods by devising new ways of measuring losses in bone mass at the organ level. Understanding the skeletal system and osteoporosis is complicated conceptually due to various parameters that describe the skeleton. Physically, there is a distinct difference for accuracy between absolute mass and relative mass. Most radiological measurements are a relative index of bone mass via bone mineral density (BMD) and are presented as a ratio, g/cm^2, g/cm^3.

Absolute bone mass is the "gold standard" because it is derived directly from a physical weighing of each whole bone, an intact anatomical unit. Prior to weighing, each bone was dried an defatted, now it is absolute weight. The rationale of measurement is (1) to determine which bones are the most sensitive in losing or gaining mass, (2) to compare them with other bones, and (3) to provide a reference standard for all bones and all ages. Human cadavers are from the Todd Collection of the Cleveland Museum of Natural History and were devoid of skeletal disease and/or generalized wasting.

Four male bones (femur, humerus, scapula, vertebrae) and ten female bones (skull, pelvis, femur, tibia, humerus, scapula, ulna, radius, fibula, clavicle). Our results demonstrate that the female bones from 20 to 90 years of age showed larger and similar losses of mass (femur 13%, humerus 16.4%, scapula 15.6%). The male bones showed small but linear losses (2–3%) starting later in the fourth decade. In contrast, the female bones showed larger linear losses (11–12%) starting in the third decade. Bone losses from ten female bones were plotted to 90 years as % losses of life span.

The graphs showed that the bones had a linear decrease. All slopes did not increase with age.

Skull and humerus showed a greater slope; femur, radius, fibula, and pelvis a smaller slope. The female bones peaked at 25–30 years of age. The final average of female losses was 40%. The average losses for 3 vertebrae were 28.5%, 2 axial bones 33%, and 5 appendicular bones 40%.

P047-Mo

Spine and Femur Bone Mineral Density in Healthy Tunisian Women

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Osteoporotic fracture risk in females is associated with peak bone mineral density (BMD) attained in young adulthood and subsequent bone loss related to menopause and aging. Determination of BMD for age in a healthy population is necessary to evaluate BMD status in patients. BMD reference data are available for a number of populations throughout the world, but reference data for the North-African population are lacking. We used dual-energy X-ray absorptiometry (DXA; GE Lunar Prodigy) to measure BMD at spine and hip in 1225 Tunisian women aged 20–89 years. Healthy women with no diseases or therapies known to affect bone were selected from two regions, Ariana and Manouba, with demographic characteristics similar to the whole of Tunisia. Mean (SD) age, height, and weight were 52.5 (14.1), 155.3 (7.7), and 70.5 (14.7), respectively. Peak BMD at spine, and femur sites were attained by about age 30–35 years. Young normal (YN) reference means and standard deviations (SD) were determined for subjects age 20–40 years, and compared with YN reference means for Middle East and USA/Northern Europe (USA/NE) (GE Lunar reference data). Tunisian female BMD was 3 to 10% higher than Middle East BMD at the spine and hip, and about 4% higher than USA/NE BMD at femur sites, but about 4% lower than USA/NE at the spine. These locally derived, population-specific reference data will allow accurate assessment of *T* scores and *Z* scores in Tunisian subjects.

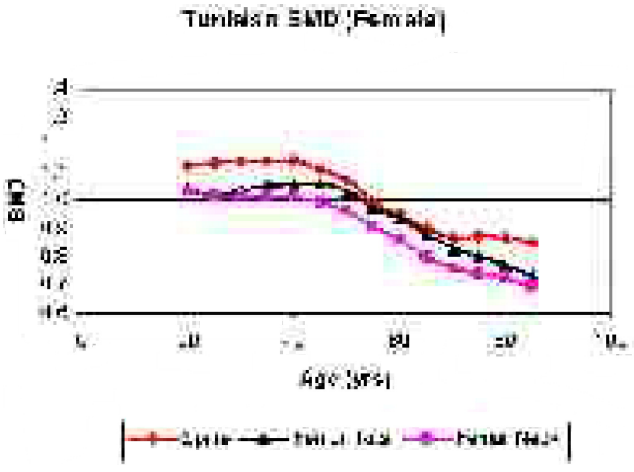

Table
Normal BMD values for hip and spine

YN BMD (SD) g/cm^2	L1–L4	Neck	Trochanter	Total femur
Tunisia	1.137 (0.12)	1.019 (0.12)	0.823 (0.12)	1.044 (0.13)
Middle East	1.101 (0.12)	0.930 (0.12)	0.755 (0.11)	0.940 (0.12)
USA/NE	1.180 (0.12)	0.980 (0.12)	0.790 (0.11)	1.00 (0.12)
Diff. vs. Middle East	3.1%	9.1%	8.6%	10.4%
Diff. vs. USA/NE	−3.9%	4.2%	4.4%	4.7%

P048-Tu

Dual Femur Densitometry in a Czech Population: Effect on Diagnosis Decisions

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The proximal femur is the best site for predicting risk of hip fracture, the most incapacitating and costly of all osteoporotic fractures. Hip fracture risk nearly triples for every standard deviation decrease of femur bone mineral density (BMD). Discordance of BMD between right and left femora is not uncommon, leading to the possibility of missed diagnosis if only one femur is measured. Recent technology enhancements allow rapid scanning of both hips in one acquisition and eliminate time-consuming repositioning. At our osteoporosis center, we measure routinely both femora to have at least baseline femur BMD values for one side in case the patient has a future hip surgery/fracture. The precision error of a dual femur BMD is also better. In this study, we evaluated if discordance between left and right femur BMD measurements exists in the Czech population and the effect on diagnosis.

We measured right and left femur BMD with the Lunar Prodigy (GE Healthcare) in 24716 Czech women (mean age 60.7 years; 20.1 to 95.1 years). Subjects had an average weight of 69.7 ± 12.7 kg and average height of 163.4 ± 6.3 cm. They were classified as osteoporotic ($T < -2.5$), osteopenic ($-2.5 < T < -1.0$), or normal ($T > -1.0$) using WHO guidelines. Paired t tests were used to identify significant side-to-side BMD differences.

There was no significant difference in average left and right total femur (TF) BMD but average femur neck (FN) BMD was significantly different between left and right side ($P < 0.001$). T scores differed by more than 0.5 units in 22% (FN) and 16% (TF) and by more than 1.0 unit in 4% (FN) and 2% (TF) of all subjects. Among the 8601 women over age 65, 23% of FN and 19% of TF T scores differed by more than 0.5 units between right and left sides, while 5% of FN and 2% of TF T scores differed by more than 1.0 unit. Diagnosis (normal, osteopenia, osteoporosis) differed with right vs. left femora in 29% of all women at one or more sites, and in 16% and 13% of subjects for FN and TF, respectively. For women over 65 years, diagnosis discordance occurred for 21% at FN and 18% at TF. In comparison to reported values in similar studies (Vargas Decamps JC Osteoporosis Int. 2004, 15, S40; Cole RE JBMR 2004, 19, S368), Czech women seem to display higher diagnosis and T-score discordance between left and right femora.

We conclude that dual femur measurements enhance accurate clinical decision-making, especially in Czech women over age 65.

P049-Su

Expression of BMP-3,-7 and CDMP-1,-2 in Normal and Osteoarthritic Human Articular Cartilage

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With aging, the responsiveness of articular cartilage to different growth factors is changed. Further, osteoarthritis is a joint disease affecting primarily the elderly. In this study, we were interested in the expression of BMP-3,-7 and CDMP-1,-2 in normal articular cartilage (young people), in macroscopically normal articular cartilage of elderly people and in osteoarthritic cartilage. All these morphogenetic proteins are members of TGF-beta superfamily and are involved in bone and cartilage homeostasis. The cylindrical cartilage–bone samples were dissected from tibial condyles of donors with no history of joint disease during authopsy and from patients with knee joint osteoarthritis. The samples were decalcified and embedded in paraffin wax. Sections were processed for histology and immunohistochemistry. In normal articular cartilage, we found the expression of CDMP-1,-2 only in the chondrocytes of all three uncalcified layers. The expression of BMP-3,-7 was negative. With aging, the expression pattern of these morphogenic proteins was changed. In the tangential layer, we found very strong pericellular reaction for BMP-7 and CDMP-1,-2. Deeper, in the transitional layer, we found strong expression intracellularly and pericellularly for the same proteins. Finally, in the radial layer, positive expression was found in the chondrocytes with very pale reaction pericellularly. The expression pattern for BMP-3 was the same but the reaction was less effective. In osteoarthritis, articular cartilage lost its morphological structure and the chondrocytes were in the form of clusters. We still found the expression of BMP-3,-7 and CDMP-1,-2 but weaker and only intracellularly in the cells of clusters. Weak positive pericellular reaction was found only for BMP-7. Our results show that, with aging, the expression pattern of these morphogenetic proteins is changed and the activity of these proteins increases. In osteoarthritis, the expression still exists but was weaker and remains as residual activity.

P050-Mo

Vitamin D3 at the 1500 IU/day Dose Comparing Daily, Weekly, or Monthly Administration to Elderly

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Appropriate vitamin D status decreases hip fracture risk by 30%, yet adherence of elderly hip fracture patients to daily calcium and vitamin D supplements is very poor. A recent consensus about the role of vitamin D3 in the prevention of hip fractures in the elderly concludes that serum 25-hydroxyvitamin D [25(OH)D] concentrations should be higher than 30 ng/mL (75 nmol/L). A monthly protocol of vitamin D during post-fracture hospitalization may be an efficient strategy for vitamin D supplementation. We wanted to find out (A) whether the daily, weekly or monthly use of the same total dose would produce the same response, and (B) whether 1500 IU/day of vitamin D would ensure that elderly Israeli hip fracture patients achieve 25(OH)D levels higher than 30 nmol/L. We randomly allocated 48 patients (age 81 ± 8 SD years) to the same total dose of vitamin D3, but given as 1500 IU daily, or 10,500 IU once weekly, or 45,000 IU once monthly for 8 weeks. The baseline mean serum 25(OH)D was 19 ± 9 SD ng/mL. By the end of the 8-week protocol, the mean increase in 25(OH)D was 17 ng/mL, and the table here shows separately, the increase for each strategy for vitamin D supplementation. The results confirm that in terms of clinical response, monthly administration of vitamin D3 is equivalent to the more laborious, daily supplementation with vitamin D. The monthly regimen for vitamin D supplementation is a simpler and practical way to provide vitamin D. By the end of 8 weeks, however, even with the equivalent of 1500 IU/day of vitamin D3, the final 25(OH)D concentration was less than 30 ng/mL in 62.5% of the patients. Thus, much more than 1500 IU/day of vitamin D3 are required to ensure that, within 2 months, all elderly have 25(OH)D higher than the minimum level desired by a panel of experts.

Table
Effects of dosing strategy on 25(OH)D response

Supplement strategy	*N*	25(OH)D ng/mL, mean increase	25(OH)D ng/mL, lower 95% CI	25(OH)D ng/mL, lower 95% CI
1500 IU/day	16	18	14	23
10,500 IU/week	17	13	7	20
45,000 IU/month	15	21	17	25

No significant differences in 25(OH)D responses.

P051-Tu

Histological Examinations on Bone Augmentation by a Combination of a Bioresorbable Plate and Bone Filling Hydroxyapatites

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Objectives: When attempting to augment defective bone, one of the primary goals is the establishment of new bone grown up to, or higher than the previous bone surface. Expecting preferable bone augmentation, we have examined histological alterations of the bone defects with the usage of a combination of a bioresorbable barrier plate and hydroxyapatite graft.

Materials and methods: Bone defects surgically-formed in rat calvariae were filled with Boneject® including atelocollagen and authentic hydroxyapatites, and then covered with a molded bioresorbable plate: DeltaSystem®. The rats at 1, 2, and 4 weeks post-operation were fixed with aldehyde solution, and the bone defects were examined for alkaline phosphatase (ALP), tartrate-resistant acid phosphatase (TRAP), osteopontin, osteocalcin, and dentin matrix protein (DMP)-1.

Results and discussion: At 1 week, new bones with characteristics of woven bones extended from the bottom of the defects, partially surrounding the particles of hydroxyapatites. ALP-positive cells were localized not only on the new bone, but also directly on the grafted hydroxyapatites, the surfaces of which revealed osteopontin and osteocalcin immunopositivity. Taken together, accumulated bone proteins on the surface of hydroxyapatites, i.e., osteopontin and osteocalcin, may be involved in osteoblastic migration and attachment, permitting osteoconductivity of this material. Young osteocytes in the woven bone did not show DMP-1 whereas osteocytes in intact bones showed intense immunoreactivity. Fibrous tissue underlying the resorbable barrier plate encapsulated these newly formed bones as well as grafted hydroxyapatites. At 2 weeks, the newly formed bone almost encompassed the particles of hydroxyapatites, reaching close to the bioresorbable plate. However, the fibrous tissue had still intervened between the plate and the newly formed bone. There were many ALP-positive osteoblasts and several TRAP-positive osteoclasts on the surface of the new bone, indicating bone remodeling. At 4 weeks, the newly formed bone became compact bone, in which osteocytes showed the DMP-1 immunoreactivity. Interestingly, the fibrous tissue had disappeared, and instead was replaced with ALP-positive cell layer. In some portions, the new bone was directly attached to the plate. Thus, the combination of hydroxyapatites and a

bioresorbable plate appears to enable preferable bone augmentation.

P052-Su

Quantitative Fluorescence Analysis of Bone Deposition After Doxycycline Administration During Endodontic Therapy—A Pilot Study

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Aim: Beside its antimicrobial properties, doxycycline is known as inhibitor of bone resorption, as well as promotor of bone formation, due to its effect on osteoclasts and osteoprogenitor cells. Doxycycline is also used as a fluorescent marker for bone deposition. As a sequel to previous studies, the aim of this research was to analyze bone deposition quantitatively during initial phases of periapical lesion healing, after root canal therapy combined with doxycycline administration.

Methods: Periapical lesions were induced in 2 mongrel dogs by exposing lower premolar pulps to the oral environment. After 35 days of pulp exposure, five root canals of each animal were instrumented and filled to the apical delta, which was confirmed radiographically. On the same day, one animal (group 1—control group, 5 roots) received intraperitoneal injection of vital dye Procion Brilliant Red. Second animal (group 2—doxycycline group, 5 roots), after endodontic procedure, received 10 mg/kg doxycycline per os daily, for 12 days. Animals were sacrificed 70 days after pulp exposure, and mandibles embedded in methylmetacrylate. Undemineralized unstained sections 5–7 μm thick were analyzed for traces of Procion Brilliant Red (group 1) and doxycycline (group 2) using fluorescent microscope. Histomorphometric index of mineralizing suface, as a fraction of resorbed bone surface circumscribing periapical lesion, was measured using computer program (Issa, VAMS, Zagreb, Croatia). Statistical analysis was performed by Mann–Whitney U test.

Results: Analysis of sections, marked with Procion Brilliant Red dye in control group (group 1), and doxycycline in group 2, showed fluorescent lines marking new bone formation in both groups. There was statistically significant difference ($P = 0.01$) in mineralizing surface as a fraction of resorbed bone surface between control group (28,40%) and doxycycline group (66.97%) (Table).

Conclusion: Administration of doxycycline enhanced healing potential. This was demonstrated by greater mineralizing surface as a fraction of resorbed bone surface, in doxycycline group. This result corresponds to the results of quantitative analysis of osteoid surface, osteoid thickness, and osteoclast index, after doxycycline administration during endodontc therapy, presented in previous studies.

Table
Mineralizing surface as a fraction of bone surface

Group	No. roots	Median	Percent. 5%	Percent. 95%
Control group 1	5	28.40%	17.39%	47.46%
Doxycycline group 2	5	66.97%	62.50%	75.46%

P053-Mo

In Vivo Micro-CT: Optimizing Image Quality, Scan Time, and Radiation Dose: What can be Realistically and Safely Achieved?

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For in vivo micro-CT to become a useful tool in bone research, the technical challenge is to achieve imaging quality necessary to obtain the required morphometric or density information, with an acceptable scan time and radiation dose. Some technical measures to help achieve these goals include use of polystyrene foam holders to optimize signal-to-noise ratio, and cone beam scanner architecture for fastest scanning of large sample areas. Also, multi-computer cluster reconstruction enables fast processing of large-format, high-resolution images. Further, image analysis and segmentation technology help to optimize data obtained from noisy images (Waarsing et al., 2004). Making use of these measures, it is possible to obtain useful morphometric images and data of both mouse and rat trabecular bone in 20 min in vivo scans (shorter for cortical bone). The radiation dose delivered locally during a 20-min hindlimb scan in a cone-beam desktop in vivo scanner (Skyscan™ 1076) is about 400 mGy. The corresponding effective dose equivalent (employing human tissue weighting factors from ICRP 60) is only 1.8 mSv. However, the critical factor is likely to be local absorbed dose and whether this causes killing or inhibited mitosis in bone forming chondrocytes. Cell biology studies suggest that chondrocyte proliferation is affected by a minimum dose of about 1000–2000 mGy. So a single 20-min scan should not significantly affect bone growth. But repeated scans might give an elevated cumulative dose depending on the interval between scans, and the DNA damage repair rate and consequent “decay” of radiation damage. Experience with in vivo scanning to date (comparing scanned with unscanned limbs) suggests that scans separated by 3–4 weeks do not significantly alter bone growth, but that scans separated by only a week do reduce it. However, more experimental work is needed to address accurately and in detail the scanning dose and frequency that can be tolerated by experimental animals without compromising bone studies by inhibiting bone growth. It is likely that overly ambitious protocols involving multiple closely spaced scans should not be recommended.

Reference:
[1] Waarsing JH, Day JS, Weinans H. An improved segmentation method for in-vivo micro-CT imaging. J Bone Miner Res 2004;19(10):1640–1650.

P054-Tu

A Comparative Study of Ultrastructure of Bone and Ceramic Hydroxyapatite by Means of X-ray Crystal Analysis

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All materials used for filling bone defects though produced in many different ways are hydroxyapatite (HA) materials by chemical structure; however, there was no comparative assessment of filling materials and bone minerals which is necessary for making better conditions of implant rebuilding. Thus, the study was aimed at comparing of crystallographic features of ceramic HA (CHA) and bone HA (BHA) by means of X-ray crystal analysis in order to improve technologies of HA production.

BHA from the study was taken from rat tibiae (all animals were with the initial mass of 130–150 g). The observation terms were from 7 to 180 days (42 animals total). CHA was kindly presented by CERHAP (Kiev, Ukraine). X-ray analysis was performed using X-ray diffraction device DRON-3,0 with goniometric attachment GUR-5. Radiation-Cu-Kα.

The sizes of BHA elementary cells during observation period along axes C and A increased, respectively, from $6.890 \pm 0.005 \times 10^{-10}$ M to $6.902 \pm 0.003 \times 10^{-10}$ M and from $9.419 \pm 0.002 \times 10^{-10}$ M to $9.419 \pm 0.001 \times 10^{-10}$ M and sizes of coherent scattering blocks calculated in reflex (110) area also increased—from $49.59 \pm 0.69 \times 10^{-9}$ M to $50.37 \pm 0.74 \times 10^{-9}$ M. Such changes may be considered as a start of normal aging processes. However, microtexture index, which characterizes uniformity of orientation of the crystals in lattice in the same terms varied in range from 0.3857 ± 0.0144 units to 0.4072 ± 0.0194 units. This provides the evidence that, in mature animals, the processes of bone formation and bone resorption still remain in dynamic balance.

The sizes of elementary cell of CHA along C and A axes were $6.883 \pm 0.002 \times 10^{-10}$ M and $9.416 \pm 0.002 \times 10^{-10}$ M, respectively, which is insignificantly lower the same values of BHA. A size of coherent scattering blocks was $44.25 \pm 0.92 \times 10^{-10}$ M which is significantly lower than BHA values (by 10.79–12.16% depending on observation term). A microtexture index was 0.5042 ± 0.0057 units which is significantly higher than in bone mineral.

The study demonstrated that BHA crystals are significantly larger than CHA crystals and microtexture index on the contrary is significantly lower. This results from simultaneous processes of bone formation and bone resorption in turn leads to an increase of sizes of the crystals due to amorphous layer growth and a decrease of degree of crystal orientation in the lattice.

P055-Su

Mandible and Maxilla Volumetric Densitometry (VBMD) in Healthy Adults Assessed by Dental pQCT

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The XCT 3000 system has been adapted in order to perform scans at the head. Maxilla and jaw can be comfortable assessed, in order to explore bone structural quality and volumetric bone mineral density. The region is of interest to odontologist and maxillofacial surgeons. Notwithstanding, the main reason for using the system is the identification of individual regional variations, reference values in healthy subjects are being requested in order to match differences with patients affected by mechanical or hormonal disturbs. A group of 85 healthy adults was studied by pQCT (XCT 3000-D system—Stratec, Germany), and positioned according to Capiglioni R et al. (Diagnóstico 1998, 7:898). Small cortical and medullar sections (167 ± 11 mm^2) were studied by threshold analysis in order to obtain statistical position and parametric values ($x \pm$ SEM) of main variables for both genders. Mean values are given in the table below.

Density inter-individual coefficient of variation was high in area (36–39%), but low in volumetric density (3.4–7.9%) explained by (r^2) 0.46 ($P < 0.01$) in cortex and 0.62 in medullar sites. Hence, patients with same bone volume may differ in their true density. The system provides individual diagnosis of bone variables and may allow the independent follow-up of densitometric and geometric parameters.

Table

Site (threshold) (mg/cm^3)	% Area (threshold/total)	Density
Cortex (900)	30.0 ± 2.2	1134.6 ± 8.1
Medullar (400)	24.8 ± 1.8	544.2 ± 8.6

P056-Mo

Identification of the Elemental Composition of Metal Particles from a Failed Joint Prosthesis by Laser Ablation Inductively Coupled Plasma Mass Spectrometry

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The purpose of this study was to investigate the potential of Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) to identify metal particles in connective tissues from a failed surgical implant.

Samples of connective tissues obtained at open operation from the area surrounding a painful, failing, shoulder prosthesis were obtained. The shoulder prosthesis was composed of a HDPE glenoid component with a cobalt–chromium alloy stem and head. In order to enhance osteointegration, the metal stem component had been coated with titanium by plasma deposition. At operation, the tissues were clearly abnormal, of a gray color and contained necrotic tissue debris. The samples were LR White (Hard Grade) embedded and sections taken for histological examination. On section and staining a macrophage response with giant cell formation was evident. The macrophages contained numerous intracellular black particles. On the surface of the block, it was apparent that there were also larger (>50 μm) pieces, of what appeared from their shiny surface to be metal. The surface of this block was used for this investigation without further treatment.

The technique of LA-ICP-MS is an analytical beam technique whereby a 4-μm diameter laser beam (213 nm) is used to vaporize the surface of any material in a controlled, precise manner. The vaporization takes place in a sample chamber which has a controlled argon flow passing through it. The entrained vaporized sample is then carried to the argon plasma (8000K) where it is ionized. The ions are analyzed in a quadrupole mass spectrometer.

Single line scans through the intracellular metal fragments show their composition to be chromium–cobalt alloy, identical to the metal composition of the implant. The elemental composition of the larger particles seen in the block surface was shown to be titanium, which was different to the intracellular debris. These findings demonstrate the ability of LA-ICP-MS to spatially resolve the elemental composition of connective tissues.

P057-Tu

Nanosims as an Analytical Tool for Trace Metal Detection in Bone and Liver from Hemodialysis Patients

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The purpose of this study is to investigate the potential of the NanoSIMS instrument in the identification of metals in bone and soft tissues at an ultrastructural level coupled with high sensitivity to PPB levels. It is the intention of this submission to demonstrate the capabilities of the instrument and not to demonstrate the presence of aluminum in bone as this has been described earlier. The technique of NanoSIMS was developed by CAMECA Paris and the first instrument installed in St. Louis, USA in 1999. There are 10 instruments in the world to date. The ability to extend the SIMS analysis to extremely small areas or volumes (50 nm beam diameter cesium, 150 nm oxygen) while maintaining extremely high sensitivity at High Mass Resolution is possible. Five masses or elements can be collected simultaneously by the instrument allowing true superimposition of ion maps.

Bone and liver specimens from an end stage renal failure patient treated by hemodialysis and receiving aluminum containing oral phosphate binders and whole blood transfusion were used as test specimens. They were formalin fixed and Araldite embedded. A block 10 mm diameter and 100 μm thick was taken, polished by diamond abrasion techniques and gold coated.

Using a 400-nm diameter primary negative oxygen beam areas 40 × 40 μm were examined by raster mapping.

There was significant deposition of iron and moderate deposition of aluminum in the liver specimen; however, there was limited co-localization of the two metals.

In contrast to the intracellular deposition of the metals in liver, the accumulation of the metals in the bone was concentrated in the matrix of bone in a lamellar or banded pattern. The metal accumulation was mainly within bone concentrating at the cement or reversal lines.

The NanoSIMS instrument offers the ability to map ions in mineralized and non-mineralized tissues at extremely low concentrations <1 ppm with a spatial resolution between 50 and 150 nm depending on the ion beam; no other instrument has this capability.

P058-Su

The Suitability of Cartilage Turnover Markers to Assess Changes in Chondral Callus Tissue during Fracture Healing

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The occurrence of delayed union during fracture healing remains a major clinical problem resulting in a prolonged healing period and a marked intervention rate. There is strong evidence that patients with delayed fracture healing most often show a regular initiation of the fracture healing cascade followed by an extension of the chondral callus phase in combination with a retardation or even total cessation of the mineralization process during endochondral ossification. The aim of this study was to analyze the suitability of cartilage turnover parameters to monitor the course of chondral callus tissue during fracture healing serologically. Therefore, markers of cartilage formation as well as degradation were analyzed over the course of fracture healing in a standardized ovine fracture model.

Three-millimeter diaphyseal bone defects were created in the tibia of thirty-two 2-year-old female sheep and stabilized with an external fixator. Cartilage oligomeric matrix protein (COMP) and the C-terminal propeptide of collagen type II (CPII) were analyzed over a 9-week healing period. The amount of cartilage within the callus was evaluated immunohistologically at 2, 3, 6, and 9 weeks following surgery. Additionally, the course of fracture healing was monitored radiographically. The histological and radiographical results demonstrated callus formation without complication for all bone defects following the path of secondary fracture healing. Cartilage formation increased until the third postoperative week and was then followed by a decrease in the cartilaginous callus area. The serological level of CPII increased during the first four postoperative weeks while the COMP level increased between weeks three and seven postsurgery. Although a standardized study design was used, the serological parameters showed broad inter-individual variations in addition to an individual response to the fracture situation. Nevertheless, the serological course of the two markers correlated with the histological course of fracture healing. The observed cartilage turnover parameters reflected the formation and degradation of chondral tissue during fracture healing. Thus, they might serve as an additional clinical tool to monitor the early course of fracture healing. Further analyses will have to verify if this new approach is suitable to discriminate between normal and delayed fracture healing and to detect disturbances during fracture healing at an early time point.

P059-Mo

Simultaneous Determination of Densitometric, Geometric, and Histomorphometric Parameters of Femoral Neck from pQCT Scans by a Dedicated Image Analysis Program. Implications for the Prediction of Fracture Risk

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Femoral neck fracture is the critical outcome of osteoporosis and the risk of its occurrence can be predicted from non-invasive assessment of the parameters defining the mechanical competence of the femoral neck. Current resolution of pQCT scanners (70 μm) might allow to obtain simultaneously geometric, densitometric (volumetric, v) and histomorphometric parameters (Banse et al., Bone 30:829–835,2002) for the whole human femoral necks, which cannot be derived by micro-CT due to sample dimensions (Jordan et al., Bone 32:86–95,2003).
The aim of this study was to develop automated image analysis software for a multiplatform public-domain image-processing package (Scion Image/NIH-Image), and determine the parameters of interest from pQCT scans of whole femoral neck sections obtained from patients (n = 14, age = 75.9 ± 9.4) undergoing hip replacement surgery for fractures and/or primary degenerative arthritis. Designed outputs included, besides standard BMDv (total, trabecular and cortical), standard histomorphometric static parameters: BV/TV, Tb.Th, Tb.Sp, Tb.N, Sp.N, and Connectivity Index (CI). A geometric criterion tailored for irregularly shaped bone samples was employed. The automated analysis was applied to all scanned samples with the aim to test the ability of the output parameters to distinguish fractured (F) from non-fractured (NF) donors. The algorithm had a high reproducibility, being nearly operator independent (CV = 2%). The maximum to minimum external diameter ratio of the sample (1.30 ± 0.06) resulted uniformly distributed in F and NF groups (no significant differences, $P = 0.1$), thus excluding biases due to different anatomical sites. Trabecular BMDv was significantly lower in F than in NF (67.25%, $P < 0.005$), whereas cortical BMDv did not significantly differ. Independent parameters of trabecular architecture such as Tb.N, CI, and BV/TV were significantly lower in F than in NF (respectively: 69.21%, $P < 0.05$; 43.41%, $P < 0.02$; 41.36%, $P < 0.01$). Tb.Sp was higher in F than in NF (191%, $P < 0.05$).
The parameters determined by the developed algorithm allowed to discriminate F from NF group. By simultaneously defining material and structural properties of the femoral neck, the current analysis method might improve the clinical estimation of fracture risk in the clinical setting when applied to QCT or NMRI scans.

P060-Tu

Quantitative Analysis of Bone and Cartilage Tissue Structures using Birefringence Imaging System

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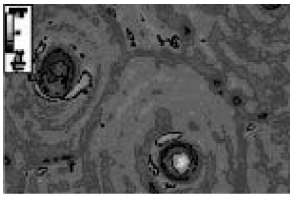
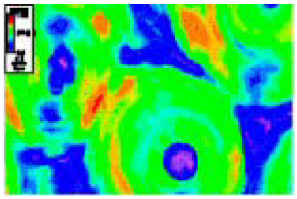
Optical microscopy is a conventional method but is often regarded as lacking necessary sensitivity and reliability for the tasks of quick, accurate, and convenient image data collection for bone and cartilage structure analysis. MetriPol, a new imaging technique, has emerged from a long period of research and development to fill the gap. MetriPol system collects multi images and resolves out hypothetical transmission, which excludes the anisotropic perturbation in real bone image. It also presents the color-coded pixelized data images of both birefringence and its orientation, neither of which can be derived by a standard microscope. Changes of bone structure, caused by growing, aging, injury, and particularly bone diseases can raise optical anisotropy, predominantly birefringence, locally or over the whole bone. The MetriPol system with a small mechanical attachment and a PC windows software can catch minute amounts of such change with its powerful resolution in retardance (best <0.02 nm). Figures (a) to (c) show the false

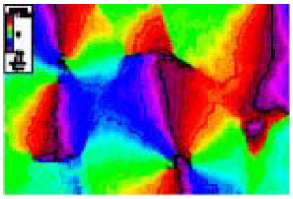
color images of normal bone structure and figures (d) to (f) show that of normal and osteoarthritic articular cartilage. These invaluable images and plots add extra data to conventional optical microscopy and these data are also significance in data mining and networked diagnosis and presentation.



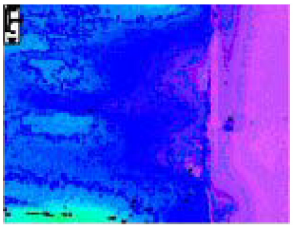
a) Transmission image of normal bone section



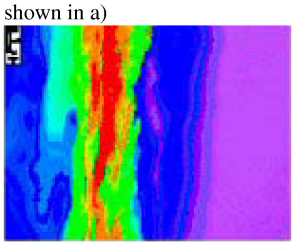
b) Birefringence image of what shown in a)



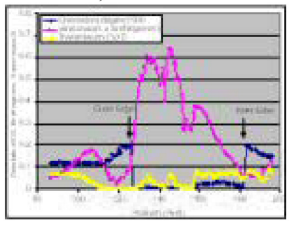
c) Orientation image of what shown in a)



d) Birefringence image of normal articular cartilage



e) Birefringence image of osteoarthritic articular cartilage



f) Quantitative analysis of the left: plots against a pixel line

P061-Su

Processes Used to Purify Human Bone Allografts Alter Matrix Surface and Cytocompatibility

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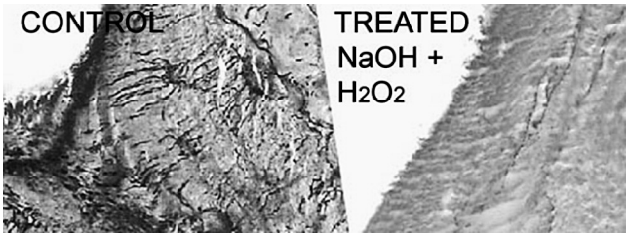
Bone allografts are used in orthopedic surgery for filling bone defects. Different industrial processes exist to purify allergenic bone, providing secured and cleaned bone blocks. However, such processes often make use of chemical regents that can be aggressive for the bone matrix and alter surface properties of the graft. The quality of chemically processed allografts has never been evaluated. We have compared the effects of maceration techniques commonly used in industry using $NaHCO_3$, H_2O_2 (for removing cells, fat, and uncalcified organic components), or NaOH (also recommended for its virus lytic properties, e.g., AIDS).

Femoral heads from osteoarthritic patients were used. The effect of one (or a combination of) chemical treatment on organic and mineral phases of the bone matrix were evaluated by histology, atomic force (AFM), SEM and TEM microscopy. Alteration of matrix proteins was searched by histochemistry. Cytocompatibility was evaluated by coculturing with human osteoblast-like cells (SaOS-2).

Collagen fibers were dramatically altered at the surface of bone treated with H_2O_2, NaOH and their combination, but not with $NaHCO_3$. A marked reduction of the number of hydroxyapatite crystals was observed in TEM, and morphological changes of bone surface were evidenced in SEM and AFM, particularly after NaOH treatment. Nevertheless, NaOH did not alter the attachment and spreading of SaOS-2 whereas H_2O_2 provoked cell death. Argyrophilic proteins of the bone matrix were removed by chemical treatments.

The use of chemical reagents dramatically altered matrix integrity by modifying collagenous and non-collagenous proteins. Whether these changes have clinical consequences on the bone bonding and osseointegration in human necessitate further investigations.



P062-Mo

Risedronate's Antifracture Efficacy is Independent of Baseline BMD Level

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Background: Several large studies have now shown that the majority of fragility fractures occur above a −2.5 *T* score. Indeed, the WHO commentary on the threshold for intervention is not the same as the diagnostic threshold for osteoporosis. Currently, there is little available information concerning the efficacy of osteoporotic therapies in *T*-score ranges above the diagnostic −2.5 level.
Objective: This study investigates the fragility fracture efficacy (vertebral and nonvertebral) of risedronate 5 mg/day in postmenopausal women over 4 ranges of femoral neck (FN) *T* scores.
Methods: This analysis included 2575 postmenopausal women from 4 phase III trials: VERT MN and NA, BMD MN and NA. Women were randomized to receive placebo or risedronate and treated for up to 3 years. Subjects were stratified into 4 subgroups based on level of baseline FN *T* score. Fractures included in the analysis were vertebral and nonvertebral (composite of 6 sites). The fracture incidence and relative risk reduction for each baseline BMD range are summarized for each subgroup.
Results: In the four BMD strata, fracture incidence in the placebo group increased with decreasing baseline BMD values, potentially confounded by increasing age and an increased percentage of patients with prevalent vertebral fractures (PVF). Risedronate, however, demonstrated significant anti-fracture efficacy in postmenopausal women, regardless of baseline level *T*-score ranges (Table 1 lists osteoporotic fracture incidence in placebo and risedronate patients with baseline FN *T*-score data < −1.5 [VERT-MN and NA, BMD-MN and NA]).
Treatment by *T*-score interaction was $P = 0.653$.
Conclusion: Although baseline BMD is an important predictor of fracture risk, Risedronate's demonstrated efficacy against fragility fractures appears to be independent of baseline BMD level.

Table

B-line *T* score	*N*	Mean age	Percent PVF	Placebo incid.	Rised. incid.	Hazard ratio	*P* value
−1.5 to −2.0	609	67	63.1%	20%	14%	0.58	<0.05
−2.0 to −2.5	822	68	68.7%	275	19%	0.63	<0.01
−2.5 to −3.0	682	69	78.4%	36%	26%	0.61	<0.005
−3.0	462	71	87.7%	46%	27%	0.44	<0.001

Osteoporotic Fracture Incidence.

P063-Tu
HPLC Determination of Pentosidine as a Molecular Indicator of Glycoxidation and Protein Cross-Linking in Diabetic Patients

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Introduction: Diabetes mellitus is a chronic disorder affecting metabolism of carbohydrates, proteins, and fats with hyperglycemia. Type 1 diabetes mellitus (DM1) is insulin-dependent autoimmune disease which occurs mainly in young patients while DM2 is not usually associated with increased blood insulin or autoimmune mechanisms and patients are usually older than 30. Nonenzymatic glycosylation of proteins belongs to one of the most important complications of DM. Glycosylation of collagen and other proteins with long biological half-life may produce irreversible advanced glycation end-products (AGEs). In this study, we have focused into determination of pentosidine (PEN), which is one of the best defined representative of the group of AGEs. PEN is a non-enzymatically formed cross-link of proteins with long biological half-life and belongs to new promising indicators of general collagen breakdown and glycoxidation loading of the organism. Because cross-linking of connective tissue proteins is not completely clear yet, determination of PEN seems to be useful contribution in this problem as well it is a good way for monitoring the intensity of glycation and oxidative processes in DM.
Aim: Utilization of HPLC for PEN determination and comparison of its serum levels in diabetic patients and control healthy group. Evaluation of these analytical results to elucidation of pathogenesis of diabetic complications.
Methods and patients: Serum samples from diabetic patients and healthy controls were analyzed for PEN content using very sensitive chromatographic method based on gradient reversed phase HPLC with fluorescence detection. The studied group consisted of 32 patients with DM1, 39 with DM2, and 16 control healthy subjects.
Results and Conclusion: Serum PEN concentration corrected to total protein in diabetic patients was increased in comparison to controls. Statistical significance ($P < 0.001$) was found in DM1 (1.02 nmol/g) vs. DM2 (1.88 nmol/g). Pentosidin determination seems to be a valuable tool for decription of metabolic pathways leading to complications associated with diabetes mellitus.
This work is supported by The Ministry of Health of the Czech Republic (grant No. NR/7895-3).

P064-Su
Coronary Artery Calcification in Dialysis Patients

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Introduction: Systemic calcifications are only one among many complications of complex pathogenic mechanism of chronic renal insufficiency. Sedimentation of calcium salts in the vascular myocardial structures, leads very early to cardiac dysfunction as well as sudden death of hemodialysis patients. Calcifications of coronary blood vessels present deposits of calcium salts in the endothelium of blood vessels and increase the risk for manifestation of coronary dysfunction. The aim of our study was to use noninvasive

computed tomography to detect arterial calcifications of coronary blood vessels.

Materials and methods: A total of 54 patients was included in our study aged 20–75 years (X = 52 ± 11.2, 22 males and 32 females). All patients were dialyzed three times per week for 4 h, and received from 4 to 8 g of calcium carbonate. The etiology of chronic renal insufficiency was diverse. Analyzed parameters showed no coronary disease in our examinees. In 30 patients, arterial hypertension was recorded, but was well regulated with adequate therapy. Heart CT was performed in all patients, as well as indirect PTH. Duration of hemodialysis treatment ranged between 4.2 ± 2.9. The second control group of patients included 60 examinees aged 25 to 80 years of age, X = 59 ± 9.3 without anginous difficulties. Statistical methods were calculated by using the Student test.

Results: Our results show calcifications of coronary blood vessels in 9 patients (6 males and 3 females) aged between 30 and 56 years. Calcification score was 0 in 47 examinees, mild in 4, moderate in 3 and severe in 2. CT showed no calcifications in the control group of patients. Age of patients was not a risk factor for the manifestation of calcification, statistical significance was recorded regarding duration of dialysis, and ratio Ca/P. Po.01. PTh. Values of triglycerides and cholesterol HDL were also not statistically significant.

Conclusion: Deposits of calcium in coronary blood vessels detected by CT can be screening for coronary heart disease of patients on hemodialysis. High level of P, a cardiotoxic factor, as well as Ca/P ratio are risk factors for the manifestation of PTH calcifications of coronary arteries causing the development of coronary syndrome. Heart CT can serve as a test in the detection of coronary disease in silent clinical picture of cardiac dysfunction in patients on hemodialysis, before deciding on coronarography.

P065-Mo

Clinical and Radiological Results Using Periosteum-Derived Osteoblasts for Sinus Augmentation

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For sinus augmentations prior to implant placement, various augmentation materials are described. This ranges from autogenous bone originated from various donor areas and numerous bone substitute materials. While the harvesting of autogenous bone is sometimes refused by the patients due to the necessary second surgery and possible problems on the donor sites, the indication for the use of commercially available bone substitutes is limited by various factors. Besides, the methods mentioned the use of periosteum-derived osteoblasts seems to be a promising alternative. In a licensed prospective study, 8 patients receiving a two-stage sinus augmentation were randomly selected and asked to participate in the clinical study. In the first stage, blood was harvested and processed in order to obtain the autogenous serum. Two days later, periosteum from the posterior mandible was harvested and brought in culture for 3 weeks in a GMP laboratory. During that time, praefibroblasts derived from the periosteum were proliferated. Then the obtained cells were seeded on a collagen matrix and differentiated to osteoblasts. In the second stage surgery, the maturated cells attached to the matrix were placed in the sinuses of the patients. 6 months after sinus augmentation, dental implants were inserted. During implant insertion using a trephine burr bone, histologies were obtained in the planned implant areas and the specimens processed in the laboratory. In light microscopy, in all cases, vital bone was observed. Using microradiography, a mineralized value of 38.2% ± 6 (female 32.6% ± 2.2, male 39.9% ± 6) could be found. The results indicate that the used method is a suitable alternative to the established augmentative methods.

P066-Tu

Electron Microscopy of the Effect of Fluoride Ions on the Apatite Crystal Formation During Osteogenesis

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The bone formation is accompanied by mineralization of carbonated apatite crystals. Although it has been generally accepted that excessive fluoride intake might affect the bone formation, however, there is little information on how to affect the apatite formation from the viewpoint of the crystal structure. In the present study, we focused on the initial stage of crystal formation in order to elucidate the effect of fluoride during bone formation. The male rats, weighing approximately 50 g, were used for this study. The rats were divided into two groups. The water containing fluoride 0.5 mg/l was given freely to rats of the experimental group. The fluoride-free water was given to rats of the control group. After 1 month later, the samples of calvaria were dissected from the rats. Then they were processed for transmission electron microscopy. Electron

micrographs have shown that the needle-shaped precursory minerals sandwiched by thin organic layers appeared at the mineralization front of the calvaria in both control and experimental rats. In the control group, we also observed that the initial lattice line of crystal appeared as nuclei within the mineral zone adjacent to the mineralization front. By contrary, no lattice image of crystallite was observed within in the experimental group. In addition, the formation of the initial lattice line could not be observed at the deeper area away from the mineralization front. Our results suggest that fluoride might inhibit the crystal nucleation at the initial stage of mineralization, although the needle-shaped precursory minerals surrounded by thin organic layers were formed normally.

P067-Su

Mineral Content and Composition of Bone: Species Variation and the Effects of Disease

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The nature of the mineral phase in bone and how it is affected by disease is still poorly understood. Bone from various species and anatomical sites can exhibit a wide range of densities but the structure and composition of the mineral is assumed to be the same. Likewise, osteoarthritic cancellous bone has been found to have a lower density than osteoporotic, but are their mineral compositions the same? This study used thermogravimetric analysis (TGA) and X-ray diffraction (XRD) to measure the composition and structure of the mineral in bone from a variety of species and from patients with osteoarthritis (OA) or osteoporosis (OP). Powdered bone was obtained from cod clythrum, deer antler, whale periotic fin, porpoise ear, whale tympanic bulla, and whale ear. Samples were heated at 10°C per minute to 1600°C using a thermogravimetric analyzer coupled to a mass spectrometer. Room- and high-temperature XRD was used to determine mineral structure and measure the unit cell dimensions. Similar studies were done on powdered human bone from the femoral heads of patients with OA or OP. The bones were found to fall into 2 clear groups: those with high organic content (cod clythrum, deer antler, whale periotic fin, human) and those with low organic content (porpoise ear, whale tympanic bulla, and whale ear). The mineral in both cases was deduced to be a carbonated form of hydroxyapatite (HAP). The carbonate and acid phosphate content of the mineral, however, differed between the two groups. The high-organic bone had greater acid phosphate, was calcium deficient, and had a smaller carbonate content than the low organic group, as determined by their breakdown products. Carbonate content was about 4% of the mineral mass in the high organic group but about 7–9% in the low organic group. Curiously, although human bone fits into the high organic group, its mineral composition fits more closely with that of the low organic tissues, with a carbonate content of about 7% and a Ca/P molar ratio close to stoichiometric HAP. The human bone used was from elderly individuals and these differences may result from maturation with aging, which is known to increase the carbonate and reduce the acid phosphate content. Supported by EPSRC, grant no. GR/L67066.

P068-Mo

The NO Donor Sodium Nitroprusside Inhibits Mineralization in ATDC5 Cells

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The hormone nitric oxide (NO) has been implicated in the regulation of bone formation and in the development of rheumatoid arthritis. Endochondral ossification is the process in which chondrocytes proliferate, become hypertrophic and induce mineralization of their extracellular matrix. This process can be studied in vitro in the chondrogenic cell line ADTC5. The aim of this study was to test whether the NO donor sodium nitroprusside has an effect in ATDC5 cells on the final stage of endochondral ossification, i.e., mineralization.

Sodium nitroprusside was shown to inhibit the mineralization of ADTC5 cells. This inhibition was not affected by inhibitors of guanylyl cyclase nor mimicked by a cGMP analog. Furthermore, sodium nitroprusside did not inhibit phosphate uptake, nor inhibited apoptosis in the ADTC5 cells.

These findings indicate that the NO donor sodium nitroprusside can specifically inhibit cartilage mineralization via a cGMP-independent pathway and that the effect was not mediated by inhibition of phosphate transport or apoptosis. These results suggest a preventive role of NO in premature or pathological mineralization.

P069-Tu

Remineralization Effects of Demineralized Apatite Crystal in Bovine Enamel

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Chewing gum which promotes remineralization is thought to enhance remineralization in caries lesions of the enamel.

There are many arguments about the mechanism which causes remineralization in enamel. The purpose of the present study is to make comparative examinations of the effects of two sorts of chewing gums, xylito+2 and Phosphoryl oligosaccharides of calcium (POsCAM) on the remineralization of artificially demineralized enamel. The enamel of bovine permanent lower incisors was used in the study. The specimens were observed using contact microradiography (CMR), an X-ray analysis microscopy (XAM, XGT-5000WR, Horiba), a scanning electron microscopy (SEM, JSM-6340, JEOL), and an electron-probe microanalyzer (EPMA, JXA-8200, JEOL). Results of CMR showed that the xylitol+2 samples were more remineralized than the POsCAM samples, and became remineralized along the bands of Hunter–Schreger. The backscattered electron image of SEM observation revealed that the calcification was high in the interprismatic enamel region of the remineralization layer with the xylitol+2. But the calcification was low in that of the remineralization layer with the POsCAM. It is thought that the xylitol+2 had an effect upon the remineralization of apatite crystals in the interprismatic enamel. Results of XAM and EPMA showed that Fe was detected in the surface layer of the xylitol+2 samples. In this surface layer, the density of Ca was high. Our results indicate that the mechanism of remineralization in enamel differs between the xylitol+2 and the POsCAM.

P070-Su

The Effect of Vitamin K2 (MK-4) on Reduced "Bone Quality" by Magnesium Insufficiency

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The insufficiency of magnesium (Mg) has been regarded as a risk factor for osteoporosis. Mg-insufficient bone reveals fragility to mechanical loading despite normal or higher levels of bone mineral density (BMD). Vitamin K2 (MK-4: menatetrenone) could recover the "bone quality" reduced by Mg-insufficiency (Kobayashi et al., Bone 35:1136, 2004). In order to verify the effects of MK-4, we have examined bone remodeling and mineral of the tibiae and femora of 4-week-old Wistar male rats fed normal (control group, 0.09% Mg), Mg-insufficient (low Mg group, 0.006% Mg), or MK-4 supplemented Mg-insufficient diets (MK-4 group, 30 mg/kg MK-4, 0.006% Mg). Mg-insufficient tibiae revealed osteoclasts with well-developed ruffled borders on the termini of the shortened metaphyseal trabeculae, as well as a complex meshwork of cement lines in the cortical bone. Therefore, bone remodeling seemed accelerated during the Mg-insufficiency, which may give rise to less "bone quality". In contrast, the MK-4 group showed poorly-formed ruffled borders of osteoclasts, relatively-elongated trabeculae and fewer numbers of cement lines. It is likely that MK-4 inhibited stimulated osteoclastic bone resorption by Mg-insufficiency, thereby normalizing bone remodeling. Concerning bone minerals, an electron probe microanalyzer verified that an increased concentration of Ca accompanied the decreased Mg in the low Mg group. X-ray diffraction demonstrated various chemical formulae of hydroxyapatites (HA) in the control group, but an extremely-elevated purity of HA $[Ca_{10}(PO_4)_6(OH)_2]$ in the low Mg group. Consistently, Mg-insufficient bone showed an overgrowth of mineralized nodules and premature mineralization of collagen fibers. These findings may attribute to the high BMD in Mg-insufficiency, but premature collagenous mineralization appears to have led to the decreased bearing to mechanical loading. In contrast, MK-4 did not affect the concentration of Mg and Ca, or HA-purity, but could prevent accelerated mineralization by Mg-insufficiency. It seems likely that Mg-insufficiency leads to Ca increments, and subsequently accelerated mineralization in bone, but MK-4 appears to regulate bone mineralization. Thus, MK-4 appears to recover the "bone quality" lessened by Mg insufficiency by two mechanisms: controlling bone turnover and mineralization.

P071-Mo

Identification of Active Phospho1 Within Matrix Vesicles: Support for a Role in Hydroxyapatite Crystal Formation

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We have previously reported a novel phosphatase, PHOSPHO1, whose expression is upregulated in mineralizing osteoblast-like cells and is located to mineralizing surfaces in bone and cartilage. PHOSPHO1 belongs to the haloacid dehalogenase superfamily of hydrolases and is capable of cleaving phosphoethanolamine (PEA) and phosphocholine (PCho) to generate inorganic phosphate (Pi). As mineral crystals are initiated, as is normal, within matrix vesicles (MVs) of tissue non-specific alkaline phosphatase (TNAP)-deficient mice and patients with hypophosphatasia, it is likely that another enzyme is responsible for elevating the intravesicular concentration of Pi for crystallization. Thus, we hypothesize that through the enzymatic action of PHOSPHO1, Pi is scavenged from PEA and PCho in order to generate the concentration required for initial

hydroxyapatite crystal formation inside the lumen of MVs. For this to occur, functional PHOSPHO1 must be present within MVs. Therefore, the aims of this study were to confirm bone-specific expression of *Phospho1* and show that PHOSPHO1 is both present and active within MVs. Real-time PCR revealed that bone had the highest levels of *Phospho1* expression compared with other adult murine soft tissues examined, and was approximately 150-fold higher in bone (tibial diaphysis) than in the gut (lowest expression). MVs were isolated from the chicken growth plate and Western blotting, using an avian-specific PHOSPHO1 antiserum, allowed identification of two forms of PHOSPHO1, 30.4 and 28.6 kDa, within the MVs relating to alternative start sites. To detect the presence of active PHOSPHO1 within MVs, we isolated MVs from cultures of murine calvarial osteoblasts obtained from TNAP null ($^{-/-}$) and TNAP heterozygous ($^{+/-}$) mice. The cells were grown in the presence of ascorbic acid and \betaGP for 15 days post confluence. Although we show here that human TNAP has a lower specific activity towards PEA compared to human PHOSPHO1, at physiological pH, we adopted this strategy to eliminate the possibility that TNAP hydrolysis of PEA may mask PHOSPHO1 activity. Using PEA as substrate, we found that the $TNAP^{+/-}$ and $TNAP^{-/-}$ MVs had a PEA hydrolase activity of 4100 ± 450 and 990 ± 86 pmol Pi released/min/mg lysate, respectively. These results show that PHOSPHO1 is highly expressed within bone and that the protein present within MVs is in an active state. These data further support our hypothesis that PHOSPHO1 plays a central role in matrix mineralization.

P072-Tu

The Expression Pattern of Vascular Endothelial Growth Factor in the Physis

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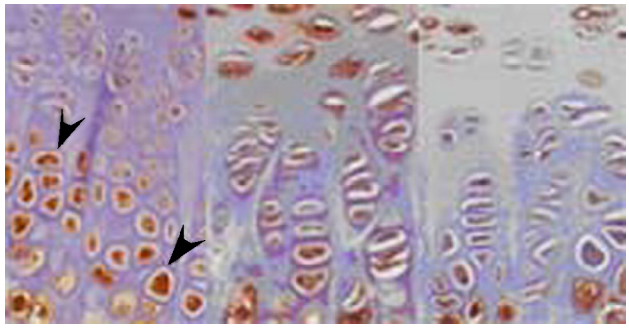
Legg–Perthes disease is a self limiting-disease characterized by ischemic necrosis of the secondary ossification center in the femoral head. Thirty percent of patients will have residual deformity such as relative overgrowth of the greater trochanter. This suggests that maturation of growth plate also is disturbed during the disease process occurring in the secondary ossification center. Angiogenesis is required for the differentation of the growth plate, and the expression of vascular endothelial growth factor (VEGF) is essential for endochondral ossification. In this study, an animal model using the Spontaneously Hypertensive Rats (SHR) is proposed to investigate any changes such as and VEGF expression occurring in the physis of the femoral head in Legg–Perthes disease.

Sixty SHRs and thirty Wistar Kyoto rats (WKYs) were sacrificed at each of 6, 9, 12, 15, and 18 weeks of age.

SHRs were divided into two groups (SHR−n and SHR+n) according to the evidence of ischemic necrosis of the seconday ossification center of the femoral head, and WKYs was used as control. TUNEL assay, immunohistichemistry, and in situ hybridization for VEGF was performed to identify the expression of apoptosis and VEGF in the growth plate. All the data were analyzed by two-way ANOVA for the comparison.

Apoptosis of growth plate in SHRs was higher than that of WKYs. Expression of mRNA and protein for VEGF was in growth plate of all three groups, with decrease in expression the course of normal ossification. Expression of mRNA and protein for VEGF in growth plate was decreased in SHRs. The expression was lowest in the group of SHR+n as compared to other groups.

We demonstrated that maturation of the physis as well as the epiphyis is disturbed in the avascular necrosis of the femoral head. This explains why the proximal femoral growth disturbances occurs regardless of the disease process of spontaneous remission of the epiphysis. In conclusion, VEGF is essential factor for the longitudinal growth of growth plate and normal ossification.



P073-Su

Role of Progressive Ankylosis Gene (ANK) in Cartilage Mineralization

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Physiological mineralization of growth plate cartilage is highly regulated and restricted to a zone close to the chondro–oseous junction. Uncontrolled (pathological) mineralization of articular cartilage leads to its destruction. Recently, the progressive ankylosis gene (ank) was identified to be a transporter of intracellular pyrophosphate (PPi) to the extracellular milieu. PPi has been shown to inhibit hydroxyapatite growth. However, high amounts of PPi can lead to calcium pyrophosphate dihydrate (CPPD) crystal deposition in articular cartilage. In this study, we demonstrate that growth plate chondrocytes undergoing mineralization express high amounts of Ank and alkaline phosphatase (APase). Suppression of Ank expression in these cells using siRNA resulted in inhibition of APase expression and activity and mineralization. On the other

hand, overexpression of Ank in non-mineralizing hypertrophic growth plate chondrocytes using a retroviral expression system led to an increase of APase expression and activity and mineralization. Enhanced Ank expression also led to upregulation of type III Na+/Pi co-transporters Pit-1 and Pit-2. Influx of extracellular phosphate (Pi) was enhanced in Ank-overexpressing growth plate chondrocytes and reduced in Ank expression suppressed cells. Transport of extracellular Pi through Pit-1 and Pit-2 resulted in upregulation of APase, MMP-13 and osteocalcin gene expression, APase activity, and mineralization of Ank-expressing, mineralization-competent growth plate chondrocytes. In conclusion, our findings demonstrate that Ank is an important regulator of mineralization in growth plate cartilage. High expression of Ank by mineralization-competent growth plate chondrocytes leads to an increased transport of intracellular PPi to the extracellular milieu. Extracellular PPi is then being hydrolyzed by APase removing the mineralization inhibitor PPi and generating Pi, which is being transported into the cell through Pit-1 and Pit-2. Pi then acts as a signaling molecule, which further stimulates APase and other mineralization-related gene expression and ultimately mineralization. Interestingly, Ank expression was also upregulated in osteoarthritic (OA) cartilage. In areas of OA cartilage with high Ank expression APase expression was also detected, suggesting that high expression of Ank in OA cartilage may lead to basic calcium phosphate crystal formation in the presence of APase or to CPPD crystal formation in the absence of APase.

P074-Mo

How Mineralization Kinetics Determine the Bone Mineral Density Distribution?

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The inhomogeneous degree of mineralization of bone and its topographical distribution on a microscopic scale are major determinants of its mechanical quality. The non-uniform mineralization is a result of the bone remodeling process, i.e., the continuous replacement of bone by new unmineralized organic matrix. The subsequent mineralization of the matrix is described by a two-step process: a rapid mineralization up to 70% of full mineralization capacity within weeks and a completion of the residual 30% lasting for several years. Quantitative backscattered electron imaging (qBEI) [1] allows a quantitative measurement of the frequency distribution of calcium concentration in bone sections. This so-called bone mineralization density distribution (BMDD) can be regarded as a parameter reflecting the age and size distribution of bone packets within the tissue. The BMDD of trabecular bone from healthy adults was shown to be almost constant independent of gender, age, ethnicity, and skeletal site [2]. Metabolic bone diseases or clinical treatment, however, results in significant deviations from the healthy reference BMDD. To yield a quantitative understanding of these deviations, we set up a computational model for the time evolution of the BMDD. The kinetics of bone tissue deposition, mineralization, and resorption are determining the distribution of mineral in the tissue as described by the BMDD. This connection between kinetics of mineralization and BMDD was cast into a set of mathematical equations. It could be shown that the shape of the BMDD histogram reflects directly the mineralization kinetics. Finally, we analyzed how changes in the turnover rate, growth and various disease scenarios influence the shape of the BMDD histogram and we compared the results with data from clinical studies.

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P075–Tu

Bone Formation—Dominant Role of Stress Changes

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The thickening/thinning of bone are regulated biomechanically and biochemically (genetically). The speeds of biochemical reactions (i.e., the speeds of intense metabolic processes) depend on the volume changes of molecular mixtures and on the stress changes in a bone element. Processes of bone thickening depend on both the dominant volume changes of molecular mixtures and stress changes. Resultant speed kj of the *j*th biochemical reaction (for example, a reaction concerning the mineralization of osteoid) is the exponential function of dominant volume changes (of molecular mixtures) and stress changes. The resultant speed of the *j*th biochemical reaction, which forms part of biochemical (metabolic) processes in the bone tissue (for example, in the remodeling limit cycle), is dependent on the product of speeds of the biochemical reaction initiated biochemically (resp. genetically) and on the speeds of chemical reaction initiated biomechanically. The *j*th biochemical reaction is influenced by the internal-primary chemical (genetical) effects and the external-biomechanical effects, i.e., the stress changes dp. The actual process of the tissue substance thickening is "determined" by stress change dp = p − pe. The resultant speed of the *j*th reaction according to derived (and proof) exponential function is influenced by signs (signum) of stress changes dp = p −

pe. The density of bone can be increasing when the stress changes in bone have the positive signum. AXIOM I (a Theorem of the Bone Thickening Retardation): Provided that the changes of mechanical stress in the tissue are negative, then the thickening in the tissue is retarded. AXIOM II (a Theorem of the Bone Thickening Acceleration): Provided that the changes of mechanical stress in the tissue are positive, then thickening in the tissue is accelerated.

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P076-Su

Fetuin and Osteocalcin Influence Calcospherite Formation in an In Vitro Acellular Model of Calcification

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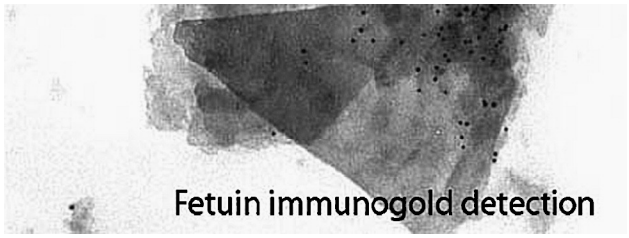
Calcification is a complex process implying numerous proteins acting as nucleator, Ca or P transporters and crystal growth regulators. Recently, it was reported that etidronate is able to impair mineralization by an indirect mechanism. It was found to upregulate the production of fetuin in the liver and the complex fetuin–osteocalcin inhibited calcification. We have developed an in vitro model of calcification based on a carboxymethylated methacrylic polymer CM-pHEMA, which acts as a nucleator for Ca/P critical nuclei. Then, calcospherites of hydroxyapatite tablets develop onto the polymer surface, identical to those observed in woven bone.

Pellets of CM-pHEMA were incubated in a synthetic body fluid during 4 days at 37° to induce the appearance of calcospherites. The pellets were transferred in a fresh medium containing fetuin (5 mg/ml) or osteocalcin (1 mg/ml) or the combination of both proteins. Pellets were incubated during 11 days, and then examined by scanning (SEM) and transmission (TEM) electron microscopy. Immunodetection of fetuin and osteocalcin was done by immunogold in TEM. Calcospherites were dissolved in HCl 0.2 M; Ca and P were dosed.

Osteocalcin did not alter calcospherites deposited on CM-pHEMA nor modified the amount of Ca, P, Ca/P ratio determined biochemically. Fetuin had a dramatic effect on calcospherite production and was associated with smaller calcospherites. The amount of Ca and P was significantly reduced, the Ca/P ratio increased considerably ($P < 0.05$). Pellets incubated with fetuin and osteocalcin also had a significant reduction of Ca and P content ($P < 0.05$) but no increased in Ca/P. Immunogold clearly identified both fetuin and osteocalcin adsorbed at the surface of hydroxyapatite tablets. This confirms the role of non-collagenous proteins to control the growth of hydroxyapatite crystal and the interaction between fetuin and osteocalcin.



P077-Mo

Physiological Death of Hypertrophic Chondrocytes

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During endochondral ossification, chondrocytes in growth cartilage undergo hypertrophy then die by a process that is morphologically distinct from classical apoptosis but has not been well characterized. The aims of the current study were to document the morphology of dying hypertrophic chondrocytes throughout fetal and postnatal growth, and to develop a culture system for studying the molecular mechanisms of physiological death of these cells. Specimens of equine articular–epiphyseal and physeal growth cartilage (AEGC and PGC, respectively) were collected from the distal femur and distal tibia during foetal and postnatal growth, and examined by transmission electron microscopy. Two types of dying chondrocyte were observed, 'dark' and 'light' chondrocytes. Dark chondrocytes were characterized by a dark nucleus with small, irregular patches of condensed chromatin; their electron-dense cytoplasm was gradually being extruded into the extracellular space. Light chondrocytes also contained a condensed nucleus but their cytoplasm and organelles appeared to be undergoing gradual disintegration within a preserved cellular membrane. The proportion of light chondrocytes was higher in fetal than postnatal specimens and greater in AEGC than in PGC. Chondrocytes were isolated by collagenase digestion from epiphyseal cartilage excised from fetal horses. These cells were centrifuged and grown as pellets for up to 28 days under a variety of conditions, then analyzed by light and electron microscopy. By day 7, pellets cultured in 0.1% or 10% fetal calf serum (FCS) were organized into a cartilage-like tissue surrounded by a perichondrium-like layer of flattened cells, and contained hypertrophic chondrocytes resembling those seen in vivo. Some light chondrocytes were present at days 7 and 14; dark chondrocytes and a small number of apoptotic cells

were present at all stages of culture. The addition of transforming growth factor-B to the culture medium resulted in an increase in the proportion of cells dying as dark chondrocytes. Triiodothyronine added to medium containing 0.1% FCS caused an increase in the proportion of cells dying as light chondrocytes, but had no effect in the presence of 10% FCS. This culture system will provide a useful model for studies on the mechanism of physiological death of hypertrophic chondrocytes.
This work was supported by a grant from the Australian Research Council and Racing Victoria.

P078-Tu

Estrogen Receptor-Related Receptor Alpha, ERRa, Inhibits the Proliferative to Hypertrophic Chondrocyte Transition in Vitro and is Dysregulated in Inflammatory Arthritis

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Estrogen receptor-related receptor alpha, ERRa, is expressed by osteoblasts and plays a functional role in bone formation. We now report a new potential function of ERRa in cartilage. We found that ERRa is highly expressed in fetal and adult rat chondrocytes in growth plate and articular cartilage and the rat chondrogenic cell line C5.18 cells in vitro. ERRa mRNA and protein were expressed from proliferating chondrocytes to mature chondrocytes with the exception of fetal hypertrophic chondrocytes. To assess a functional role for ERRa in chondrocyte proliferation and differentiation, we blocked its expression by antisense oligonucleotides in C5.18 cell cultures and found significant inhibition of cell growth with concomitant downregulation of Sox9 and Ihh (Indian Hedgehog), both master genes in cartilage formation and cyclinD1, a cell cycle regulator. We also found a proliferation-independent inhibition of cartilage formation, concomitant with a dramatic decrease in Sox9, Ihh, Aggrecan, Link, and Col2a1 expression compared to control cultures. Consistent with the decrease of Sox9 and Ihh, the hypertrophic chondrocyte markers Col10a1 and PTHrP receptor increased and the anti-apoptotic marker Bcl2 decreased, suggesting that ERRa is involved in the acceleration of maturation of proliferating chondrocytes into hypertrophic chondrocytes in vitro. We therefore next asked whether ERRa dysregulation may play a role in conditions in which cartilage integrity is lost such as in inflammatory arthritis. Erosive arthritis was induced by injection of type II collagen into the joints of DBA-1 mice. Semi-quantitative RT-PCR of RNA from joints 7 weeks after injection revealed a dramatic decrease in expression of ERRa and mature chondrocyte markers; the decreases were even more striking when mice were boosted 5 weeks after the first injection. Notably, expression of ERRa and markers of bone formation was also decreased in the subchondral bone of the same treated mice. Taken together, these results suggest that ERRa plays a role both in regulation of cartilage formation and the proliferating to hypertrophic chondrocyte transition, and in the destruction of joints in erosive arthritis. They also suggest that agonists and antagonists of ERRa may be useful as therapeutic agents in skeletal diseases affecting bone and cartilage.

P079-Su

Melanocortin and Linear Growth

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Clinical and in vitro data point to a positive correlation between the excess production of pro-opiomelanocortin (POMC) peptides and linear growth. POMC is the precursor to the melanocortin family of peptides, which includes ACTH, alpha-MSH, beta-MSH, and gamma-MSH. Both obesity and Familial Glucocorticoid Deficiency (FGD) are linked to increased circulating levels of POMC peptides and increased pre-pubertal growth and advanced bone age. In vitro, ACTH increases the differentiation of chondrogenic cells along the endochondral pathway. To test the hypothesis that melanocortin peptides, signaling through endogenous melanocortin receptors in the growth plate, are involved in linear growth, we used the obese (ob/ob) mouse as a model. In this animal model of obesity, POMC expression in the pituitary is low and the ob/ob mice are typically 5–10% shorter than their lean littermates (+/?). ob/ob and wild type littermate (WT) mice were adrenalectomized (ADX) or ADX and administered gamma^2-MSH (gamma^2), 50 μg/day s.c., for 3 weeks. gamma^2-MSH is an endogenous agonist of the MC3-R. 10 animals were included in each group. All ADX animals were supplemented with minimal corticosterone (2.4 μg/day) to maintain basal metabolism but retain elevated ACTH levels. ADX increased nose-to-anal length (ADX, 91.84 ± 1.40 mm vs. sham, 90.22 ± 1.36 mm, $P < 0.05$) and tibial length in the WT animals (ADX, 17.14 ± 0.274 mm vs. sham 16.92 ± 0.109 mm, $P < 0.05$). Nose-to-anal length and tibial length of WT ADX treated with gamma^2-MSH were also significantly longer than WT sham, but were not significantly greater than WT ADX alone. ADX of the ob/ob animals also significantly increased tibial length (ADX, 16.57 ± 0.332 mm vs. sham, 16.19 ± 0.298 mm, $P < 0.05$) but no significant increase in nose-to-anal length was observed. ADX ob/ob animals treated with gamma^2-MSH also experienced an increase in both nose-to-anal length (ADX + gamma^2, 91.77 ± 1.59 mm, $n = 9$,

vs. sham, 88.76 ± 1.24 mm, $P < 0.01$) and tibial length compared to the sham ADX animals (ADX + gamma^2, 16.83 ± 0.29 mm vs. sham, 16.19 ± 0.298 mm, $P < 0.001$). Interestingly, nose-to-anal length in the ADX ob/ob animals treated with gamma^2-MSH was significantly greater than the WT sham (91.77 ± 1.59 mm vs. 90.22 ± 1.36 mm, $P < 0.05$) and no significant difference in tibial length was observed between these groups. These data together with the recent identification of the MC3-R in cultured chondrogenic cells suggest a role for the melanocortin signaling at the level of the growth plate in the regulation of linear growth.

P080-Mo

Cellular and Molecular Characterization of Primary Rat Chondrocytes Differentiated in Culture

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Chondrocyte differentiation is a key event in growth of the appendicular and axial skeleton, embryonic patterning of skeletal elements and endochondral ossification. Many regulatory pathways involving endocrine, cytokine, and mechanical signals all impact this complex process. These include, among others, growth factors (FGFs, IGFs, TGF-\beta, BMPs) and the regulatory loop involving parathyroid hormone related protein (PTHrP) and Indian hedgedhog (Ihh). Defects in the progress of chondrocyte differentiation lead to several skeletal abnormalities including chondrodysplasias and achondroplastic dwarfism. In our laboratory, we focus on osteopetrotic mutations in rats and mice, and our observation of severe and progressive postnatal growth plate dysplasias led us to develop an in vitro method to study chondrocyte differentiation in greater detail. We therefore modified a method for the culture of primary costochondral chondrocytes from neonatal rats which exhibit many characteristics of differentiating chondrocytes seen in vivo. Differentiation was induced over a period of 20 days using a combination of insulin and other factors and decreasing serum concentrations. Absence of contamination by other connective tissue cells has been confirmed by the lack of detection of specific cell markers. Chondrocytes isolated and cultured with this method secrete and mineralize matrix as demonstrated by increased Alcian blue and Alizarin Red staining and strong expression of type II collagen and aggrecan observed by in situ hybridization and real-time PCR. During differentiation, cells exhibit many features of hypertrophic chondrocytes as clearly shown by electron microscopy, immunomicroscopy and increasing type X collagen expression. Real-time PCR analysis was also carried out for SOX9, TGF-\beta, MMPs and BMPs, as well as for PTHrP, Ihh and their respective receptors, parathyroid hormone receptor I and patched. These molecules show distinct patterns of expression throughout the differentiation process. In summary, we present a new and simplified method to culture and differentiate chondrocytes that retain morphological and molecular features observed in vivo. This approach complements information gained by in vivo studies in a system more easily amenable to experimental manipulation, and should increase our understanding of chondrocyte differentiation and skeletal growth regulation.

P081-Tu

Cervical Chemonucleolysis Associated to Ventral Slot in Dogs: Clinical–Surgical, Radiological and Histological Evaluation

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The present study has as objectives to evaluate the clinical–surgical, radiological, and histological aspects of canine cervical intervertebral disks after chemonucleolysis with chymopapain associated to the ventral slot. For that, it was used 24 dogs randomly divided into six equal groups. The ventral slot was accomplished in the area of the intervertebral disk C2–C3 and chemonucleolysis in the other cervical disks. Each group of four animals was submitted to euthanasia with an anesthetic overdose 24 h, 8, 30, 60, 90, and 120 days after the enzyme injection and sagittal sections of the treated disks, associated to adjacent cartilaginous endplates and bony structures were obtained for histological analysis. The dogs were also monitored clinically and radiographically for up to 120 days, according to the groups. In this study, all dogs tolerated the surgical procedure, without detectable postoperative pain, neurological deficit or alteration of the conscience state. In the radiographic evaluation, it was observed consistent disk space narrowing 24 h after the procedure and total absence of space from the eighth to the 90th day after chemonucleolysis in the area of all treated disks. However, at 120 days postoperative, there was an increase in disk height, corresponding, on average, to 59.13% of the preinjection value. Also, on the 30th day postoperative, it was noted absorption of the vertebral bodies adjacent to the treated disks that progressed to healing, with evidences of vertebral fusion 120 days after the treatment. In the histological evaluation, it was observed 24 h after disk injection nuclear digestion, characterized by cavitations and decrease of safranin-O staining intensity, indicating loss of proteoglycans. At 8 days, the nuclear content was still vacuolated but more fibrillar. At 30 days, it was noted lesions and hemorrhage in the cartilaginous

endplates. An irregularly defined mass in the nuclear space was observed on days 60th and 90th, but it seemed fibrocartilaginous tissue on day 120. Microfractures and bone necrosis were also observed on day 90, which were healed by day 120. Chemonucleolysis with chymopapain associated to the ventral slot in the cervical column of dogs determines lysis of intervertebral disks, cervical instability, and lesions of cartilaginous and bone adjacent structures that tend to repair with fibrocartilaginous tissue along the time.

P082-Su

Development of a Mesenchymal Stem Cell Based Therapy for Repair of Growth Plate Cartilage in an Ovine Model

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Injury to growth plate cartilage can result in premature growth arrest and limb angularity. Current clinical treatment uses corrective surgery to address the manifested deformity, as opposed to regenerating the damaged tissue. As yet, there is no biologically based therapy to repair growth plate injury. One potential cell type for growth plate repair is the mesenchymal stem cell (MSC) due to its chondrogenic potential. MSCs can be isolated from the connective and supportive tissues and can give rise to cells of, and beyond, those of mesodermal origin. As MSC can give rise to cartilage-producing cells, we are interested in the application of MSCs to repair growth plate injury, using an ovine model. As a popular large animal model for orthopedic research, we have characterized the in vitro properties and differentiation potential of sheep MSC. Bone marrow aspirates were harvested from the iliac crest and subjected to density gradient centrifugation to isolate the mononuclear cell fraction. The MSC population was then collected according to their plastic adhering characteristic. Proliferation of MSC was strongly induced by addition of growth factors FGF-2, TGF\alpha, IGF-1, PDGF, and EGF but not by TGF-\beta1, TGF-\beta3, BMP-2, and BMP-7. We have demonstrated sheep MSC are multipotential in vitro and can be directed to differentiate toward cells of the osteogenic, adipogenic, and chondrogenic lineage. Furthermore, we have optimized in vitro culture conditions for differentiation of MSC to chondrocytes. We have examined in vivo cartilage formation using both a gelatin sponge and PGLA fleece, and seeded MSC using agarose, alginate, or fibrin glue. When transplanted subcutaneously into immunocompromised mice with an appropriate scaffold, sheep MSC can separately form bone and cartilage. Recently, in lambs, we have evaluated the therapeutic potential of autologous MSC seeded within a gelatin sponge containing TGF-\beta1 and hyaluronate, to repair growth plate cartilage following injury.

P083-Mo

Bone Morphogenetic Protein Signaling in Chondrocyte Proliferation and Hypertrophy During Endochondral Bone Formation

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Bone morphogenetic proteins (BMPs) are believed to enhance both proliferation and hypertrophy of chondrocytes. BMP signaling is mainly mediated by Smad proteins, but other pathways are also known to transduce BMP signals. Here, we analyzed roles of these pathways in chondrocyte proliferation/hypertrophy-induced by BMPs.

Materials and methods: Metatarsal rudiments were prepared from transgenic mice expressing Smad6 in chondrocytes (Tg) and wild-type littermates (WT) at 14.5 d.p.c. The rudiments were organ-cultured in the medium containing recombinant human (rh) BMP2 (control medium). A p38 MAP kinase inhibitor (SB203580), a mitogen-acivated protein kinase kinase inhibitor (PD98059), or a Pi3-kinase inhibitor (LY294002) was added to the control medium. After 7 days culture, zones of proliferative and mineralized hypertrophic cartilage were assayed morphometrically. Expression of the marker genes were analyzed by in situ hybridization.

Results and discussion: Metatarsal rudiments prepared from Tg at the start of culture were predominantly composed of proliferative cartilage and not distinguishable from rudiments from WT. Rudiments from WT showed expansion of zone of proliferative cartilage and formation of zone of mineralized hypertrophic cartilage after 7 days culture in the presence of rhBMP2. Both rudiments from Tg cultured in control medium and rudiments from WT in medium with SB 203580 showed similar expansion of proliferative cartilage and decreased formation of hypertrophic cartilage compared with rudiments in control medium. Rudiments from Tg cultured in media with SB203580 exhibited comparable expansion of proliferative cartilage, but lacked hypertrophic zone. In situ hybridization analysis showed complete absence of Col10a1 and Mmp13 expression in these rudiments. These results suggest that Smad6 overexpression and addition of SB203580 additively inhibit chodrocyte hypertrophy induced by BMP2. Thus, p38 MAP kinase pathways may mediate BMP signals independently from Smad proteins during chondrocyte hypertrophy. Addition of LY294002 to medium did not affect chodrocyte proliferation and down-regulate formation of zone of hypertrophy

to limited extent compared with SB203580 application. Addition of PD98059 did not affect either chondrocyte proliferation or hypertrophy. Future studies should determine signaling pathways which transduce BMP signals for chondrocyte proliferation.

P084-Tu

The Accumulation of Mutant Fibroblast Growth Factor Receptor 3 (FGFR3) in the Endoplasmic Reticulum of Chondrocytic Cells and their Apoptosis

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Objective: Thanatophoric Dysplasia (TD) shows severe chondrodysplasia; chondrocyte proliferation is inhibited due to a point mutation of fibroblast growth factor receptor 3 (FGFR3). In addition, chondrocytic apoptosis has been reported in TD. Recent reports indicate that the altered localization of mutant proteins in the endoplasmic reticulum (ER) causes "inner stress of ER" linked to apoptotic signaling. We have examined intracellular localization of mutant FGFR3 in the chondrocytic cell line (CFK2) and Chinese hamster ovary (CHO) cells transfected with cDNAs encoding mouse wild type FGFR3, $FGFR3^{R248C}$ and $FGFR3^{K644E}$ (corresponding to human $FGFR3^{K650E}$) and the occurrence of apoptosis in the transfected cells.
Materials and methods: Transient transfection with cDNAs encoding mouse $FGFR3^{R248C}$ or $FGFR3^{K644E}$ corresponding to human $FGFR3^{K650E}$ in CFK2 and CHO cells was employed for the assessment of intracellular localization of mutant proteins and actin, as well as the TUNEL reaction. In addition, paraffin and epoxy resin sections of 18-week-old human fetal tibiae were employed for TUNEL and ultrastructural analyses.
Results and discussion: CFK2 cells transfected with cDNAs encoding $FGFR3^{R248C}$ or $FGFR3^{K644E}$ showed an accumulation of mutant protein in ER and cytoplasmic vacuoles, whereas the CFK2 cells expressing intact FGFR3 revealed this molecule on the cell membranes. The CFK2 cells synthesizing these mutant FGFR3 proteins displayed condensed cell bodies and less extension of stress fibers, sometimes revealing nuclear pyknosis with TUNEL positivity. In contrast to CFK2, CHO cells transfected with mutant FGFR3 cDNAs did not show ER accumulation of mutant proteins, nor apoptosis. Consistently, tibial epiphyses of TD type II, rather than TD type I, demonstrated pyknotic and TUNEL-positive nuclei of chondrocytes. Thus, ER accumulation of mutant FGFR3 may cause apoptosis of chondrocytes, but not other cell types such as CHO cells.

P085-Su

Cartilage Tissue Formation from Bone Marrow Derived Cells Using Rotating Wall Vessel (RWV) Bioreactor

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Establishment of a cartilage tissue regeneration technique is needed to treat bone diseases such as osteoarthritis. However, problems such as necrosis of cells due to high-density cell culture and shear stress by gravity have not yet been solved. Thus, we examined an RWV (Rotating Wall Vessel) bioreactor that simulates a micro-gravity environment with low shear stress for cartilage tissue regeneration. An RWV bioreactor generates stress by horizontal rotation of a cylindrical vessel equipped with gas exchange membrane to compensate the effect of gravity, resulting in homogenous cell growth and differentiation without sinking, and cells aggregate and form a three-dimensional tissue. In this study, we established a three-dimensional culture technique for construction of large and homogenous cartilage tissues without necrosis by culture of bone marrow cells using RWV bioreactor. Moreover, we attempted to repair large osteochondral defects in rabbit knee joints with cartilage aggregates formed by the rotation culture.
Bone marrow cells were collected from rabbit long bones. The cells were cultured for 3 weeks in DMEM with 10% FBS. The cells were subcultured by trypsinization, and resuspended in DMEM supplemented with TGF-b, etc. The cell suspension was seeded in the cylindrical vessel of an RWV reactor, and rotatory culture was performed for 4 weeks. Cartilage function of the cultured tissues was examined by quantitative RT-PCR of aggrecan and type II collagen mRNA, safranin-O staining of paraffin-embedded sections, immunostaining, and measurement of the marker of matured cartilage, ALP activity. Large cell aggregate with a longer diameter of about 1.5 cm was formed by rotatory culture of bone marrow cells in an RWV bioreactor. The mRNA expression of aggrecan and type II collagen was detected, and homogenous production of the two proteins was confirmed in whole tissue by immunostaining. The cylindrical osteochondral defects (5×5 mm in width and 4 mm in depth) were created on the patella groove of the femur in 32 rabbits (10-week-old) and the aggregates were implanted into the defects. Histochemical study at 4, 8, 12 weeks postimplantation suggested that transplantation of allogenic cartilage aggregates is effective in repairing large osteochondral defects.
Large and homogenous three-dimensional cartilage tissues were successfully generated by culture of bone marrow cells in an RWV bioreactor, which were effective in repairing osteochondral defects.

P086-Mo

Sonic Hedgehog Promotes Proliferation and Chondrogenic Differentiation of Bone Marrow-Derived Mesenchymal Stromal Cells in Vitro

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Sonic Hedgehog is known to be an important signaling protein in the early embryo development. Shh is involved in induction of early cartilaginous differentiation of mesenchymal cells in the limb and in the spine. To test the impact of Shh on adult stem cells, we treated bone marrow-derived mesenchymal stem cells (MSCs) with either recombinant Sonic Hedgehog protein (Shh) or Transforming Growth Factor-beta-1 (TGF-b) in vitro for 3 weeks and investigated cartilaginous differentiation and proliferation. MSC treated with TGF-b or Shh showed expression of cartilage markers Aggrecan, CEP-69 and Collagen II after 3 weeks compared to controls as shown by RT-PCR. A proliferation assay as well as cell cycle analysis showed enhanced proliferation in the Shh-group. In one assay, we observed the development of a large three-dimensional cell organization complex with matrix production that stained immunohistochemically positive for Collagen II. Those results suggest a particular role for Shh in the tissue organization of cartilage in vitro since it increases cell number, promotes chondrogenic differentiation, and is able to induce three-dimensional cartilage formation.

P087-Tu

Zones of Epiphyseal Cartilage Classified Due to Cellular Morphology and Functional Activity State

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In any task in applied osteology, one of the main requirements is to assess adequately the morphology and functional state of the most reactive parts of the skeletal bones—epiphyseal cartilages and periosteum.
There are several accepted classifications of epiphyseal cartilage zones (J. Trueta, I. Morgan, 1969; E. Gardner, 1978; S. Y. Ali, 1983 et al.) which are widely used today. Most authors define four zones in epiphyseal cartilage:

1) A zone of inactive (indifferent) cartilage cells;
2) A proliferation zone (it consists of young cells);
3) A zone of B mature (fully differentiated) cells;
4) A zone of dying cells and calcification.

Some authors divide the epiphyseal cartilage into three zones: a superficial zone of inactive chondrocytes, a zone of vesicular (hypertrophic) cells, and a cell destruction zone. But the authors did not take into account a functional activity of each cartilage zone.

As far as longitudinal growth of the bone is possible due to epiphyseal cartilage, we consider it to be necessary to add to known four zones the fifth zone—the zone of the primary osteogenesis. In this case, we classify cartilage zones from both morphological and functional points of view and thus define five zones:

1) A zone of indifferent (inactive) cells. It lies adjacent to epiphysis. In the places where it contacts with subchondral bone, there is a thin winding line, which forms because blood vessels that feed the cartilage penetrate there. In this zone, cell proliferation is not observed.
2) A proliferation zone. There are many cells lying on each other so that they form 'coin stacks'. The cells divide actively and numerous mytotic figures can be observed.
3) A zone of definitive (mature) cells. It consists of large cubic cells which also form the "stacks" as in the previous zone. These cells are at the terminal stage of differentiation and unable to divide.
4) A destruction zone. It features destruction of the large cells and matrix calcification.
5) A zone of primary osteogenesis, the deepest part of the epiphyseal cartilage. It is a place where blood vessels and osteogenic cells grow into the growth plate. The osteogenic cells form the trabeculae of the primary spongiosa surrounded by the calcified cartilage cells.

The offered classification allows designing an algorithm of longitudinal bone growth assessment depending on functional activity of separate growth plate zones.

P088-Su

The Production of Pro-Inflammatory Cytokines by Activated Human Articular Chondrocytes is not Influenced by Bisphosphonates

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Background: Pro-inflammatory cytokines such as IL-6 are known to play an important role in osteoclast development and may be involved in the subchondral, periarticular, and generalized bone loss in rheumatoid arthritis (RA).
Bisphosphonates are strong inhibitors of osteoclast-mediated bone resorption. According to several reports, these compounds have a down-regulating effect on pro-inflammatory cytokines as IL-6 in RA. The aim of this study is to investigate the influence of bisphosphonates on the in vitro production of pro-inflammatory cytokines in activated human chondrocytes.
Methods: Human articular cartilage explants were incubated during 48 h with clodronate, pamidronate, or risedronate (10^{-6} and 10^{-8} mol/L each time), dexamethasone (10^{-8} mol/L) or medium as a control. Subsequently, cultures were stimulated with IL-1 10 ng/mL ($n = 6$) or 1 ng/

mL (n = 10) for 48 h. Co-incubation was performed with or without the bisphosphonates or dexamethasone. A flow cytometric microsphere-based immunoassay was used for the detection in the supernatants of the pro-inflammatory cytokines IL-6, IL-8, TNF-a, and the regulatory cytokines IL-12p70 and IL-10.

Results: Stimulation with IL-1 resulted in a production of IL-6 and IL-8 in a dose-dependent way. There was no induction of the other cytokines measured. This production of IL-6 and IL-8 was not inhibited nor enhanced by the bisphosphonates.

Conclusion: This study showed that IL-1 can stimulate articular chondrocytes to produce IL-6 and IL-8. As IL-6 is known to promote osteoclast development, this means that in conditions of joint inflammation cartilage itself can contribute to subchondral bone resorption. However, bisphosphonates do not interfere with this mechanism of osteoclast activation.

P089-Mo

Altered Cellular Kinetics and Expression Pattern of Type X Collagen and Indian Hedgehog in the Physis of Femoral Head of the Spontaneously Hypertensive Rats

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Legg–Calvé–Perthes disease is characterized by the occurrence of ischemic necrosis of the secondary ossification center in the femoral head. Although metaphyseal growth plates are assumed to be affected by the disease process, the pathologic changes within the growth plate remains to be unknown. Expression of type X collagen and Indian hedgehog (Ihh) is essential for endochondral ossification. In this study, an animal model using spontaneously hypertensive rats (SHR) is proposed to investigate any changes in the physis, such as cell kinetics, type X collagen and Ihh expression in the experimental group were compared with those in Wistar–Kyoto rats (WKY) of the control group.

60 SHRs and 30 WKYs were sacrificed at each of 6, 9, 12, 15 and 18 weeks of age. Experimental SHR groups were divided into two groups (SHR − n and SHR + n) according to the evidence of ischemic necrosis of the second day ossification center of the femoral head and WKYs were used as control. 5-Bromo-2′-deoxydiuridin (BrdU, sigma) was injected and BrdU immunohistochemistry was performed for the cell kinetic analysis. Immunohistochemistry for type X collagen (Quartett) and Ihh (Santa Cruz) was performed to identify expression in the growth plate and epiphysis. All data were analyzed by two-way ANOVA for the comparison.

The cell proliferation analysis of the growth plate of SHR group showed lower activity in resting zone than those of the WKY group at earlier growth stages. Type X collagen was expressed in hypertrophic chondrocyte of secondary ossification center and hypertrophic zone of the growth plate of all three groups, which decreased in the course of normal ossification. Type X expression in epiphysis and growth plate was decreased in SHR. The expression was lowest in SHR + n when compared with other groups. Ossification was found around type X collagen expressed areas. Expression of Ihh was prehypertrophic chondrocyte in the growth plate of all three groups which showed lowest in SHR + n when compared with other groups.

In conclusion, expression of type X collagen and Ihh in the groups of SHR were essential factors for the longitudinal growth of growth plate.

P090-Tu

Elevated Expression of Hypoxia-Inducible Factor-2alpha in Terminally Differentiated Growth Plate Chondrocytes

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The growth plate of long bones is a constitutively avascular and hypoxic tissue. The ability of cells to tolerate lowered oxygen levels is dependent on their ability to initiate adaptive responses such as shifting metabolic catabolism to the anaerobic glycolytic pathway and/or inducing vasculogenesis and angiogenesis. Acquisition of the terminally differentiated (hypertrophic) phenotype by growth plate chondrocytes is essential for normal bone development. Vascular endothelial growth factor (VEGF) is clearly involved in this process but the molecular basis of the control of growth plate differentiation and vascularization remains poorly understood. Using Percoll density gradient centrifugation, chick chondrocytes were separated into populations of different maturational phenotype. A differential display analysis of the populations showed highly upregulated expression of hypoxia-inducible factor-2\alpha (HIF-2\alpha) mRNA during chondrocyte differentiation. HIF-2\alpha is a homologue of the HIF-1\alpha transcription factor, both of which play a role in the activation of a number of genes, including VEGF. HIF-1\alpha mRNA was also found to be expressed although the levels of expression were found to be similar in all of the chondrocyte fractions. The elevated expression of HIF-2\alpha during chondrocyte differentiation was accompanied increased VEGF gene expression. Analysis of the murine chondrocyte cell line, ATDC5, which undergoes ordered maturation indicated that HIF-2\alpha gene and protein expression also increased in parallel with chondrocyte differentiation. These results therefore suggest that HIF-2\alpha may be involved in the initiation of blood vessel formation in the growth plate, a process crucial for endochondral ossification and bone growth.

P091-Su

Influence of Protease Inhibitors on Chondrogenic Differentiation of Mesenchymal Stem Cells

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Introduction: Chondrogenically differentiated mesenchymal stem cells derived from bone marrow (BMSC) or adipose tissue (ATSC) appear to be attractive sources for cell therapeutic applications like the treatment of cartilage lesions. Unfortunately, hypertrophic markers like collagen type X are induced by common in vitro protocols for chondrogenic differentiation of mesenchymal stem cells (MSC) and these occur in osteoarthritic but not in healthy articular cartilage. For clinical application of MSC-derived chondrocyte-like cells a stabilization of a nonhypertrophic differentiation stage like in healthy articular cartilage is desired.
Aim of study: The aim of this study was to evaluate whether inhibitors of proteases or matrix metalloproteinases are able to delay or inhibit hypertrophic differentiation of MSCs.
Methods: Chondrogenic differentiation of BMSC and ATSC was induced by TGF-β, or TGF-β/BMP6 and spheroids were differentiated in the presence of the inhibitors leupeptin, pepstatin, aprotinin and hydroxamate for 28 days. Gene expression was analyzed by RT-PCR and differentiation of the spheroids was evaluated by alcian blue stain for acid proteoglycans and immunohistology for collagen types I and II.
Results: After leupeptin-, pepstatin- and aprotinin treatment deposition of collagen type II protein and proteoglycans was reduced in ATSC but not BMSC derived spheroids. Hydroxamate completely inhibited proteoglycan and collagen type II staining in both groups, although transcripts for COL2A1, besides transcripts for COL10A1, MMP3, MMP13 and AGC1 were detectable.
Conclusion: The evaluated inhibitors of proteases and especially the MMP3-inhibitor hydroxamate modulated the chondrogenic differentiation of MSCs. These inhibitors, however, did not specifically downregulate transcription of the hypertrophic markers COL10A1 and MMP13 which was desired to reach a molecular phenotype typical for healthy articular cartilage.

P092-Mo

Human Chondrocytic Lines Provide a Model for Achondroplasia and Thanatophoric Dysplasia

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Achondroplasia (ACH) and thanatophoric dysplasia (TD) are human skeletal disorders of increasing severity accounted for by mutations in the fibroblast growth factor receptor 3 (FGFR3) gene. We generated six immortalized human chondrocyte lines that express a constitutively heterozygous mutant of FGFR3. Mutation analyses showed that the chondrocytic lines carried, respectively, the G380R mutation (ACH phenotype), the S249C, R248C, G370C, Y373C mutations (TDI phenotype) and the K650E mutation (TDII phenotype).
Chondrocytes were isolated from human fetal growth cartilage and immortalized by transfection of the SV40 large T antigen gene. The cell lines were characterized and analyzed for factors controlling chondrocyte differentiation. Cell lines were subcloned according to the following parameters: cell morphology, mRNA and protein levels of extracellular matrix molecules to confirm a cartilage-specific and stable phenotype. We selected cell lines associated with an expression of extensive extracellular matrix components including proteoglycans (aggregan, byglican, decorin), collagens type II and type IX, MMP3 and signaling molecules (Ihh, Pthrp, FGFR3). Here we show the constitutive phosphorylation of FGFR3 and the activation of the STAT pathway in these immortalized cells. The cell lines provide a good model for ACH, TDI and TDII phenotypes, in addition we show for the first time the excessive activation of signaling cascades mediated by the FGFR3 mutants in human chondrocytic cell lines. Availability of this model will permit rational strategies for targeting the FGFR3 signaling pathways and to address new strategies in the treatment of achondroplasia.
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P093-Tu

Identification of Acid-Sensing Ion Channels (ASICS) in Bone

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It is well known that bone balances serum pH variations to maintain a stable physiologic systemic pH and that osteoclast and osteoblast activity is tightly regulated by subtle changes in extracellular pH [1,5]. However, yet the means by which bone can sense changes in pH are still ill defined. Acid sensing ion channels (ASICs), members of the ENaC/DEG (epithelial sodium channel/degenerin) gene family of amiloride-sensitive, proton-gated cation channels, are almost ubiquitous in the mammalian nervous system. The ASIC subfamily comprises ASIC1 (with the splice variants ASIC1a and ASIC1b), ASIC2 (with isoforms ASIC2a and ASIC2b), ASIC3 (with three variants) and ASIC4 (two variants). ASICs are activated by ion exchange, during which multiple protons are displacing Ca2+ from high-affinity binding sites [4]. Knowing the importance of mechanical loading for bone metabolism,

ASICs are the more so interesting as in various organisms members of the ENaC/DEG gene family have been linked to mechanical sensation [2,3] and degenerin can interact with the extracellular matrix [6].
Firstly, we showed that small changes (0.1–0.2) in pH dramatically changes mineralization and also affects osteopontin expression. Secondly, using real-time PCR we demonstrated ASIC1a, ASIC3 and ASIC4 in human bone as well as cartilage biopsies. ASICs mRNA expression was also detected in isolated human osteoblasts. Immunostaining and Western blotting further demonstrated the presence of ASICs in osteoblasts. In osteoclasts we only detected ASIC1a and ASIC3. Interestingly, transcription of ASIC1a was restricted to mature osteoclasts and was absent in monocytic precursors.
In conclusion, we demonstrate for the first time using human bone and cartilage biopsies that ASICs are expressed in human skeletal tissues and that ASICs are thus not exclusively expressed in nervous tissues. The presence of ASIC channels may explain how bone cells can respond to environmental changes in pH. ASICs thus open new venues for the regulation of bone metabolism (e.g., mechanical sensation, regulation of hormone activity) and may provide a mean by which metabolic acidosis can directly affect bone cell function.
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P094-Su

Differential Gene Expression of Bone Anabolic Factors and Trabecular Bone Architectural Changes at a Distal Skeletal Site in Primary Hip Osteoarthritis

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Evidence is accumulating for the role of bone in the pathogenesis of osteoarthritis (OA). Human OA subchondral bone is sclerotic, yet mechanically weak due to hypomineralization, increased collagen metabolism and the presence of collagen type I homotrimer. This study examined whether gene expression of bone anabolic factors and trabecular bone architecture and turnover are altered at a skeletal site distal to subchondral bone, the intertrochanteric (IT) region of the femur, in primary hip OA patients compared to age- and sex-matched controls. IT trabecular bone cores were obtained from 16 primary OA patients at total hip arthroplasty surgery (8f, 8m, mean age 65 [48–85] years) and 16 non-OA controls at autopsy (8f, 8m, 64 [44–85] years). RNA isolated from each bone sample was used for semi-quantitative RT-PCR analysis of alkaline phosphatase (ALP), osteocalcin (OCN), osteopontin (OPN), IGF-I, IGF-II, TGF-β1 and the collagen type I genes COL1A1 and COL1A2, mRNA. For 9 OA and 11 control cases, undecalcified bone histology was prepared for histomorphometry. Expression of ALP ($P < 0.004$), OCN ($P < 0.004$), COL1A1 ($P < 0.0001$) and COL1A2 ($P < 0.002$) mRNA were all significantly elevated in OA bone, suggesting increased osteoblastic biosynthetic activity and/or bone turnover at the IT region in OA. However, static indices of bone turnover did not differ between OA and controls. Interestingly, the ratio of COL1A1:COL1A2 mRNA was 2-fold greater in OA bone compared to control ($P < 0.0001$), suggesting the possible presence of collagen type I homotrimer in IT OA bone. Analysis of the collagen type I alpha1:alpha2 protein ratio is required to confirm these mRNA data. Levels of OPN, IGF-I, IGF-II and TGF-β1 mRNA were similar between OA and control bone. Although bone volume fraction and trabecular thickness were not different between the groups, OA bone had significantly increased surface density of bone ($P < 0.004$), decreased trabecular separation ($P < 0.004$) and increased trabecular number ($P < 0.004$). This architectural distinction between OA and control IT trabecular bone is consistent with our previous studies. The differential gene expression and trabecular architectural changes observed at a distal skeletal site in OA implicate the generalized involvement of bone in the pathogenesis of OA. If OA is caused or exacerbated by altered bone structure, treatment strategies may be identified to prevent the bone changes and thus delay joint degeneration.

P095-Mo

Human (FETAL) Cartilage-Gene Expression Profiles and Specific Gene Analysis

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To characterize the gene expression pattern of human cartilage, we determined the sequences of 4478 ESTs isolated from a cDNA library of pooled cartilage tissue (20th week to 2nd year of life). The average read length of the ESTs was 500 bp. To obtain this number of quality-controlled ESTs, we had to prepare more than 15,000 templates due to the high number of short inserts within the

library. Extensive and recently updated examination revealed that about 77% of EST sequences match to known genes/RNAs, 10% to genomic sequences, whereas 9.5% still match to anonymous ESTs, only 3.5% were contaminations. The most abundantly expressed transcript was derived from type II collagen gene (COL2A1), comprising 2.7% of all ESTs (120 clones). Furthermore, the list of characterized genes included 16 different collagen genes, underscoring the importance and diversity of collagen expression in cartilage.

Recently, two reports on large-scale generation of ESTs from human fetal cartilage have been published. Here we present a detailed comparison of our results with those generated from an 8- to 12-week (Zhang et al., 2003) and an 18- to 20-week human fetal cartilage cDNA library (Pogue et al., 2004), respectively. These data cover profiles of human cartilage from 8 week of gestation until the 2nd year of life and their spectrum will help to guide further research to identify genes essential for cartilage development and provide new chondrodysplasia candidate genes. However, evaluation of the data also shows the large variety of expressed transcripts in the tested tissue, resulting in the identification of new genes yet not characterized beyond their nucleotide sequences. Some of these clones are currently under detailed scrutiny by further bioinformatic tools and experimental investigation. Actual results demonstrating cartilage-specific involvement of these genes will be presented.

P096-Tu
Abstract Withdrawn

P097-Su
Transcriptome Analysis and Comprehensive Expression Profiling in Human Tissues and Cells for Identification and Characterization of Relevant Drug Targets in Bone Metabolism

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Target selection is a strategic decision point in the drug discovery process. The use of comprehensive transcriptome analysis is a powerful tool to characterize and take decisions on target selection and prioritization. Axxam has developed a platform based on gene expression profiling, including technologies like high density oligonucleotide microarrays, real-time quantitative PCR and in situ hybridization as a tool to identify and characterize genes encoding targets relevant in bone metabolism. We have focused our attention on genes encoding druggable targets like proteases and GPCRs.

Whole transcriptome analysis using HG-U133 GeneChip (Affymetrix®) has been performed through a panel of 22 human tissues and cells comprising bone, bone marrow, adipose tissue, human Osteoblasts and human mesenchymal stem cells (hMSC) primary culture samples in different culture conditions. Probesets intensity was calculated by GCRMA and Quantile normalization has been applied. The data obtained have been analyzed by Spotfire® Decision site for Functional Genomics and by in house developed programs. Pathways and bone markers have been inspected. Several high level statistical analysis and pathway analysis have been applied in order to identify genes specific to bone and skeletal tissues versus other tissues.

In house generated and curated databases for "druggable" targets has been used in order for additional filtering of the generated gene lists.

Quantitative real-time PCR has been applied in order to validate GeneChip® data and to generate extensive expression profiling of selected targets in a panel of more that 50 human non-pathological tissues through Axxam proprietary GeneTrawler®.

Some relevant examples will be shown in more detail.

P098-Mo
The Prevalence of Ochronosis in a Romani (Gypsy) Population in Tamil Nadu, South India

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Background: Alkaptonuria is a rare hereditary metabolic disorder, characterized by the absence of the enzyme homogentisic acid oxidase. This defect leads to the accumulation of homogentisic acid in articular cartilage and soft tissues leading to ochronotic spondyloarthropathy. We observed a significant correlation in the frequency of joint pains, effusion, deformity and ochronosis in patients from the Romani community who occasionally came to our hospital. The aim of the study was to document the prevalence of osteoarthritis and alkaptonuria in the local Romani community.

Methods: Symptoms for osteoarthroses were evaluated in 806 gypsies, living together in the Romani community in and around Vellore. Detailed clinical examination was done for arthralgia, low patients with signs of arthritis and deformity. Classical cutaneous and extra-articular markers of ochronosis were documented along with urine high profile liquid chromatography (HPLC) for homogentisic acid. Radiological examination was done in sixteen patients who had advanced arthritic symptoms. Skeletal scintigraphy was done for four patients.

Results: Eight hundred and six subjects participated in the study. The mean age was 32.8 + 12.19 years. The majority were women 458 (56.8%). A quarter of the subjects (26.4%) were symptomatic and had clinical signs of osteoarthritis. The knee was the most commonly involved joint (77%), followed by the spine (41.3%), ankle (15.0%)

and shoulder (14.1%). Tendo Achilles rupture was clinically diagnosed in six patients. Three patients had pigmentation of the palm. This soft tissue ochronosis associated with pathognomonic signs of AKU was further confirmed by salient radiological features. Skeletal scintigraphy showed a significant uptake of technitium99 in the intervertebral disc spaces of the lower dorsal and lumbar spine.

Conclusion: Urine HPLC for homogentisic acid was done in 111 (13.8%) subjects. 21 (18.9%) of samples tested positive by this method. This is very high prevalence for any ethnic group, the usual being one in a million.

P099-Tu

Other More Logical Approach to Osteoporosis with Teriparatide

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Background: FDA has approved teriparatide, which is a man-made form of parathyroid hormone, for the treatment of osteoporosis in postmenopause, demonstrating validity of increasing bone mass in men. Over and above hominum errare, that is, man emblem is to be mistaken again and again.

Statement of purpose: Determining if teriparatide is in the right way or wrong to osteoporosis.

Statement of method: We have performed a review in a worldwide basis on drugs increasing bone mass.

Summary of results: The effects of teriparatide on fracture risk have not been studied in men. In animal studies with teriparatide, there was an increase in the number of rats developing osteosarcoma. Therapy for more than 2 years is not recommended. Knowing osteoporosis is knowing mineral homeostasis. Treatment with teriparatide is recommended for 18 months in a continued basis. The first approved agent that stimulates new bone formation was the sodium fluoride other strontium ranelate. The risk of teriparatide is finishing as sodium fluoride. There are a lot of studies ongoing. It is necessary to perform a study knowing the calcium metabolism, intake and loss, vitamin D and hormone D metabolism. Intestinal malabsorption syndromes, and nephropathies, but the most important is to know the human development which reach bone peak 25 through 30 years old, not in 18 months, and mineral homeostasis. Parathyroid hormone plus alendronate is a combination that does not add up, but alendronate plus HRT add up in women and teriparatide increase 9.7% at end. So, the way is the bone cycle homeostasis, bone mineral homeostasis, bone growth and mineralization during years. The failure of sodium fluoride was due to do not give well the calcium. The HRT was in the summit, but dropped because of Million Women Study and WHI study and others with biases, and the results cannot be extrapolated to all oestrogens and gestagens. Equine oestrogens are not for women but for mares, progesterone is not medroxyprogesterone acetate. The WHI and the one million sank HRT without logical rationale. This can be the way for Forteo.

Conclusions: Giving teriparatide for 1 to 3 months and after an light antiresorptive for 1 to 3 months in a cycled basis is precise to assay and to think if there are any peak in parathyroid hormone day cycle and so do, and to evaluate calcium, vitamin D and hormone D metabolism. Without that teriparatide has the same way open as sodium fluoride.

P100-Su

Fat Content and Fatty Acid Profile of Osteoarthritic and Osteoporotic Bone

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Osteoarthritis (OA) is linked with obesity and anecdotal evidence from surgery and the laboratory indicated a high fat content in femoral heads. It has also been hypothesized, however, that osteoporosis may result from a switch by osteoblasts to an adipocytic phenotype. This should result in a high fat content in the more porous bone. Measuring the total lipid content and fatty acid profiles of OA and OP femoral heads might be expected to yield interesting information, and that was the aim of this study. Lipid content was measured from 5 femoral heads from patients undergoing total hip replacement for OA and 5 following osteoporotic fracture of the femoral neck. Bone tissue, containing marrow, was powdered in a liquid nitrogen freezer mill and weighed. Lipids were extracted using chloroform-methanol and weighed. The remaining (hydrated) bone powder was also weighed. Lipid was then converted to fatty acid methyl esters (FAMEs) and the fatty acid profile determined using a Varian 3800 Gas Chromatograph with a J and W Scientific DB-225 Column. The mass of lipid per unit mass of bone tissue, mean (SD), was 0.24 (0.04) g/g in the OA group and 0.21 (0.05) g/g in the OP group. Because the apparent density of OA bone is greater (0.71 g cm^{-3}) than OP bone (0.38 g cm^{-3}) the fractional volume available for the fat is considerably smaller, porosity 59% in OA, 80% in OP. Expressed as mass of lipid per unit volume of bone tissue (by dividing bone mass by apparent density) resulted in 0.22 g cm^{-3} in OA, 0.10 g cm^{-3} in OP ($P = 0.002$, t test). There were also significant differences in the fatty acids present in OA and OP, expressed a percentage of the total lipid mass (Table 1). Despite having a lower porosity OA bone contains twice as much lipid as OP bone per volume of bone tissue. There are also significant differences in fatty acid profiles. Notable is arachidonic acid, a major pro-inflammatory mediator. This comprised twice the fraction of lipid in OA compared with OP. These data support the hypothesis of altered lipid metabolism in OA. The relative lack of lipid in OP was unexpected.

Table
Significantly different fatty acids in OA and OP bone

Fatty acids	OP %mass ± SD	OA %mass ± SD	*P*
C16:1 Palmitoleic	4.1 ± 1.1	7.0 ± 1.2	0.005
C18:0 Stearic	4.67 ± 0.30	3.26 ± 0.48	<0.001
C20:2n6 Eicosadienoic	0.102 ± 0.023	0.151 ± 0.023	0.011
C20:4n6 Arachidonic	0.245 ± 0.029	0.479 ± 0.067	<0.001
C20:3n6 Dihomo-gamma-Linolenic	0.117 ± 0.028	0.166 ± 0.019	0.012
C22:4n6 Docosatetraenoic	0.119 ± 0.017	0.178 ± 0.020	0.001

P101-Mo
Calcified Tissues Involved in Tooth Ankylosis

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The interactions between the alveolar osseous tissues and the dental tissues are determinant factors for the viability of both the ankylosed deciduous or permanent teeth, and the eventual permanent teeth situated below the deciduous one. However, the studies aimed to apprehend the chronology of the tissular events in the ankylosis process around the teeth have been scarce until recently.

We present the first results of a series of studies dealing with the tissue interactions established between the alveolar tissues and the teeth in an experiment in which a forced teeth reimplantation was surgically obtained. Twenty adult female dogs were operated, under general anesthesia. The periodontal ligaments of both the second maxillary molars were removed after a surgical teeth extraction. Afterwards, the natural teeth were replaced and secured by way of a surgical suture of the gingiva. The ensuing dental ankylosis was evaluated clinically. The sacrifice of the animals was performed by anesthesia overdose 2 to 18 weeks after the intervention. The alveolar bone samples were submitted to a scheduled procedure of embedding in plastic polymers without prior decalcification, in order to perform ultra-structural studies: scanning microscopy with secondary and backscattered electrons (BS-SEM).

All our samples showed that the calcified tissues involved in the ankylosis process show a very similar pattern that has been previously described for the processes of sutural fusion, fracture healing, teeth eruption and osteointegration of various biomaterials. It involves a first phase of osteoclastic activity, followed by the formation of thin trabeculae of chondroid tissue. After that, the bone apposition occurs, starting with woven bone, then followed by lamellar bone. These tissues seem to be responsible for the adherence of the ankylotic teeth to the dental socket. The ubiquitous presence of chondroid tissue in the samples strongly suggests that the mechanisms involved in the dental ankylosis are related to the endomembranous ossification process, rather than to the endochondral ossification.

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P102-Tu
The Unique Growth Process of the Ultrathin Mineralites in Bone-Apatite

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Bone is composed by very small hydroxyapatite particles embedded in collagen fibers. Measurements of the solid particles were the subject of numerous research projects using electron microscopy since 1952, low angle X-ray, neutron diffraction and lately by atomic force microscopy. Recently the apatite particles were appropriately termed mineralites. They are tabular, typically several tens of nanometer long, slightly less wide and ultrathin; their thickness vary between 5 and 2 nm. A study in 1996 using transmittance electron imaging determined the thickness as 2 nm and this value was confirmed by AFM in 2001. In 2003, a comparison between young and mature bovine bones showed that the thickness of a mineralite in a mature bone was 0.7 nm. This dimension brings the mineralite into the crystallographic range: 0.7 nm = 7 A is the height of hydroxyapatite crystallographic unit. It means that one crystallographic layer forms a separate sheet.

Usually crystal growth from solution starts with the generation of a three-dimensional, hydrated nucleus. Spontaneous growth on the nucleus involves deposition of mobile ions into an array of 3-dimensional crystal organization through growth steps. This feature of solution growth does not apply to the formation of the monolayered apatite. The rectangular habit of mineralites, composed of one layer of hexagonal crystallographic units of apatite, indicates an external control by constrains of the accommodating space and/or by hetero-nucleation.

A new model of hydroxyapatite nucleated by aspartic acid molecule will be presented. The process in the solution starts with attraction of dehydrated calcium ions to an ionized aspartic acid molecule and subsequent attraction of carbonate ions and phosphate ions. A carbonate ion and a terminal oxygen of the acid fill up the space of a phosphate ion in a hydroxyapatite crystallographic unit. Four such clusters and two phosphates form a nucleating unit of apatite. Six regular apatite units may be assembled from its six sides forming a monolayered "flower". The assemblage of such flowers in surface covering manner form the ultimately thin mineralite of 0.7 nm thickness. Joining of 20 regular units in 3 layers all around the nucleating unit and its six-side extensions generate the usual bone mineralites of 2 nm thickness.

P103-Su

Immunohistochemical Localization of Perlecan and Heparanase in Hertwig's Epithelial Root Sheath During Root Formation in Mouse Molar

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Epithelial–mesenchymal interaction is required for tooth development. In cementogenesis, mesenchymal cells in dental follicle are known to penetrate the rupture of Hertwig's epithelial root sheath (HERS) and differentiate into cementoblasts. Although epithelial–mesenchymal interaction with dental basement membrane is a key process for cementogenesis, the mechanisms such as degradation of the dental basement membrane have not been clarified. Perlecan, a large heparan sulphate proteoglycan (HSPG), is an important component of basement membranes. Perlecan can preserve growth factors and cytokines by heparan sulphate chains and modulate their biological functions. On the other hand, heparanase is an endo-beta-D-glucuronidase capable of cleaving heparan sulphate chains of perlecan. Cleavage of heparan sulphate chains is involved in release of heparin-binding growth factors. The aim of this study was to elucidate the immunolocalization of perlecan and heparanase in developing mouse molar to clarify their roles in cementoblast differentiation by light and electron microscopy. At the initial stage of root formation, perlecan was present throughout the dental basement membrane. In contrast, weak heparanase expression was detected in the cells of HERS. During root formation, perlecan was disappeared from the dental basement membrane faced on dental follicle. Intense immunoreactivity for heparanase was detected in the cells of HERS during tooth root development. Some cells of HERS migrating into the periodontal ligament also showed positive immunoreactivity for heparanase. After root formation, the HERS cells formed cell clusters in the periodontal ligament and became the epithelial rests of Malassez. Perlecan localization was detected on the basement membrane of epithelial rests of Malassez, whereas these cells exhibited no immunoreactivity for heparanase. These results suggest that the degradation of perlecan in the dental basement membrane is closely related with the localization of heparanase in the cells of HERS. Heparanase may contribute to cementoblast differentiation by liberating heparin-binding growth factors in the dental basement membrane.

P104-Mo

Bone Densitometry in Moderate and Severe Asthmatic Patients in Khuzestan Province—Iran

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Background: Osteoporosis is an important complication of asthma disease whose secondary fracture is very problematic for such patients.
Objective: This study whose purpose is to determine the rate of bone density in moderate and severe asthmatic patients and compare that to normal people.
Methods: This study was conducted for the duration of one year on 71 male asthmatic patients with the average age 38.2 and 60 other, that were include in the control group with the average age of 37.6. In this investigation bone measurement was done using dual energy x-ray absorptiometry (DEXA) in both lumbar spine (L2–L4) and femoral neck.
Results: Patients in the two groups of control group and asthmatic patients (moderate and severe) were compared. The rate of bone density was found to be 1.142 g/cm^2 in lumbar spine in control group versus moderate asthmatic patients that was 0.913 g/cm^2 as well as the severe group with the rate of 0.858 g/cm^2. The cross-sectional results of the comparison revealed a significant statistical value of $P < 0.005$.In femoral neck the rate of bone density was 0.915 g/cm^2 in the control group and 0.857 g/cm^2 in the moderate asthmatic patients and 0.801 g/cm^2 in the severe ones. In this comparison the asthmatic patients who were found to be more prone to osteoporosis were those who used more steroid. On the control group versus asthmatic patients with and without the use of steroid in the lumbar spine. The rate of bone density in the control group was 1.145 g/cm^2 and in the patients without steroid was 0.948 g/cm^2 in the asthmatic patients with steroid was 0.842 g/cm^2.The results indicated a significant difference of $P < 0.005$. Further results in the femoral neck showed a bone density of 0.914 g/cm^2 in the control group, 0.896 g/cm^2 in the asthmatic patients without steroid and 0.801 g/cm^2 in asthmatic patients with steroid. These results again revealed another significant difference of $P < 0.005$ in all cross-sectional groups.
Conclusion: This study showed that asthmatic patients even without steroid use are predisposed to osteopenia and osteoporosis. This involvement was more obvious in the lumbar spine.

P105-Tu

Implantation of Octacalcium Phosphate (OCP) Stimulate Both Chondrogenesis and Osteogenesis in the Tibia, but Only Osteogenesis in the Mandibular Bone of Rats

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Background: It is not known whether long bones and calvaria have distinct biological characteristics. Octacalcium phosphate (OCP), which is precursor of the hydroxyapatite, has been reported to stimulate bone formation if implanted in the rat parietal bone defects. The present study was designed to investigate how the long bone and calvarium

respond to OCP implantation and to compare their biological characteristics.
Methods: The synthetic OCP was implanted into the rat tibiae and mandible bone defects. The biological response was examined histologically identify bone and cartilage formation.
Results: Both chondrogenesis and osteogenesis were initiated in the tibia 1 week after implantation of OCP and most of the cartilage was replaced by bone at week 2. However, the mandible bone only show osteogenesis responding to OCP implantation at week 1, and no cartilage formation was associated with the osteogenesis.
Conclusions: The present study demonstrated the distinct characteristics of biological response to OCP implantation between the long bone and calvarium defects in rat.
Keywords: Octacalcium phosphate, Implantation, Long bone, Calvarium, Osteogenesis, Chondrogenesis, Rat.

P106-Su
Study on Bone Mineral Density (BMD) Among the Male Behcet's Disease (BD) Patients in Khuzestan Province—Iran
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Background: Osteoporosis is an important health problem in patients involved with rheumatic disease with and without steroid use.
Methods: For study of BMD of lumbar spine (L2–L4) and femoral neck, 69 BD patients with mean ages of 26.6 years old were selected. For the control group, 70 males with mean ages of 25.2 years old and without any predisposing factors were selected. All patients were on colchicine.
In this study bone measurement was done by dual energy X-ray absorptiometry (DEXA) in lumbar spine (L2–L4) and femoral neck.
Results: In present study, 69 BD patients showed low BMD of femoral neck compared to 70 cases of control group ($P < 0.001$).While BMD of lumbar spine among 69 BD patients did not show significant compared to control group ($P > 0.05$).
Conclusion: The reduction of BMD of femoral neck showed significant among the BD male patients.

P107-Mo
Environmental Effects on the Bone Tissue
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The bone and cartilage cells, the connecting tissue, the adipose and smooth muscle cells are differentiated from fibroblasts. These processes are determined by genetical and environmental effects (e.g., extracellular receptor). From our earlier study we know that the modifications of the extracellular environment determine the formation and eventually the functionability of special connective tissue cells. Thus environmental effects (chemical and/or physical) play a very important role among the factors that induce osteoporosis.
The chronic effects of subtoxic doses/(1 ppm/bw/kg) of hexa- and tri/chlorobenzenes were studied on bone tissue. The cellular mechanisms of bone tissue destruction were investigated in model experiments.
Wistar rats (male, 120–280 g/bw) were treated orally with chlorobenzenes (hexachlorobenzene–trichlorobenzene = 1:1; 1 ppm/bw/kg) for 30, 60 and 90 days through gastric tubes. Samples were taken from the bone tissues of the treated, control and solvent-treated control animals. Tissue structure and Ca^{2+} content of the bone and the liver function (gamma-GT, SGOT, SGPT) and structure were investigated. Monolayer cultures were generated from the fibroblasts of treated and control animals, and the cell proliferation, collagen synthesis (protein content in the presence and absence of collagenase-E) and structure (morphometry) were examined.
As a result of the chronic chlorobenzene treatment, bone tissue destruction was observed in the animals. This general \structural damage in the bone tissues was correlated to significant change in the Ca^{2+} content of the bone matrices.
This work was supported by: ETT 61/2003 and ETT 270/2003.

P108-Tu
Behaviour of Osteosarcoma and Primary Osteoblastic Cells on FITC-Labelled Fibrillar and Monomeric Collagen Layers
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Background: The interaction of cells with extracellular matrix collagens can be divided to (1) collagen fibril recognition and attachment via integrins, (2) extracellular collagen degradation of by matrix metalloproteinases (MMPs) and (3) phagocytosis and intracellular collagen degradation by cathepsins, as cathepsins B and L. Phagocytosis is the principal pathway for collagen degradation in physiological turnover of connective tissues. Extracellular degradation dominates in pathological situations, where large amount of collagen is degraded, e.g., in inflammation or cancer.
Objective: To visualize these three events of cell behaviour. To compare the extracellular collagen degradation between

osteosarcoma cells and primary osteoblastic cells and also between monomeric and fibrillar collagen layers.
Methods: We labelled monomeric and fibrillar collagen coats on glass cover slips with fluorescein-5-isothiocyanate (FITC) and cultured MG-63 and ROS 17/2.8 osteosarcoma cells and primary human osteoblastic cells on them. We observed the cell responses with confocal microscope, total internal reflection fluorescence microscope (TIRFM) and field emission scanning electron microscope (FESEM). The collagen coats were studied with atomic force microscope (AFM).
Results: The osteosarcoma cells attached to collagen and produced holes, ruptures, paths and tracks and dragged stripes of collagen behind them as their moved on the collagen layer. 2-Integrin were not co-localized with a focalα. The attachment sites and adhesion protein paxillin. The typical tracks made by the cells were inhibited by the wide-spectrum MMP-inhibitor galardin. The monomeric collagen was much more effectively degraded than the fibrillar collagen. We also noticed that in some conditions the entire monomeric coat was disappeared by osteosarcoma cells, but not by primary osteoblastic cells. By TIRF microscopy, phagosomes full of FITC-labelled collagen were seen.
Conclusions: The role of collagen detecting integrins in cell attachment needs to be further investigated. Primary osteoblasts, unlike the osteosarcoma cells, do not destroy the monomeric collagen layer, even though it is highly susceptible for degradation.

P109-Su
Enhanced Bone Formation in Hyaluronan-Based Scaffold (Hyalograft 3D®) with Bone Marrow-Derived Stem Cells
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Among the various biodegradable scaffolds, a hyaluronan-based biodegradable polymer (Hyalograft 3D®) has already been proved to be effective scaffold for skin and cartilage. However, the application of Hyalograft 3D® for bone formation has been reported in very few literatures. And recently, bone marrow-derived stem cell (BMSC) has been applied in various tissue regeneration. The aim of this study is to evaluate of the usefulness of Hyalograft 3D® in bone formation and the effectiveness of BMSC. In vitro test, we evaluated the osteogenic potential of Hyalograft 3D®, where rat BMSCs were cultured. In the MTT assays at 6 h, 3 and 7 days post-plating to test, whether the Hyalograft 3D® could support rat BMSC-derived osteoblastic cell growth, the cells were well proliferated. The cells were well attached to the scaffold, which was investigated by scanning electron microscopy after 7 days in culture. In the analysis with RT-PCR at 7 days after seeding, it was demonstrated that the cells expressed differentiation-specific markers (alkaline phosphatase, collagen type I, osteopontin and fibronectin) as like normal differentiation process. In animal experiments, Hyalograft 3D® alone (n = 4) and Hyalograft 3D® loaded with BMSCs (n = 4) will be compared in rat calvaria defect model (4 and 8 weeks). The new bone was well formed in both groups and it was enhanced in Hyalograft 3D® loaded with BMSCs compared with Hyalograft 3D® alone. These results suggest that Hyalograft 3D® can be used as a scaffold for bone regeneration besides present usual applications in skin and cartilages, together with the advantage that it degrades spontaneously after implantation without detectable inflammatory response unlike other biodegradable polymer such as polyglycolic acid.

P110-Mo
Effects of Continuous Brain Infusion of Leptin on Bone Metabolism in Rats
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Central role of hypothalamus in the control of bone remodeling might involve leptin, a hormone secreted by adipocytes, that has recently emerged as a modulator of bone metabolism through multiple pathways. From conflicting data it is tempting to hypothesize that the response to leptin is not uniform throughout the skeleton but may vary depending upon structural components (trabecular vs. cortical), location (axial vs. appendicular) and skeletal maturity. The present study was designed to analyze possible differences in bone remodeling, mass and density following a continuous intracerebroventricular (i.c.v.) infusion of leptin (1.5 μg/rat/day) to male rats (80 days old) for 28 days. The treatment produced a body weight loss (401 + 12 g vs. 455 + 16.8 g of controls, $P < 005$) and a food consumption decrement. Total and metaphyseal femoral BMC and areal BMD by DXA were significantly lower in the leptin treated group vs. controls (total BMC −20%, BMD −25%; metaphysis BMC −51%, BMD −49.4%) whereas no changes were detected in the femoral diaphysis and in lumbar vertebrae (L1–L4). Also, no changes in planar femoral area were detected, suggesting that the treatment did not affect appendicular bone growth. At the end of the experiment, excised femurs and tibiae were examined by peripheral quantitative computed tomography (pQCT, resolution 70 μm). A decrease in the trabecular volumetric BMD was detected between leptin treated and control rats, whereas no significant differences were observed in the cortical volumetric BMD. Femoral and tibial cross-sectional areas by pQCT were slightly lower in the treated group vs. control but the difference was not statistically different. The excretion of lysylpyrydinoline

(LP) and serum levels of osteocalcin (OC) were decreased with time in the controls (LP −32%; OC −30%), as expected since the rats were approaching a steady-state level of bone maturity. On the contrary, both markers remained elevated in the leptin treated group as those of the basal values suggesting the maintenance of a high activation frequency of bone remodeling units.
This study demonstrates that the effects of the central infusion of leptin vary upon the different skeletal sites and structural components. The effects involve a modulation of bone remodeling at the activation frequency level of a faster growing state. It is therefore conceivable that bone size was maintained, despite the lower body weight, being the potential expression of this activated state.

P111-Tu

Neurotransmitter, Serotonin, Affects Cortical Bone Architecture in Rat Femur

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Recently, more and more evidence is found for a control of bone mass by the central nervous system. Many studies indicate a role for the nervous system in embryonal skeletal development, during fracture healing and during remodelling after insertion of implants. Immunohistochemical experiments demonstrate the existence of nerve fibers in bone tissue. Furthermore, earlier we showed the presence of functional serotonin receptors in bone cells. Proliferation of osteoblast precursor cells was found to be stimulated by α-methyl-5-HT, a serotonin-analogue selective for 5-HT2 receptors. However, the exact mechanisms and involvement of neurotransmitters still have to be elucidated.
In this study we investigated the effects of serotonin on bone metabolism in vivo. We injected 2- to 3-month-old female Sprague–Dawley rats daily subcutaneously with serotonin (5 mg/kg) during 3 months. DEXA scans were made after 2 months and 3 months of treatment. Total body BMD was significantly increased after these time points in the treated animals.
μCT analysis of femurs of 3-month-old rats showed apparent differences in the metaphysis of the femurs. In treated animals cortical thickness was significantly increased. Trabecular bone volume, however, was significantly decreased in serotonin treated animals. Interestingly, the perimeter and cross-sectional moment of inertia (MOI), a proxy for geometrical bone strength, were the same in both groups. These data suggest a reduced endosteal bone resorption in serotonin treated animals. This is supported by the observed decrease in bone marrow cavity.
This study shows that serotonin significantly alters bone mass and cortical bone architecture in rats. Serotonin treatment seems to decrease endosteal bone metabolism c.q. resorption, leading to the observed increased cortical bone thickness, and decreased trabecular bone volume. As a result MOI at this time point remained the same in the two groups. However, changed endosteal bone resorption may have very profound effects on bone strength at older ages.

P112-Su

Physicochemical Characterization and Histologic Analysis of Different Xenografts in the Repair of Critical Size Defect in Calvaria of Rats

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The aim of this study was to evaluate the physicochemical properties of mixed bovine bone (MBB) and analyze the osteoconductive potential of MBB compared to woven inorganic bovine bone (Bio-Oss® e Gen-Ox®), having as a control the blood clot, in rat calvariae. MBB was analyzed by means of thermogravimetry, infrared spectroscopy, differential exploratory calorimetry, pore size and porosity determination, scanning electron microscopy, X-ray diffraction and cristalinity. Critical size defects (9-mm diameter) were made in rat calvariae (n = 5/period-material), filled according to the experimental group and covered with bovine cortical bone membrane (Gen-Derm®). At the end of the study periods (1, 3, 6 and 9 months postoperatively), the skulls were collected, fixed in 10% formalin, radiographed and processed for histological analysis. Physicochemical analysis showed characteristic collagen and hydroxyapatite groups in the MBB. The comparative microscopic evaluation showed that (a) there was no complete closure of the defects in any study group; (b) in all groups, the membrane was resorbed before 1 month; (c) in the control group, ossification took place on the periphery of the defect, and the central region of the defect was filled with connective fibrous tissue; (d) in the group treated with Bio-Oss there was ossification on the periphery of the defect and dense fibrous tissue surrounding the graft particles; (e) in the group treated with Gen-Ox there was bone formation surrounding the biomaterial particles; (f) in the group treated with MBB the inflammatory infiltrate persisted in the first month and was then substituted by fibrous connective tissue

surrounding the particles. In conclusion, (a) MBB is composed by organic (15%) and inorganic (75%) fractions; (b) none of the tested materials was able to induce the complete closure of the critical size defects; (c) Gen-Ox was the most osteoconductive material among those tested; (d) neither MBB nor Bio-Oss, in this experimental model, showed osteoconductive properties; and (e) none of the materials was resorbed in 9 months. Keywords: biomaterials, osseous implant, bone repair, guided bone regeneration, bovine bone, xenografts. Financial Support: CNPq (National Council for Scientific and Technological Development) and Fapesp (The State of São Paulo Research Foundation).

P113-Mo

An Adsorption Model Explains the Differences in Hydroxyapatite Binding Affinities of Important Bisphosphonates in Clinical Use

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Differences in bone mineral binding affinities of clinically utilized bisphosphonates (BPs) have been described previously with a decreasing rank order of zoledronate > alendronate > ibandronate > risedronate > etidronate, and with calculated hydroxyapatite (HAP) kinetic affinity constants (KL) of 3.47, 2.9, 2.3., 2.0 and 1.1 × 106 L/mol, respectively. Although all of these contain the central hydroxyl group, characteristic of high mineral affinity which imparts low dosage use, significant differences in affinity among these drugs exist. When compounded over multiple doses, this may lead to differences in uptake and release, and perhaps in bone quality. Work with early generation BPs showed that a tridentate binding mode to calcium in HAP explained most of their strong mineral chemisorption property. Similar modeling on this mineral surface with the nitrogen (N) containing BPs allowed a computer-aided 3-D analysis of the potential orientation of the N functionality of these agents. Once a low energy conformation of each BP was oriented in a tridentate binding mode on the trigonal prismatic column of calcium atoms in HAP, the N side chain conformations of the BPs were examined for their interaction with the [001] surface. The 4-amino group of alendronate can form an excellent N–H–O hydrogen bond (132°, 2.7 Å N–O distance) to the labile –OH oxygen on HAP, where carbonate and fluoride are known to intercolate. The ring N of zoledronate can only form a weaker electrostatic interaction with this labile –OH site; however, it forms two additional strong hydrogen bonds to a bifurcated network, between two P–O oxygen atoms closely oriented within the crystal lattice (above and below the plane) with a 130° and 131° angle and a 2.6 and 2.7 Å N–O distance, respectively, explaining its high affinity. In the case of risedronate, steric hindrance of the pyridyl ring prevents its N from hydrogen bonding in either fashion and it may only form weaker electrostatic interactions, such as at the labile –OH (N–O distance 3.0 Å, 102°). This affords weaker affinity compared to alendronate and zoledronate, but does produce higher affinity than etidronate (no N functional group). Comparative modeling of multiple benchmark BPs also demonstrate this affinity/H-bonding correlation. There is increasing evidence that the mechanism of action of each BP combines a differing ratio of biochemical activity vs. bone mineral interaction, which can lead to different pharmacology over extended dosing periods.

P114-Tu

Biotoleration of Cycloolefin Copolymer and its Blends in Vivo

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Introduction: Cycloolefin copolymer (COC) has similar mechanical features to biomechanical characteristics of organic tissues. Previously we have shown its toleration by synovial fibroblasts and osteoblasts in vitro. In this study we monitored the biotoleration of this material in vivo in rats belonging to the stem Wistar.

Materials and methods: COC coated with collagen type I or without surface treatment and high density polyethylene (HDPE) as control material were implanted under skin of rats. For better adhesion of collagen, the surface of COC was modified with N and O ions. After incubation period (1, 4 and 12 weeks), rats were sacrificed and newly generated pellicle was frozen in TissueTec. Later immunohistochemistry for macrophages, adhesive molecule (ICAM) and interleukine (IL-1 beta) were performed. To establish the cell types in extracted pellicle FACS analysis for the inflammatory cells (T and B lymphocytes) was done.

Results: Macrophage production was the lowest in the newly generated tissue on COC coated with collagen (15%) which was much lower than on HDPE (50%), detection of IL-1 beta was again lower on COC coated with collagen (30%) in comparison to HDPE (70%). The number of cells positive for ICAM molecules was on all types of COC materials higher (15%) than in HDPD (5%).

Using FACS analysis we determined the highest frequency of T lymphocytes in newly generated tissues grown on COC

with ions modified surface and the lowest in COC material; however, the inflammatory response was not very dramatic. **Conclusion:** All abovementioned tests in vivo showed high biotoleration of COC material. From performed experiments we conclude that cycloolefin copolymer is a material very well tolerated by living organisms. Furthermore, coating with collagen type I helps to increase the biotoleration of this material.

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P115-Su

Periodontal Tissue Regeneration by the Application of Phosphophoryn/Collagen Composite

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Objective: The aim of this study was to examine the regeneration of periodontal tissue after the application of phosphophoryn/collagen composite to horizontal 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 min. The distance between the bone crest and cemento-enamel junction (CEJ) 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, the animals were sacrificed and sections were prepared in a bucco-lingual direction.

Results: At 4 weeks post-surgery, calcified tissue formation was observed in the healing connective tissue of the test sites. At 12 weeks in the test sites, new cementum with Sharpey's fibers was observed on the treated root surface. Histometrical analysis revealed that the amount of new bone was significantly higher in the test sites than in the control sites at 12 weeks post-surgery ($P < 0.05$).

Conclusions: These results suggest that phosphophoryn/collagen composite has potent roles in promoting periodontal tissue regeneration during the healing process and could readily achievable methods of treatment for periodontal disease.

P116-Mo

Collagen Regulates Growth and Differentiation of Human Osteoblasts

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Bone is a specialized connective tissue that together with cartilage makes up the skeletal system. Seventy percent of the bone is composed of calcium phosphate in the form of hydroxyapatite and the remaining 30% consists of organic components that are produced by cells of the osteoblastic lineage. The most abundant protein component of the bone is collagen. Type I collagen constitutes 90% of the total protein of bone and types III, V and VI collagens are also present, though in much lower amounts and their functions are not known. In order to investigate mechanisms by which dihydroxy vitamin D3 (VD3), and L-ascorbic acid 2-phosphate (Asc 2-P), a long-acting vitamin C derivative, regulate the growth and differentiation of human osteoblasts; we cultured human osteoblasts-like cells in the presence of VD3 and/or Asc 2-P. Cell growth as assessed by cell number and DNA content was increased in the presence of Asc 2-P. Alkaline phosphatase (ALP) activity and collagen synthesis, which are early osteoblast differentiation markers, were stimulated by the presence of Asc 2-P. Synthesis of type III collagen was closely associated with growth of the cells. Inhibition of collagen biosynthesis attenuated growth-stimulating activity of Asc 2-P and culture of the cells on type III collagen-coated dishes stimulated growth of the cells, indicating that Asc 2-P stimulates growth of the cells through stimulation of type III collagen synthesis of the cells. On the other hand, VD3 stimulated type I collagen synthesis and ALP activity of the cells. Inhibition of collagen synthesis by inhibitors of collagen synthesis attenuated the stimulative effect of VD3 on ALP activity and the activity was significantly increased and the growth rate was decreased when the cells were cultured on type I collagen-coated dishes. These results indicate that collagen mediates the regulatory effects of Asc 2-P and VD3 on the growth and differentiation of human osteoblastic cells. VD3 also increased the levels of mRNA for Cbfa1/Runx2 and Osterix, transcription factors critical for osteoblast differentiation as well as those of differentiation markers such as ALP, type I collagen and osteocalcin. These results suggest that VD3 control the growth and differentiation of human osteoblastic cells by regulating the gene expression of osteoblast-related transcription factors as well as that of type I collagen.

P117-Tu

The Extracellular Matrix 1 Gene is Essential for Early Mouse Development

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Introduction: The human extracellular matrix 1 gene (*ECM1*) encodes a 85-kDa glycoprotein with a cysteine distribution (CC(X7-10)C) comparable to that of the serum albumin proteins. Three *ECM1* splice variants are identified, *ECM1a* (10-exon gene), *ECM1b* (lacks exon 7) and *ECM1c*

(contains an additional exon 5a within intron 5). The mechanisms by which ECM1 proteins exert their biological function(s) are still unknown. Based upon the *Ecm1* expression pattern and the effects of recombinant ECM1 protein on different in vitro model systems Ecm1 is likely to play a role in endochondral bone formation, epidermal differentiation and angiogenesis. The significance of ECM1 in human skin biology has been highlighted further by the identification of loss-of-function mutations in *ECM1* in patients with lipoid proteinosis, a rare human recessive autosomal skin disorder and presence of autoantibodies to ECM1 in lichen sclerosis, a chronic inflammatory disease of the skin. In addition ECM1 interacts with perlecan that plays an important role in skin and bone development (1).

Method: To investigate the in vivo role of Ecm1, we used homologous recombination in mouse embryonic stem cells to produce *Ecm1* null mice by deleting the first two exons of the mouse *Ecm1 gene* (thus deleting the transcription and translation start). Two independent *Ecm1* KO mice lines were generated.

Results: Mice homozygous for the *Ecm1* null mutations (*Ecm1*−/−) are not viable and mutant embryos die around implantation, before the onset of gastrulation. Heterozygous mice (*Ecm1* +/−) are fertile and indistinguishable from wild type littermates. Expression studies (rt-pcr) revealed that embryonic Ecm1 is already expressed from mouse preimplantation development (E4.5) onwards.

Conclusions: The *Ecm1 null* phenotype demonstrates an unexpected and crucial role for Ecm1 during early stages of mouse development. Experiments are being performed to elucidate the role of Ecm1 during pregastrulation development. The early embryonic lethality prevents however to study in this mouse model the later function of Ecm1 in, e.g., skin or bone development.

Reference: (1) Chan (2004) The role of extracellular matrix protein 1 in human skin. *Clin. Exp. Dermatol.* **29 (1)** 52–6.

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P118-Su

Real Time Quantitation of Syndecan 1–4 Expression in a Non-Critical Rat Tibial Defect Model

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Bone healing is a process of structural reconstitution through regeneration in which a number of cellular events occur including cell proliferation, chemotaxis and differentiation. During this process, mesenchymal progenitor cells are recruited to the osteoblast lineage and progressively differentiate into osteoblasts that produce a mineralized extracellular matrix (ECM). Although most of the organic component of ECM is comprised of collagen, growing evidence suggests the most bioactive element of the developing ECM is its heparan sulfate proteoglycan (HSPG) complement. Attached to core proteins are linear, unbranched gylcosaminoglycan sugars that contain protein-binding domains that regulate the flow of an astonishing number of mitogenic influences that coordinate tissue repair. Among the HSPG-binding factors known to be important to this process are FGF1 and FGF2, the FGFRs, BMPs 2, 4 and 7, collagen 1 and fibronectin. Syndecans (Syd-1, Syd-2, Syd-3, Syd-4) are one of the major HSPGs implicated for growth factor binding, cell adhesion and migration and are expressed by developing osteoblasts; however, their expression during bone healing remains unclear. In this study we examined Syd 1–4, osteopontin (OP) and osteocalcin (OC) expression over a 14-day period using a non-critical sized tibial defect model in adult male Wistar rats ($n = 4$ drilled, $n = 5$ control). Defects were created (1.0 mm diameter) through both cortices immediately distal to the tibial crest. Tissue was collected from the defect site 14 day post-surgery (un-drilled tibiae were collected as control) and total RNA extracted. Real-time quantification of Syd 1–4, OP and OC mRNA was then performed and normalized to hypoxanthine guanine phosphoribosyl transferase. Efficiencies of >95% were recorded for all primer sets. Our results show that Syd-1, -2, -3 and -4 are expressed in both drilled and control tibia; albeit at differing levels, with Syd-3 being the most abundantly expressed (>5-fold higher than all other syndecans), irrespective of treatment. However by 14 days post-injury, only Syd-1 (>2-fold) and OP (>1.5-fold) levels had increased significantly compared to controls with Syd-2, -3, -4 and OC levels remaining unchanged, suggestive of early remodeling bone tissue. Together, these results support a possible role for Syd-1 during osteoblastogenesis involving both cell–cell and cell–matrix interactions. However the differential expression and distribution remains to be examined.

P119-Mo

Changes in the Collagenous Matrix of the Bones from Tartrate-Resistant Acid Phosphatase Knockout Mice

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Tartrate-resistant acid phosphatase (TRAP) is an iron containing protein expressed by osteoclasts, macrophages and dendritic cells. The enzyme is secreted by osteoclasts during bone resorption and serum TRAP activity correlates with the level of resorptive activity in certain diseases of bone metabolism. Although its precise role is unknown we have shown that mice lacking TRAP have developmental deformities of the limbs and axial skeleton. Bones from these mice were wider and shorter than normal with thickened cortices and disorganized, enlarged growth plates. Osteo-

clasts from knockout animals demonstrate defective bone resorption in vitro. These mice also have defective osteoblast function leading to increased mineralization contributing to the observed increased mineral density. Type I collagen, the major structural protein of bone provides its high tensile strength. Changes in the collagenous structure in mice lacking TRAP could result in bone abnormalities.

Aims: To investigate the biomechanical and biochemical properties of bones lacking TRAP with particular reference to the collagenous matrix.

Methods: Femurs from 8-week-old TRAP knockout and wild type mice were investigated for their mechanical strength by the 3-point bending technique. Powdered bone samples were analyzed for their collagen cross-link content and matrix metalloproteinase (MMP) activity.

Results: The bones from the TRAP knockout mice required greater ultimate stress (MPa) ($P < 0.05$) to break, contained more ($P < 0.05$) total immature and mature collagen cross links and MMP2 expression (pro and active), as a percentage of the standard, compared to the bones from wild type animals.

Discussion: The observed increased mechanical strength of TRAP knockout mice bones may be related to both the increase in mineral density and intermolecular collagen cross-linking. The latter plus increased MMP2 activities are also consistent with enhanced matrix metabolism. This suggests a previously unconsidered role of TRAP in bone collagen synthesis as well as degradation.

P120-Tu

Conserved Features of Matrix and Bone Gamma-Carboxyglutamic Acid Proteins in Vertebrates-An Evolutionary Point of View

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The evolution of calcified tissues is a defining feature in vertebrate evolution. Investigating evolution of proteins involved in tissue calcification should help elucidate how calcified tissues have evolved. The purpose of this study was to collect and compare sequences of matrix and bone γ-carboxyglutamic acid proteins (MGP and BGP, respectively) to identify common features and determine the evolutionary relationship between MGP and BGP. Thirteen cDNAs and genes were cloned using standard methods or reconstructed through the use of comparative genomics and data mining. These sequences were compared with available annotated sequences – a total of 48 complete or nearly complete sequences (28 BGPs and 20 MGPs) have been identified across 32 different species representing most classes of vertebrates – and evolutionary conserved features in both MGP and BGP were analyzed using bioinformatic tools and Tree-Puzzle software. We propose that (1) MGP and BGP genes originated from two genome duplications that occurred around 500 and 400 Myr ago before jawless and jawed fish evolved, respectively; (2) MGP appeared first concomitantly with emergence of cartilaginous structures and BGP appeared thereafter along with bony structures; and (3) BGP derives from MGP. We also propose a highly specific pattern definition for the Gla domain of BGP and MGP.

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P121-Su

Osteocalcin and Matrix Gla Protein in Developing Teleost Teeth. Identification of Sites of Expression and Protein Accumulation

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In this study the tissue distribution and accumulation of osteocalcin or bone Gla protein (BGP) and matrix Gla protein (MGP) were determined during tooth development in a teleost fish, *Argyrosomus regius*. In this species, the presence of BGP and MGP mRNA in teeth was revealed by in situ hybridization. mRNA for BGP was detected in the odontoblasts as well as in cytoplasmic processes emerging through dentinal tubules, while mRNA for MGP was expressed in the apical portion of cells adjacent to the root of the teeth, in the enamel portion, as well as in cells within the pulpal space. Immunolocalization of BGP and MGP demonstrated that these proteins accumulate mainly in the mineralized dentin or enameloblastic processes, confirming in situ hybridization results. This study demonstrated that BGP and MGP gene expression and protein accumulation in *A. regius* teeth were basically similar to other vertebrates like rats or rodents. However, some species-differences in patterns of gene expression and protein accumulation were detected between fish and higher vertebrates.

P122-Mo

Anabolic Effects of Physiological Strain Regimen in a New Cancellous Bone Explant Tissue Culture-Loading System

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To study coordinated bone cellular responses and the resulting tissue response while avoiding complexity of in vivo situation, we used a new organ culture model (named "Zetos"; Jones et al., ECM 2003). It provides the ability to culture cancellous bone cores over long periods (until now difficult due to rapid degeneration inside the organ) and to apply specific compressive strains to bone cylinders. A regimen that mimics a jump pattern (4000 μS, 1 Hz, 300 cycles/day) is applied. Cylindrical bovine biopsies (10 mm diameter, 5 mm height) from sternum were precisely machined, fitted in chambers and fed with culture medium in conditions ensuring uniform double fluorochrome labelings within the whole sample. Biomechanical (through Zetos itself), tomographic (μCT40, Scanco Medical) and cellular evaluations were performed on basal control (BC) samples immediately processed, loaded (L) and non-loaded (NL) 3-week cultivated samples. Bone alkaline phosphatase activity (ALP) in culture medium increased by 30% and 35% in L vs. NL after 5 and 8 days, respectively, and no longer differed afterward. Sandwich ELISA for Runx2, osteocalcin and ALP were performed in bone samples. In L, Runx2 and osteocalcin showed increased levels after 7 and 14 days (Runx2: 322 and 285%; osteocalcin: 90 and 27%, respectively), which normalized thereafter. ALP activity increased (47%) only after 7 days in L. Meanwhile we showed that marrow stromal cells from bovine sternum plated on type I collagen-coated-silicon membranes and submitted to cyclic strain (6000 μS, 1 Hz, 10 min/day, FLEXCELL FX-3000 unit) over 14 days showed an increased proliferation the first 3 days of stretch, increased levels of ALP and osteocalcin (147 and 76%, respectively) at 14 days and enhanced Runx2 level (67%) at 3 days. In conclusion, mechanosensitive bovine osteoblast lineage cells retain their sensitivity in the Zetos organ culture loading system. Such cellular adaptation results in tissue response evidenced by thicker trabeculae arranged in a more plate-like pattern.

Table

	BC	NL	L
Young modulus (PA)	159.53 ± 57.9	157.72 + 40.9	184.1 + 66.80
Plate/Rod Index	1.25 ± 0.2	1.37 + 0.22	0.87 + 0.20[a]
Tb.Th (μm)	84 ± 16	84 ± 10	104 ± 16[a]
OS/BS (%)	7.05 ± 1.64	9.72 ± 3.67	14.37 ± 2.98
O.Th (μm)	11.43 ± 1.93	10.38 ± 2.85	13.6 ± 3.70[a]
BFR ($\mu m^2/\mu m^3$/day)	/	0.79 ± 0.30	1.16 ± 0.42[b]

[a] Significant vs. NL + BC.
[b] Significant vs. NL.

P123-Tu

Osteon Swelling Induced by Loading

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Cosserat and micromorphic theories predict independent processes such as dilation and microrotation of microstructure elements following loading (1). Turning exercise causes different strain magnitudes between horse forelimbs (2) and ultrasound velocity showed a transient reduction post-loading at a specific site on the first phalanx of one limb (3) which may be related to an increase in osteon diameter.

Methods: Four adult horses were exercised for 5 min twice daily in a 16 m diameter round yard. When daily ultrasound velocity measures of the dorsomedial first phalanx showed a reduction on one forelimb the horses were euthanized. Bone samples of this site from each horse were embedded in LR-White and 5 μm sections stained with van Kossa. The diameter of 30 osteons in each sample were measured and the mean difference between left and right limbs calculated for each horse.

Results: The correlation coefficient between the difference in osteon diameter and the difference between the limbs in the ultrasound velocity change from pre- to post-exercise was −0.97 ($P = 0.03$). Ultrasound velocity showed a mean reduction of 104 m/s (SE = 67), in one bone compared to an increase of 25 m/s (SE = 91), in the contralateral pair. The mean difference between limbs in osteon diameter was 8 μm (SE = 8.8), with a mean size of 235 μm in the bone that showed the ultrasound velocity change compared to 224 μm in their pair.

Discussion: The transient ultrasound velocity reduction appears to be related to an increase in osteon diameter. It may be associated with an increase in osteon interlamellar space and thus osteon swelling. The size of the change is similar to the 10 μm cement line displacement shown in bovine bone (4). These findings suggest that site-specific elasticity changes due to microstructure alteration can be determined by ultrasound velocity as predicted in generalized continuum theories.

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P124-Su

Trabecular Bone of Proximal Human Femur: Dependence of Mechanical Compressive Strength on Local Variations in Bone Morphometry

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The mechanical properties of trabecular bone depend both on bone mass and bone architecture. The aim of this study was to correlate the compressive mechanical properties of trabecular bone determined experimentally to histomorphometric parameters obtained by microcomputed tomography (muCT) and to assess if failure can be correlated to detectable local variations in the latter parameters. Twenty human femoral head bone samples were used in this study. From each femoral head, one trabecular bone specimen of cylindrical shape, height 26 mm, diameter 10 mm, was extracted, aligning the milling tool to the main direction of the trabeculae. The samples were examined by muCT and the histomorphometric parameters as bone volume fraction (BV/TV), trabecular separation (Tb.Sp) and trabecular thickness (Tb.Th) were calculated. Then they were submitted to uniaxial compression testing. Endcaps were cast to both bases of each specimen to reduce end artefacts during the mechanical test. An extensometer was fixed to the central part of each specimen to permit the calculation of elastic modulus (E) and ultimate stress (S_u). After mechanical testing the samples were rescanned by muCT and then submitted to ashing procedure. A linear dependence of ash density on BV/TV ($r^2 = 0.90$) was found, supporting the use of BV/TV as a predictor for the ash density. Regression analysis showed linear dependence of E on BV/TV ($r^2 = 0.64$) and of S_u on BV/TV ($r^2 = 0.80$). Linear relationships were also found between S_u and Tb.Sp ($r^2 = 0.70$) and between S_u and Tb.Th ($r^2 = 0.49$). Comparing muCT images collected before and after the mechanical test, it was found that failure was almost located at a specific region of the bone sample (up to one-half of the total height), which also had the lowest BV/TV of the specimen. Linear regression analysis between S_u and the lowest BV/TV showed an improvement in the coefficient of determination ($r^2 = 0.87$), as for S_u vs. Tb.Th calculated in the same region ($r^2 = 0.54$), while the coefficient of determination for S_u vs. Tb.Sp remained nearly unaffected ($r^2 = 0.69$). These preliminary results confirm that the compressive properties of trabecular bone of the human femoral head, when loaded along the main axis of the structure, are significantly related to the histomorphometric parameters BV/TV, Tb.Th and Tb.Sp. In particular, the variations in S_u showed a better prediction when local minima of BV/TV were considered, rather than the BV/TV representing the bone sample as a whole.

P125-Mo

Integrin Signaling is a Major Pathway for Intracellular Response to Low-Intensity Pulsed Ultrasound

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Introduction: Strong evidence exists for ultrasound stimulation of fracture repair. Ultrasound is a pressure wave that passes through soft tissues and bone, and generates a mechanical force on cells. Despite known clinical effects, the pathway that converts an ultrasound-generated force into a biological response remains unknown. We tested the hypothesis that ultrasound-mediated pressure waves activate integrin-mediated signaling in chondrocytes.

Materials and methods: Chondrocytes were isolated from late-term rat pups from cartilaginous distal femur and proximal tibia. On day 3 of culture, cells were exposed to ultrasound for 15 min/day, generated with a commercially available transducer (Smith and Nephew, Inc.). For evaluation of 35SO4 incorporation or RNA synthesis cells were harvested after 7 ultrasound exposures. For evaluating protein phosphorylation cells were harvested at multiple times up to 2 h after ultrasound exposure. 35SO4 incorporation was normalized to DNA content. RNA was extracted using standard techniques and gene expression was evaluated by RT-PCR. Protein phosphorylation was evaluated by immunoprecipitation followed by western blotting with an anti-phosphotyrosine antibody. Phosphorylation was normalized to protein concentrations.

Results: Exposure of chondrocytes to ultrasound caused a significant increase in aggrecan mRNA levels and increased 35SO4 incorporation. Ultrasound exposure also increased phosphorylation of multiple intracellular proteins, including FAK, IP3, Src, Rho and MAPK. To confirm integrin activation, these experiments were repeated in the presence of reagents that blocked the interaction between integrin and the intracellular cytoskeleton or extracellular matrix proteins. Pretreatment of chondrocytes with cytochalasin-D, with RGDS, or with anti-β1 integrin antibodies, eliminated FAK phosphorylation and decreased aggrecan synthesis. Additionally, pretreatment with antibodies against type II procollagen, eliminated FAK phosphorylation.

Discussion: Our results suggest that the ultrasound-generated pressure wave activates integrin-mediated intracellular signaling. Concordance between the pathways activated by ultrasound and those activated by mechanical forces in other cell types supports the hypothesis that the ultrasound induced pressure wave initiates mechanically sensitive cellular pathways in the chondrocytes that are highly similar to the mechanically sensitive cellular pathways present in other cells.

P126-Tu

Generation of Micromotion in Soft Tissue Adjacent to Fractured Bone by Pulsed Ultrasound-Generated Pressure Waves

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Introduction: Clinical studies support that pulsed ultrasound accelerates repair of bone fracture. Several cellular mechanisms have been identified as contributing to this effect of ultrasound. How a responding cell detects ultra-

sound pressure wave remains unknown. In this study we mathematically modeled pressure wave intensity and radiation force as the wave passed through tissues, then compared this model to direct measurements of the pressure wave and tissue micromotion in cadaver specimens.

Materials and methods: Mathematical modeling was performed using published data on the density and speed of sound in various tissues. Fractures were generated by transverse saw cuts of the distal radius and midshaft tibia. Ultrasound transducers produced about 30 mW/cm^2 intensity with 200 μs pulses of 1.5 MHz every millisecond. Pressure wave measurements were made with a membrane hydrophone. Micromotion of soft tissues and bone was measured directly by laser interferometry.

Results: We generated dynamic images of the mathematical model as AVI files and calculated pressure wave at various points around the radius and the tibia. The model predicts reflection of the pressure wave, but the wave passes through the bone cortex and there is no penumbra behind the radius or tibia. This model predicts that the pulsed pressure wave amplitude decreases 75% and is delayed 20 μs across the tibia or radius; direct measurement shows a 50% decrease in signal intensity and a 20-μs delay. Micromotion velocity was 50 μm/s in tissues adjacent to the bone or fracture site, 30 μm/s in bone fragments at the fracture site and 1–5 μm/s in the tibia and radius. We calculated that ultrasound pressure from this signal would generate 40–500 microstrain on periosteal cells at the fracture site.

Discussion: Our results from the mathematical model and direct measurements of tissue pressures and micromotion in cadaver specimens confirm that pulsed ultrasound generate a dynamic radiation pressure on bone and soft tissue that results in tissue motion with a velocity in the micrometer per second range (1–50 μm/s). The amplitude of the pressure wave is decreased approximately 50% as it passes through the bone, but this did not eliminate motion of the soft tissues. The microstrain in periosteal and callus cells predicted by this model is at the level associated with mechanical stimulation of other cells.

P127-Su

Expression of a Novel Alternatively Spliced UCP2 mRNA in Osteosarcoma

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Introduction: Most tumor cells are genetically programmed to withstand the onslaught of anti-cancer reagents. Although many high-grade osteogenic sarcomas (OGS) are resistant to the current regimen of chemotherapy, current knowledge provides limited understanding of the mechanisms underlying chemoresistance in OGS. Many anti OGS drugs act through generation of reactive oxygen species, and it has been shown that uncoupling protein 2 (UCP2) may protect tumor cells from the cytotoxic action of such anti-tumor drugs. We investigated if OGS specimens posses a high level of UCP2.

Materials and methods: Total RNA was extracted with Trizol by following the protocol supplied by the manufacturer. UCP2 mRNA expression level was assessed by microarray analysis or by reverse transcription of total RNA followed by polymerase chain reaction (RT-PCR). The nucleotide sequence of amplified products was confirmed by direct automated sequencing. Quantification of mRNA expression was performed by RT-PCR using radioactivity. Quantification experiments were carried out in duplicate and repeated three times. Institutional IRB approval was obtained for genetic analysis of patients' tumor samples.

Results: RNA was extracted from 19 tumor specimens, 8 OGS-derived cell lines and 6 normal bones. Microarray analysis showed that the level of UCP2 mRNA ~50% less in normal bones compared to OGS specimens. Sequence analysis of PCR amplification products showed a previously unreported alternatively spliced UCP2 mRNA (as-UCP2). We did not detect expression of the as-UCP2 mRNA in normal bone, whereas the transcript was expressed at high levels in six of nine OGS cell lines and in four of seven tumor specimens. Expression of the as-UCP2 transcript was greater in high grade than in low-grade tumors.

Discussion: The results suggest that the expression levels of wild-type UCP2 mRNA do not differ between normal bone and OGS. Interestingly, expression of alternatively spliced UCP2 appears to be limited to tumor cells. Expression of the as-UCP2 mRNA in OGS-derived specimens offers a novel mechanism for drug resistance in which as-UCP2 mitigates the cytotoxic effect of anti-tumor agents. It is evident that this potential mechanism for drug resistance involving the as-UCP2 needs to be validated at the protein level and in animal studies.

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P128-Mo

Peak Strains in Equine Locomotion

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Racehorses allow the study of bone loaded maximally. Radiographic measures of the relative thickness of the midshaft dorsal cortex (RI = ratio of cortex to medulla × ratio of dorsal to palmar cortex) of their third metacarpal bone (MC3) can be reliable and repeatable (1). The RI is a ratio so removes the effect of absolute differences in bone size and magnification effects from the radiography as long as the radiography machine and plate are aligned correctly. Peak microstrain (q) at this site at 12 m/s (y) is proportional to the RI [$y = -5985 + 944$RI] (2). RI was measured (1) weekly for 12 weeks in 35 racehorses (2–6 years old) training at racing speed (16–19 m/s). All horses had previously adapted to fast exercise and had no significant

change in RI during the study. The mean RI was found to be 3.7 (SE = 0.1), range was 2.4 to 5.9. This equated to a peak strain at 12 m/s of $-2488q$, with a range from -415 to $-3719q$. The linear relationship between peak strain at this site and exercise speed (s) [strain = $-55-244$ s] shown previously (3) suggests that strains in the order of $3960q$ may be present in mature experienced racehorses at 16 m/s. Strains as large as $-7862q$ have been measured on a yearling MC3 at a treadmill speed of 16.5 m/s (4) and $-4841 \pm 572q$ in four 2-year-olds exercising at 16 m/s on a dirt track (5). The consistency of the RI through months and years of racing in horses being monitored for soundness (6) suggests that the strains associated with very fast exercise may not be sufficient stimulus to induce further bone modelling in these horses. The size of strains required to induce bone modelling may be much greater than the -2500 to $-3000q$ suggested by Frost's mechanostat under some conditions at least.

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P129-Tu

Fibroblast Growth Factor Receptor-3 is Up-Regulated by the Inhibition of ERK1/2 Phosphorylation in Mechanically Stimulated Osteoblasts

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The maintenance of bone homeostasis depends on the ability of cells to detect and transduce extracellular signals that direct changes in tissue architecture. Mechanical stimulation in the form of fluid shear is crucial to the maintenance of bone homeostasis and, as yet, the precise mechanism by which osteoblasts convert mechanical signals into cellular responses is still unknown. This study sought to investigate the role of fluid shear on the time-dependent differentiation of pre-osteoblast MC3T3-E1 cells over a 30-day time period. Since fibroblast growth factor receptors (FGFRs) are known to be vital to the successful development of the skeleton during embryogenesis (1, 2), we also investigated the expression of FGFR 1–4 and the two prototypic ligands, FGF-1 and-2, and the activation of MAPK at regular intervals across the 30 days. When exposed to recurrent fluid shear, osteoblasts demonstrated increased cell proliferation, differentiation and maturation compared to unstimulated controls over the 30-day time period, supporting previous observations that fluid shear increases osteogenesis (3). Concomitant with this increase in osteoblast differentiation, we observed a time-dependent increase in the expression of FGFR-3 in fluid shear-stimulated cells, while observing a down-regulation in FGFR-1, FGFR-4 and FGF-1 expression. In addition, recurrent fluid shear inhibited the phosphorylation of ERK1/2, especially at the later time points, without affecting p38 MAPK activation. We observed similar findings by inhibiting ERK1/2 phosphorylation over 25 days with the specific MEK1/2 inhibitor U0126, confirming that ERK1/2 inhibition led to the increases in FGFR-3 expression and osteoblast differentiation observed. Taken together, we conclude that recurrent fluid shear over 30 days causes the inhibition of ERK1/2 phosphorylation that in turn acts to increase the expression of FGFR-3 and several osteogenic genes involved in osteoblast differentiation.

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P130-Su

Altered Cellular Kinetics and the Expression of Heat Shock Protein in the Growth Plate of Unloaded Rats

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Bone mass is the results of the balance between bone formation and resorption. Among the multiple factors that regulate bone cell activities, mechanical environment plays a critical role. There will be a reduction of bone mass and even irreversible changes in the skeleton when human is exposed to the situation where normal weight bearing is prohibited due to medico-surgical diseases. Previous studies regarding the effects of non-weight bearing on the cortical or cancellous bone have shown the impairment of osteoblast activity at the cell level and/or lack of osteoblast recruitment at the tissue level. However, growth and remodeling of bone are the results of endochondral ossification as well as periosteal bone formation. This study was conducted to reveal any histopathological changes occurring in the growth plate when the rats were subjected to be deprived of normal weight bearing using the model of hindlimb unloading.

Thirty male Sprague–Dawley rats, aged 6 weeks, were acclimatized with standard conditions. They were divided into two groups according to periods of Hind limb Unloading 3 weeks (n = 5) and of Reloading 1 week (n = 5), and each control groups was maintained for an identical period in the same housing conditions. The animals were double labeled with 5-Bromo-2'-deoxydiuridin (BrdU) and BrdU immunohistochemistry was performed for the cellular kinetic analysis. Transferase-mediated deoxyuridine triphosphate–biotin nick end labeling (TUNEL) assay was done for the investigation of apoptotic changes in the growth plate, and the positive cells were counted in each zones of the growth plate in both TUNEL and BrdU immunohistochemistry. Heat shock protein (HSP) 47, 70 were immunolocalized to assess the chondrocytic activities in terms of production of stress protein. The length of each zone of the growth plate – resting, proliferation and hypertrophic zone – was measured by histomorphometric analysis, for the investigation of any changes occurring in the various zones of weight bearing cartilage.
Non-weight-bearing induced a reduction of height of growth plate, reduced cellular proliferation of chondrocytes and altered expression of stress protein. When reloading 1 week applied the group showed the cellular activities in terms of cellular proliferation and the production of heat shock protein. These results suggest that application of reload for the restoration of chondrocytic activities when the normal weight bearing is deprived of.

P131-Mo

Femoral Bone Tissue Strains During a Fall to the Side

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Bone mineral density (BMD) is a commonly used predictor of proximal femur strength. However, local stresses and strains in the cortical and trabecular bone-tissue structures in fact determine the onset of failure. The objective of this study was to document these local values during a fall to the side, in a normal and an osteoporotic femur, using micro-finite element analysis (μFEA) [van Rietbergen et al., J Bone Min Res 18:1781–1788, 2003]. Specific purposes were to determine the safety factors against fracture for both cases and to establish locations of failure initiation.
Based on close matched age, body weight and length, a healthy (T score: −0.5, neck BMD 0.917 g/cm^2) and an osteoporotic (T score: −4.0, neck BMD 0.496 g/cm^2) human proximal femur were selected for the creation of two large-scale, 3-dimensional μFEA models. Falls to the side onto the greater trochanter were simulated for both femurs, applied with forces on the femoral head and reaction forces at the trochanters. Maximal principal strains were calculated in each of the 96-71 million elements.
The tissue stresses and strains were higher in the osteoporotic femur than in the healthy one (Fig. 1). Safety factors against permanent deformation of 6.4 and 4.5 were found for the healthy and osteoporotic femurs, respectively. In both cases tensile yield was reached before compressive yield.
The proximal femur fails during a fall due to excessive tensile strains in the medial cortex. Hence, although osteoporosis is a trabecular condition predominantly, its effects on fracture risk is expressed indirectly, by increasing cortical strains.

P132-Tu

Simulation of Trabecular Bone Remodeling and its Regulation by Mechanical and Biological Factors

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Living trabecular bone is continuously remodeled by the action of bone removing osteoclasts and bone depositing osteoblasts. The famous Wolff–Roux law states that the cell action is coupled to a local mechanical stimulus resulting in a higher probability for bone deposition (resorption) at sites under high (low) mechanical load, respectively. For the nature of this mechanical stimulus, stress, strain, strain rate, elastic energy, microdamage, etc., have been proposed. The probability for local bone resorption/deposition (i.e., what we call the remodel law) is further crucially influenced by biological factors like hormone levels, drugs and nutritional supplements. A mathematical formulation of the remodel laws, however, is still out of reach. Using computer simulations we have studied the influence of different remodel laws on the time evolution of trabecular bone microstructure inside a human vertebra in different scenarios. Employing an approximate, but fast algorithm to assess the local mechanical load in the network-like structure of trabecular bone [1] instead of Finite Element methods, we could focus our attention on both the mechanical and biological factors influencing bone remodeling. Without assuming a direct coupling between osteoclasts and osteoblasts, a balance between bone resorption and deposition was reached resulting in a steady state bone volume fraction in all simulations [1]. While the bone volume fraction remained constant, the

microstructure coarsened. Since trabeculae along the vertical main loading direction thickened faster, the anisotropy between vertical and horizontal trabeculae became more pronounced. Depending on the implemented remodel law, we observed differences in measurable quantities like trabecular thickness distributions. Also, the behavior to disturbances (e.g., an increased osteoclast activity) is qualitatively different for different remodel laws.

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P133-Su

Mineralization Vector Quantifies Differences in Fracture Callus Due to Loading

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During fracture healing the mineralization of newly formed bone varies temporally and spatially. We hypothesized that an analytical description of this mineralization could be developed to characterize successful healing and distinguish between loading treatments. We defined a mineralization vector (*MV*) to provide a 3D quantitative description of the mineralization. Nineteen 8- to 10-month-old male rats were divided into two groups: femoral fracture alone (Fx, $n = 10$) or femoral fracture with patellar tenotomy (FxTen, $n = 9$). Femurs were harvested at 2, 4, 6 and 8 weeks and imaged with synchrotron microtomography (12-μm voxels) at the ALS in Berkeley, CA. Each callus was divided into four axial quadrants: Q1, Q2, Q3 and Q4 (Figure). MV was then calculated for each quadrant using this generalized equation:

$$\mathrm{MV} = [\text{weighted sum } (\beta \times \text{location})_i\ x,\ y,\ z]_{i>\phi} - [\text{weighted sum } (\beta \times \text{location})_i\ x,\ y,\ z]_{i<\phi}$$

where MV = mineralization vector, β = attenuation value of voxel, x, y, z, = unit vectors, i = individual voxel and ϕ = mean attenuation of bony tissue. The difference in magnitude between in Q4 and the other quadrants was significantly larger in the FxTen group than the Fx group ($P < 0.05$, single factor ANOVA). The vector magnitude approximates the spatial relationship between the cortical bone centroid and the callus centroid. On the posterior femur, Q4 is an area of intact musculature for the FxTen group as opposed to Q1 and Q2, which are adjacent to the functionally silent quadriceps group. These data show that MV analysis is a promising approach for quantitative assessment of healing. Further studies will include kinetic/kinematic gait analysis to correlate MV analysis with in vivo loading.

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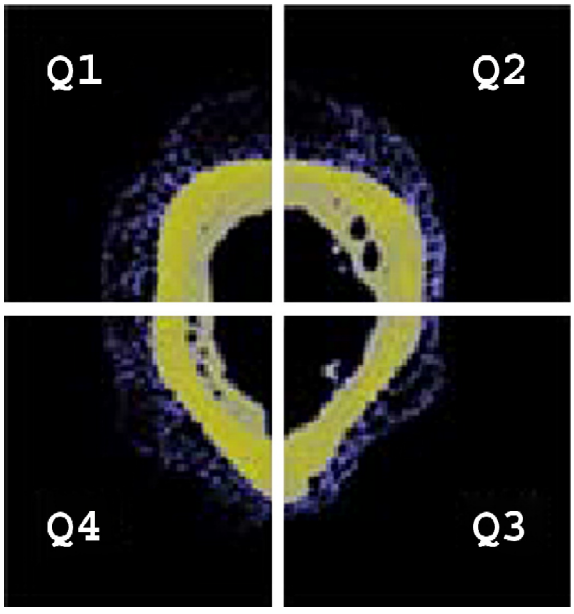
Figure: Cross section of rat femur just proximal to fracture site 2 weeks post-fracture. Quadrants for MV analysis indicated.



P134-Mo

Cathepsin K Deficient Osteoclasts of Long Bone Use MMPs to Resorb Bone

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The cysteine proteinase cathepsin K (cathK) is considered an essential enzyme in the resorption of bone matrix by osteoclasts. The enzyme is highly expressed in osteoclasts and selective interference with its activity strongly inhibits bone resorption. Yet, pycnodysostosis patients and mice deficient for the enzyme are characterized by a relatively mild osteopetrotic phenotype, suggesting that other proteolytic enzymes compensate for the lack of cathK. Proteolytic enzymes that have the capacity to digest bone matrix are other members of the group of cysteine proteinases (CPs) and those belonging to the matrix metalloproteinases (MMPs). In the present study we investigated participation of these two classes of enzymes in osteoclastic bone resorption by culturing bones from wild type and

cathK−/− mice in the presence of selective inhibitors of either class of enzymes. Metatarsals were isolated from 5-day-old mice and cultured in M199 and 2.5% FCS for 5 h with or without the CP-inhibitor E-64 and/or the MMP-inhibitor CT1166. Following culturing the explants were processed for microscopic analysis. The volume density of non-digested demineralized bone matrix (DBM) adjacent to the ruffled border of the osteoclasts was analyzed (see Everts et al., J. Cell. Physiol., **150**:221, 1992).

The data demonstrated relatively large areas of non-digested bone matrix adjacent to osteoclasts in cathK−/− bones but not in control bones. The CP-inhibitor had no additional effect on the amount of DBM in the K−/− bones whereas in control bones this parameter was strongly increased. In control bones the MMP-inhibitor had no effect, thus supporting the view that MMPs are not essential for resorption of bone by long bone osteoclasts (Everts et al., FASEB J., **13**:1219, 1999). Blocking the activity of MMPs in cathK−/− bones, however, strongly affected bone matrix resorption. In the presence of the inhibitor a more than two-fold increase in the amount of DBM was found compared to cathK−/− bones cultured in control medium. The presence of both inhibitors resulted in an effect similar to that seen with the MMP-inhibitor alone.

Our data demonstrate that osteoclasts lacking active cathK may compensate for this by employing MMPs. This may explain the relatively mild osteopetrotic effects in the absence of cathK as seen in patients suffering from pycnodysostosis.

P135-Tu

Serum Cathepsin K Measurements: Repeatability, Intra-Subject and Postprandial Variability

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Cathepsin K, a cysteine protease, plays a major role in bone matrix degradation. Recently, an enzyme-linked immunosorbent assay for cathepsin K measurement in serum and cell culture (Cathepsin K ELISA BI-20432, Biomedica, Austria) was developed using a polyclonal sheep antibody. In this study we assessed the repeatability and intra-subject and postprandial variability of cathepsin K measurements in human sera.

Serum samples from nine healthy postmenopausal women aged 65.9 ± 6.5 years (mean ± SD) were collected (a) in the fasting state on two occasions separated by 1–4 weeks, and (b) 120, 240 and 360 min after a standard breakfast (1385 kcal, 37 g protein, 88 g fat, 104 g carbohydrate). Samples were kept frozen at −20°C up to 6 months and at −80°C up to 12 months before being analyzed in batch duplicates by a single investigator.

Measurement of sera obtained on two separate occasions yielded mean (±SD) cathepsin K values of 3.20 ± 1.41 pmol/L and 2.65 ± 1.29 pmol/L, respectively, with no statistical difference between the two sets of data (P = 0.39). Mixed-effect analysis of variance suggested that the intra-subject variance of cathepsin K was 1.81 ± 0.90 pmol/L2 of which biological and inter-assay variance accounted for 68% (1.28 ± 0.87 pmol/L2) and analytical variance accounted for 32% (0.58 ± 0.28 pmol/L2). The postprandial cathepsin K levels at baseline, 120, 240 and 360 min were 2.65 ± 1.29, 2.79 ± 1.07, 2.37 ± 1.00 and 2.75 ± 0.96 pmol/L, respectively; no statistically significant difference was observed between these results (P = 0.79). Cathepsin K levels did not appear to be affected by hemolysis of the blood sample (2.73 ± 1.15 vs. 2.28 ± 1.24 pmol/L, respectively, P = 0.12, n = 5).

We conclude that serum cathepsin K levels as measured by the Biomedica assay show good intra-subject repeatability and low postprandial variability.

P136-Su

Proteases Control Chondrocyte Late Differentiation and Apoptosis During Endochondral Ossification and Degenerative Joint Diseases

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Late chondrocyte differentiation is important for endochondral ossification, but also may occur illegitimately in degenerative joint diseases. The process is subject to environmental negative control at several checkpoints by soluble mediators derived from the cartilage or surrounding tissues. Blood vessel formation is an essential event during endochondral ossification. Thus, we hypothesize that cells from the blood stream or the vessel walls are likely to produce relevant factors that act in concert with autocrine cartilage factors or systemic hormones. The chick embryonic sternum contains cells with different propensities to differentiate and thus represents an excellent model to investigate the molecular control of chondrocyte maturation. In serum-free suspension culture, cells of the cranial part mature after stimulation with thyroid hormones, insulin or IGF-I and secrete markers for hypertrophic development whereas caudal cells do not. In coculture with endothelial cells, however, caudal cells also produce collagen × and alkaline phosphatase. This effect is mediated by an activation cascade of proteinases exclusively produced by the endothelial cells (Babarina et al. 2001). The hypertrophic development of insulin or IGF-I stimulated cranial cells is abrogated by several types of protease inhibitors in a dose dependent manner (serine-, cysteine-, aspartate or metalloproteinases). This indicates that hypertrophic differentiation of cranial chondrocytes is dependent not only on anabolic effects

elicited by insulin or IGF-I but additionally requires proteolytic enzymes activating or inactivating extracellular signaling mediators. However, thyroxin-induced hypertrophy did not require proteolytic action. Monocytes or macrophages produce but do not activate the pro-form of gelatinase B (MMP-9). By contrast, chondrocytes have the capacity to activate macrophage derived MMP-9 via MMP-3 or a cascade of MMP-14 and-13 under certain conditions, e.g., osteoarthritis (Dreier et al. 2004). Incubation of chondrocytes with MMP-9 containing conditioned medium of a chick monocyte cell line led to apoptotic cell death. This indicates that proteases may additionally induce chondrocyte apoptosis, necessary for the replacement of cartilage by bone. Our investigations strongly point towards the importance of proteases not only in cartilage breakdown but also in the control of chondrocyte late differentiation and apoptosis during endochondral ossification or joint diseases.

P137-Mo

Collagen Fibril Diameter at Different Cortical Bone Sites

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Bone is a specialized form of connective tissue made up of hydroxyapatite crystals within a matrix of collagen. The protein in the bone matrix is mostly type I collagen. Connective tissue greatly contributes to the strength of the bone. There is evidence that bone loss and bone collagen abnormalities are seen frequently in association with osteoporosis.

The mechanical role of collagen in bone is important. This importance is becoming increasingly more clear as evidence mounts on the detrimental effects of altered collagen on the mechanical properties of bone. Collagen fibril diameter is one of the parameters that influence these properties. It has been shown that Ca/P ratio values, which provide a sensitive measure of bone mineral content, between different bone sites are highly significant ($P < 10^{-3}$) demonstrating a dependence upon lifestyle and bone use. Hence, the collagen fibril diameter at different bone sites was measured.

For this study, 4 male Wistar rats 17 months old were used and cortical right femoral as well as front and rear tibia samples were analyzed. Samples were prepared for electron microscopy and were examined on a JEOL JEM-100 CX-II. Measurements of the diameter of collagen fibrils were performed on areas of cross-sectional collagen by the use of an algorithm developed in the laboratory. Mean diameter values of collagen fibrils (M ± SD) for cortical femoral, front and rear tibias are: 51.4 ± 11.8 nm, 47.1 ± 11.6 nm and 49.5 ± 13.0 nm, respectively. Differences between different bone sites are highly significant ($0.05 < P < 10^{-3}$). Different bone use for each bone site exerts differing pressure conditions to each site and as a consequence at least their strength has to be, respectively adapted. Collagen fibrillar parameters could easily be one of these adaptations. These results are in agreement with the Ca/P ratio values obtained for the same bone sites.

P138-Tu

Collagen Bone and Skin Structure in Health and Disease. Image Processing and Quantitative Ultrastructural Studies

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The non-mineral portion of bone is mainly collagen and most of the body's collagen is found in the bone and skin. The predominant collagen of bone is type I. Dermal skin is also largely composed of type I collagen, although type III is also present. Connective tissue greatly contributes to the strength of the bone. Bone without collagen is brittle. It has been demonstrated that structural abnormalities in type I collagen may explain some cases of severe osteoporosis. We reasoned that factors that affect type I collagen might affect both the skeleton and the skin. Thus, we studied the structural changes in the bone and skin in response to inflammation-mediated osteoporosis and ovariectomy.

Collagen structural changes (fibril architecture and diameter) were detected, at the ultrastructural level, in bone (trabecular and cortical) and skin specimens from inflamed rabbits and ovariectomized rats. In treated animals the arrangement of fibrils was anarchic. The overall collagen fibril architecture was disturbed compared to normal. Treated collagen fibrils' mean diameter values were significantly altered than those from controls, in all tissues examined. The banding patterns of fibrils were normal in all cases; however, measurements by a computerized method of measuring axial periodicity of fibrils indicated significantly lower values for treated than untreated samples pointing to an alteration of tissue mechanical properties upon osteoporosis. Results show a correlation between the effects induced either by inflammation or ovariectomy in bone and skin collagen. The question of whether these changes play a role in the pathogenesis of osteoporosis remains to be demonstrated.

P139-Su

Bone Marrow Percutaneous Autograft in Segmentary Defect Produced in Rabbit Radii

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In order to study integral and concentrated osseous bone marrow grafted percutaneously in reparation of experi-

mentally produced osseous defects, it was used 36 White New Zealand male rabbits, aging between 5 and 6 months and weighting 3.5 kg. 20 animals were submitted to bone marrow collection from the iliac crest and the samples were processed and submitted to nucleated cell count. In the 16 remaining animals that formed groups I and II, of 8 rabbits each, it was produced bilateral osseous defects in radii, removing 1.0 cm of osteoperiosteal segment in all its diameter, 3.0 cm proximal of the radial–carpal articulation. In group I each animal received 1.0 ml of integral bone marrow in one radius (treatment) immediately after iliac crest aspiration. In group II, after 2.0 ml bone marrow aspiration and centrifugation, it was injected 1.0 ml of the sediment. In all groups it was injected 1.0 ml of physiological saline solution in the contralateral radius (control). Grafting was performed 5 days after producing the osseous defect and bone marrow was percutaneously injected through a 21-G needle, which was inserted at the site after direct palpation until finding ulnar resistance. It was also performed total alkaline phosphatase enzyme serum dosage every 72 h after grafting but it was not efficient to demonstrate osseous activity. Cellular count wide variability was observed among the aspirated. Radiographic evaluation was performed every 7 days for 5 weeks; it showed precocious radiopacity at the site of percutaneous bone marrow graft with circumscribed and well defined areas in comparison to the control. In the radii that received saline solution, osseous formation initiated from the extremities towards the middle of the defect. The concentration of bone marrow cells by centrifugation does not interfere negatively in the osteogenic potential of the sample. This study confirms that the osteogenic efficiency of the bone marrow graft occurs mainly after the first and second weeks of grafting, showed by the difference of radiographic images and tendency to uniformity after this period. This fact supports the hypothesis that the grafted radii were in a different phase of reparation in relation to the controls although at 5 weeks after grafting there were more histological similarities than differences.

P140-Mo

Type of Bone Formation Induced by BMP in Distraction Osteogenesis: Intramembranous, Endochondral or Both?

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Distraction osteogenesis (DO) is a surgical procedure with numerous clinical applications in bone lengthening and replacement of bony loss. Following an osteotomy, the bone ends are subjected to controlled distraction using an external fixator and new bone forms in the distracted gap. It is believed that bone formation in DO is mostly intramembranous. However, we have previously shown that exogenously applied BMP7 (which is both osteogenic and chondrogenic) may accelerate bone formation in DO. Therefore, to investigate whether BMP induced bone formation in DO is intramembranous or endochondral, we studied the effects of BMP7 injection during DO on the temporal and spatial expression of the chondrogenic targets; Hedgehog ligands (Indian, desert and sonic) and receptors (smoothened and patched), PTHrP, Cox2, FGF ligands 1, 2 and 18, FGF receptors 1, 2 and 3, BMP 2, 4 and 7 and receptors BMPRIA, BMPRIB, BMPRII as well as the transcription factors Runx2, Osterix and Sox 9. The study was performed by lengthening the right tibia of 24 rabbits using a uniplanar fixator. One week after distraction, 75 micrograms recombinant BMP7 was administered to one group of rabbits while the other received placebo. Rabbits were sacrificed 10 min, 1 day, 2 days and 2 weeks following the injections. The expression of the proteins was studied in the distracted tissue by immunohistochemistry. Results showed that all proteins were normally expressed during DO, however, their expression was upregulated at various degrees by exogenous BMP7 for all time periods analyzed. The proteins were also co-localized to the same cells that expressed BMP7 and its receptors. Chondrocyte and fibroblast-like cells were mostly stained. The most notable upregulation was in the Hedgehog ligands and receptors—almost 400% increase in comparison to the control. These results suggest that, under BMP7 influence, bone formation during DO could be modulated to adopt a chondrogenic pathway (possibly besides an intramembranous one). This may help address one of the main problems pertaining to the technique of DO, which is the long duration the external fixator needs to be kept on until the newly formed bone consolidates. Results from this study may lead to the development of novel therapeutic strategies and alternatives to accelerate bone formation during DO by using exogenously applied substances that stimulate endochondral bone formation, therefore allowing the fixator to be removed earlier.

P141-Tu

Demineralized Homologous Bone Matrix Associated with Autogenous Bone Marrow in the Repair of Radial Segmental Bone Defects in Rabbits

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The demineralized bone matrix (DBM) implantation enhances the new bone formation that will fill a bone defect. In early periods, its association with bone marrow increases the bone formation significantly, demonstrating the spurring effect on the marrow osteoprogenitor cells. The purpose of this study was to evaluate the osteoinductive function of DBM associated with autologous bone

marrow (ABM) in bone defects made by surgically removal of a radius bone segment in 12 adult White New Zealand rabbits (separated in 3 groups of 4). The DBM grafts were prepared in the same dimensions of the defects. Immediately before the DBM grafting, it was soppy in ABM, previously collected. Radiographs were taken just after surgery and every week until the moment of sacrifice. Four animals of each group were sacrificed 3, 6 and 9 weeks after the procedure and submitted to macroscopic evaluation. Two animals of each group were submitted to histological evaluations and the other two animals were submitted to tetracycline fluorescence exam. This study showed, at 3 weeks, a total fill of the defects in 78.6% animals, a partial fill of the defects in 21.4% animals and histological evidences of bone maturation in many different phases. At 3 weeks, the tetracycline fluorescence exam showed amorphous and irregular bone formation areas. At 6 and 9 weeks, the same exam showed fluorescence rings representing osteons. At 9 weeks all defects were totally filled and there was bone remodeling. It was concluded that DBM was osteoinductive. The removal of the mineral portion led the osteoinductive proteins more available and the repair capacity was enhanced with its association with marrow stroma cells.

P142-Su

Expression of Bone Morphogenetic Proteins in Human Metastatic Prostate and Breast Cancer

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The prostate and breast cancer frequently metastasize to bone. Human prostatic adenocarcinoma produces osteoblastic metastases in bone, whereas the majority of bone secondaries from breast cancers are osteolytic lesions. The mechanisms of the metastatic process to bone are poorly understood. Recent research showed different findings about BMPs expression in both prostate and breast carcinoma. The present study was centered on the BMPs expression in prostate and breast carcinoma cells with established bone metastases, as confirmed by a bone scan. The aim of this study was to explore the difference of the expression of BMP-2, -3, -4, -5, -6 and -7 between those two tumors, as prostate carcinoma induces osteoblastic metastatic lesions in bone, whereas breast carcinoma metastasizes inducing osteolytic lesions. Primary tumor specimens from 20 patients with prostate cancer and 15 with breast cancer were studied immunohistochemically for BMP-2/4, -3, -5, -6 and -7. All patients had multiple bone metastases as proven by bone scan. Laboratory findings and clinicopathologic parameters were obtained from clinical data. Immunopositive cells were counted on 15 randomly chosen high power fields and expressed as percentage (minimum 1000 cells per specimen). Our results demonstrated a different pattern of BMPs expression in human metastatic prostate and breast cancers. In prostate cancer, BMP-2/4 and BMP-5 expression were positive in all examined cases, whereas BMP-3, -6 and -7 were found positive in 15%, 55% and 40% of cases, respectively. The percentage of positive cells varied among individual BMPs. BMP-2/4 showed the highest level of expression (83% of positive cells in all cases). All samples of breast cancer cells expressed only BMP-7, with high percentage of positive cells too (86% positive cells). The pattern of BMPs expression in prostate cancer cells was typically cytoplasmic, whereas in breast carcinoma the staining was exclusively nuclear. Alkaline phosphatase concentration was higher in patients with prostate cancer and differed significantly from patients with breast carcinoma. In conclusion, the results show different BMP expression in prostate and breast cancer cells, tumors with different types of bone metastasis. The prostatic carcinoma expressed all BMPs while breast carcinoma only BMP-7 independently of tumor differentiation. This may be relevant for metastasizing to bone and causing osteolytic or osteoblastic reaction.

P143-Mo

Pro-Osteogenic Effects of Follistatin on Bone Morphogenetic Proteins in Mesenchymal Progenitor Cells

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Bone morphogenetic proteins (BMPs) are responsible for the regulation of many biological processes including cell growth, differentiation, morphogenesis and embryonic patterning. Certain BMPs induce the differentiation of mesenchymal cells along a chondro-osteogenic lineage and are capable of promoting endochondral bone formation at extraskeletal sites. BMPs are negatively regulated by the antagonists noggin and chordin, which prevent BMP interaction with its receptors. Follistatin is a glycoprotein that can bind and neutralize activins and inhibins. It has been reported that follistatin can also antagonize BMP signalling, but in a manner different to that of noggin and chordin, and these observations have been based largely on analyses using developmental models. Unlike noggin and chordin, follistatin-bound BMP can interact with the BMP receptor to form a trimeric complex and we have recently discovered that follistatin-bound BMP appears to enhance the osteogenic activity of BMPs in vitro. The aim of this study was to determine the pro-osteogenic effects and cellular mecha-

nisms underlying BMP-dependent activity of follistatin on mesenchymal progenitors.

We demonstrated that follistatin enhanced the ability of BMP-2, -4, -5, -6 and -7 to induce alkaline phosphatase activity in C2C12 premyoblastic cells, MC3T3-E1 preosteoblastic cells and W-20-17 bone marrow stromal cells, causing a 2- to 5-fold increase compared to BMP treatment alone. Follistatin had no effect in the absence of BMP. Expression profiles of osteogenic and myogenic genes in C2C12 cells treated with follistatin and/or BMP-2 were determined using quantitative real-time PCR and macroarray analysis. Follistatin was shown to modulate the expression of multiple genes involved in BMP signalling and enhanced BMP-induced osteogenic differentiation, indicated by the upregulation of Cbfa1/Runx2, osterix, alkaline phosphatase and osteocalcin and downregulation of MyoD1 mRNA expression. Follistatin was also shown to augment BMP-induced osteogenesis in an ex-vivo mouse calvarial model. This was demonstrated by a 2-fold increase in calvarial thickness compared to BMP-2 treated controls, with elevated osteoblastic activity and deposition of new bone matrix.

These data suggest strongly that follistatin enhances the osteogenic effect of BMPs in mammalian mesenchymal progenitor cells. Consequently, follistatin could act as a bone anabolic agent with broad therapeutic applications in orthopedic medicine.

P144-Tu

BMP-2 and BMP-7 Gene Transfer Using in Vivo Electroporation Induces Rapid Bone Formation Accompanied by BMP-4 Expression

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Gene therapy using bone morphogenetic proteins (BMPs) is expected to promote bone healing and regeneration. The initial studies on bone regeneration using BMPs have been mainly focused on whether new bone could be induced. The current requirements before clinical application of BMPs for bone regeneration or healing are to control the volume, quality and time course of bone formation. Gene transfer using in vivo electroporation is a simple and inexpensive method that only requires a plasmid and an electroporation device. In this study, we examined simultaneous gene transfer of BMP-2 and BMP-7 to induce bone induction, and assessed the volume, quality and time course of bone regeneration after in vivo electroporation. We constructed BMP-2 and BMP-7 gene expression plasmid vectors (pCAGGS-BMP-2 and -BMP-7). We initially evaluated the ALP activity of cultured mouse myoblast C2C12 cells after transfection with each plasmid. Simultaneous transfer of pCAGGS-BMP-2 and BMP-7 achieved much higher ALP activity than the transfer of either pCAGGS-BMP-2 or pCAGGS-BMP-7. Then we injected each plasmid (25 microgram) into the calf muscle of 9-week-old Wistar rats, and performed electroporation at 100 V for 50 ms/s × 8. At 10 days after treatment, soft X-ray revealed that the calf muscles injected with both pCAGGS-BMP-2 and-BMP-7 had better-defined opacities than after single gene transfer. On histological examination, simultaneous treatment also tended to induce rapid bone formation. Moreover, ALP activity in the injected areas was much higher than after single gene transfer. pQCT analysis showed that the bone density induced by simultaneous treatment with pCAGGS-BMP-2 and-BMP-7 was higher than that in the single gene transfer groups. Furthermore, BMP-4 mRNA was expressed and increased up to 5 days in a time-dependent manner, and then decreased at 9 days in the combined BMP-2 and BMP-7 gene transfer groups. On the other hand, BMP-4 expression was weak in the BMP-2 or BMP-7 single gene transfer groups. These results indicate that simultaneous transfer of the BMP-2 and BMP-7 genes is superior to single gene transfer for bone induction along with endogenous BMP-4 expression.

P145-Su

Homologous Demineralized Bone Matrix Associated or Not to Autologous Bone Marrow in a Rabbit Lumbar Spinal Fusion

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The present study evaluated the behavior of demineralized bone matrix (DBM) associated or not to autogenous bone marrow on lumbar spinal fusion in 48 New Zealand White rabbits. 24 animals (group 1) received the DBM over the previously decorticated L5-L6 transverses processes, 24 animals (group 2) received the association of DBM to autogenous bone marrow and, in 9 rabbits (control group) only the decortication was made. Eight animals from both treated groups and 3 animals from control group were killed at 5, 7 and 9 weeks after surgery. Only the treated groups were submitted to biomechanical evaluations. In control group, radiographic and microscopic evaluations demonstrated minimum periosteal reaction without fusion. Among those who received only the DBM, at 5 weeks, the fusion rate on manual palpation was 37.5% followed by 50% at next weeks. The radiographics analysis demonstrated a fusion rate of 25% at 5 weeks, 0% of fusion at 7 weeks and 33.3% of fusion at 9 weeks. Microscopic evaluation showed predominantly the DBM fragmentation followed by its reabsorption that was substituted almost entirely by fibrous connective tissue. In the cases that fusion was observed, the endochondral ossification demonstrated that DBM was osteoconductive only in the interface transverse processes/DBM. In the group that received DBM associated to bone marrow, at 5 weeks, the union rate on manual palpation was 87.5%, followed by 75 and 100% at 7 and 9 weeks, respectively. The radiographic analysis demonstrated a fusion rate of 50%, 62% and 75% on the 3 evaluations. Microscopic evaluation, at 5 weeks, showed

incorporation between DBM and the transverses processes. It was observed the presence of cartilaginous tissue in the graft center. In the following weeks the endochondral ossification continues and the DBM was almost entirely substituted by trabecular bone tissue, forming a mature bone bridge between and over the adjacent transverses processes. The biomechanical test showed a significant difference referred to force and resistance between the operated and adjacent segments in both groups. Besides the osteoinductive actions over the stem cells, the matrix is responsible from the organized and restricted bone deposition to local environment, acting as osteoconductive scaffold. The results permitted to conclude that the association of these compounds might be used with success as a promoter agent of lumbar spinal fusion.

P146-Mo

Manipulation of the Anabolic and Catabolic Responses in a Critical Defect Model with OP-1 and Zoledronic Acid

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Bone repair involves both anabolic and catabolic responses. Therapeutic strategies may be optimized by targeting both of these responses. Osteogenic Protein 1 (OP-1 = BMP-7) and other BMPs stimulate the anabolic response, inducing osteoblast recruitment, differentiation and bone production. However, BMPs also directly up-regulate catabolism by the stimulation of osteoclasts through BMP receptors and indirectly via osteoblasts through RANK/RANKL. We hypothesized that if such osteoclastic up-regulation were modulated by zoledronic acid (ZA), the combination of OP-1 and ZA should produce increased callus over OP-1 alone.

A rat 6 mm critical size defect model was utilized. The animals were divided into five groups: Carrier, Carrier and ZA, OP-1, OP-1 and ZA and OP-1 with ZA administered 2 weeks post-op. Doses were OP-1 50 micrograms, and ZA 0.1 mg/kg as a single subcutaneous dose. Femora were harvested at 8 weeks post-surgery for radiographic, quantitative CT, mechanical and histomorphometric analyses.

Carrier alone and Carrier ZA groups had not united by 8 weeks. Radiological union occurred in all OP-1 groups, but was tenuous in some animals treated with OP-1 alone. BMC in the 6 mm defect increased by 45% in the OP-1 ZA group and 96% in the OP-1 ZA 2 weeks group over OP-1 alone ($P < 0.01$). Callus volume increased by 45% in the OP-1 ZA group and 86% in the OP-1 ZA 2 weeks group over OP-1 alone ($P < 0.01$). The increases in callus volume in the OP-1 ZA 2 weeks group translated to increases in callus strength and stiffness of 107% and 148%, respectively ($P < 0.05$).

Histomorphometry revealed increases in BV/TV and trabecular number with ZA treatment ($P < 0.05$). Both mineral apposition rate and mineralizing surface were equivalent between OP-1-treated groups, regardless of administration of ZA. This indicates that the increased bone volume was due to decreased catabolism with ZA treatment—no additional anabolic effect of ZA was seen.

Zoledronic acid significantly increased the mineral content, volume and strength of OP-1-mediated callus formation in this rat femoral critical size defect model. Modulation of both anabolic and catabolic responses may optimize the amount, mineral content and strength of callus produced, which could be of clinical benefit in obtaining initial bone union.

P147-Tu

Sensory Neuropeptide Inhibits Adrenergic Stimulation of Osteoclastogenesis in Mouse Bone Marrow Cells

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Human osteoblastic and osteoclastic cells have been demonstrated to be equipped with adrenergic receptors and neuropeptide receptors. Pharmacological stimulation of beta-adrenoceptor was demonstrated to increase the synthesis of interleukin (IL)-6 and prostaglandin (PG) E2, well known to modulate bone metabolism by regulating the development and function of osteoclasts and osteoblasts, in cultured osteoblastic cells. In vivo experiment, we also demonstrated that the activation of sympathetic nerve fibers modulates the osteoblastic activity in mouse calvaria via noradrenergic nerve terminals. These findings suggest that sympathetic and sensory innervations are required for regulating bone metabolism under physiological conditions. The present study was designed to elucidate the mode of action of isoproterenol (Isp; adrenergic beta-agonist) and to characterize the effect of the calcitonin gene-related peptide (CGRP; sensory neuropeptide) on osteoclast formation induced by Isp in a mouse bone marrow culture system. Treatment of mouse bone marrow cells with Isp generated tartrate-resistant acid phosphatase-positive multinuclear cells capable of excavating resorptive pits on dentine slices, and caused an increase in receptor activator of NF-kappa B ligand (RANKL) and a decrease in osteoprotegerin (OPG) production by the marrow cells. The osteoclast formation was significantly inhibited by OPG, suggesting the involvement of the RANKL-RANK system. CGRP inhibited the osteoclast formation caused by Isp or soluble RANKL but had no influence on RANKL or OPG production by the bone marrow cells treated with Isp, suggesting that CGRP inhibited the osteoclast formation by interfering with the action of RANKL produced by the Isp-treated bone marrow cells without affecting RANKL or OPG production. Since the mRNA encoding the calcitonin receptor-like receptor has been reported to express in mature osteoclasts, it may be thought that CGRP is working on the osteoclast precursors,

pre-osteoclasts and mature osteoclast. This in vitro data suggest the physiological interaction of sympathetic and sensory nerves in osteoclastogenesis in vivo.

P148-Su

A Possible Preferential Effect of Salmon Calcitonin-Nasal Spray (CT-NS) on Reduction of Lumbar Spine Compression Fractures in Elderly Osteoporotic Women: Results from the Proof Study

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A greater impairment in health related quality of life (HRQOL) may occur with lumbar spine (LS) compression fracture (CF) than with thoracic spine (TS) CF (Silverman, 2001); such impairment would presumably be even greater in elderly (>70) individuals, a age for which antiresorptive (AR) osteoporosis (OP) therapy has recently been shown effective (Boonen, 2004; Hochberg, 2005). A potential interrelationship of site-specific (TS vs. LS) and age-specific (<or> than age 70) effects of such therapy has not been studied. To define a possible preferential effect of the AR therapy CT-NS at the LS in women >age 70 a post-hoc data analysis from the 5-year PROOF study of 1255 postmenopausal (PM) women was performed. Relative (RRR) and absolute (ARR) risk reduction for incident TS and LS CF, as stratified for age, were examined in the 200 IU CT-NS dosage as compared to the placebo (P) group.

For the overall cohort (age 50–80) (n = 557), 200 IU NS-CT RRR was 33%, $P < 0.03$, and ARR was 8.2%, $P < 0.02$. For TS alone ARR was significant (ARR 6.4%, $P = 0.04$), and RRR was 33%, $P = 0.06$. For LS alone neither RRR or ARR were significant (RRR 33%, $P = 0.13$) For women >-age 70 (n = 238), however, not only was there a significant RRR and ARR for the overall cohort (RRR 44%, $P = 0.026$; ARR 14.3%, $P = 0.01$), but the RRR and ARR were also significant at the LS (RRR 64%, $P = 0.017$; ARR 10.4%, $P < 0.01$). As well, ARR increased in magnitude from years 50–80 in not only the overall cohort and the LS group, but also in the TS group.

These data then demonstrate therapeutic benefit of CT-NS for both RRR and ARR for CF in PM OP women of all ages, with particular and perhaps preferential benefit for older women >age 70 at the LS. Such findings, when combined with the known analgesic effects of CT-NS (Pun 1989), are of clinical importance in considering choice of a therapeutic agent for OP women of all ages but particularly for older women. Furthermore, this is the first study to show with increasing age a preferential therapeutic benefit at a given spine site; further research is needed with other AR agents to confirm whether such effects are unique to CT-NS, or a general effect of all AR OP therapies.

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P149-Mo

Diminished Osteoclast Response to Calcium Stimulus in Postmenopausal Osteoporotic Women

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We have previously shown that immediate secretory response of endogenous calcitonin (CT) to intravenous calcium load is responsible for rapid initial decrease in the bone resorption in healthy young subjects (Calcified Tissue Int, 74:377–381, 2004). To analyze whether acute changes in the serum concentration of C-terminal telopeptide of colagen type I (CTX) and secretion of CT in response to calcium challenge is reduced in postmenopausal osteoporotic women, 12 late postmenopausal osteoporotic (OP) women, 10 normal late postmenopausal (PM) women and 10 healthy premenopausal women were given intravenous infusions of 1.7 mg/kg elemental calcium over a 10-min period. In addition 8 postmenopausal thyroidectomized (TE) women were studied. A similar increase in serum ionized calcium and a decrease in plasma intact parathormone was observed in all groups. However, only in the premenopausal women plasma CT increased significantly by 13 min and serum CTX decreased as early as at 30 min as compared with the baseline (P less than 0.05). In healthy PM women a significant decrease of serum CTX was observed as early as at 60 min as compared to the TE women (P less than 0.05). In OP women, the time course and an extent of suppression of CTX after calcium infusion was not significantly different to the TE women. In healthy women, linear regression analysis demonstrated a significant (P less than 0.01) negative correlation (r = 0.62) between CTX response at 60 min and age. This observation may reflect decreased responsiveness or reserve of aging C cells to a calcium stimulus. We conclude that PM women with osteoporosis do have decreased acute skeletal responsiveness to calcium stimulus compared with a healthy women.

P150-Tu

Novel Mutations of the Calcium-Sensing Receptor in Patients with Familial Hypocalciuric Hypercalcemia and Autosomal Dominant Hypocalcemia

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The calcium-sensing receptor (CaSR) is a member of the G-protein-coupled receptor family. It regulates the secretion of parathyroid hormone (PTH) in response to changes in extracellular calcium concentrations as well as the reab-

sorption of urinary calcium. Heterozygous gain-of-function mutations cause autosomal dominant hypocalcemia (ADH) whereas loss-of-function mutations of the receptor cause familial hypocalciuric hypercalcemia (FHH).
The clinical differentiation between ADH and idiopathic hypoparathyroidism (IHP) as well as between FHH and primary hyperparathyroidism (pHPT) is not always possible. Therefore we screened 8 patients with hypocalcemia and 140 patients with hypercalcemia for mutations of the CaSR gene. Genomic DNA was extracted from peripheral blood lymphocytes and mutations of the CaSR were detected after specific amplification of the six coding exons by PCR and direct sequencing of the PCR products.
Within the group of patients with hypocalcemia we identified one novel (A844T) and three already described (Q245R, T151R, P221L) mutations. The serum calcium concentrations of the patients with mutations were 1.91–1.98 mmol/l and the PTH levels were slightly reduced or within the normal range.
We also detected six novel mutations of the CaSR (W530G, W718X, M734R, L849P, Q926R and D1005N) and two already described mutations (E250K and P55L) in the patients with hypercalcemia. The patients with mutations of the CaSR had serum calcium concentrations of 2.68–3.05 mmol/l. One of these patients showed a reduced urinary calcium excretion.
The differentiation between FHH and primary hyperparathyroidism is important to avoid unnecessary parathyroidectomy in patients with FHH. As hypocalciuria is not always detectable, the molecular genetic analysis of the CaSR is a useful tool to differentiate FHH from pHPT in patients with mild hypercalciuria and slightly elevated PTH. It is also important to distinguish between ADH and idiopathic hypoparathyroidism because of the incidence of renal complications found in ADH patients when treated with high doses of vitamin D to raise serum calcium concentrations.

P151-Su
Experimental Hypercalcemia in Horses Induces Hypomagnesemia, Hypokalemia, and Hyperphosphatemia with Increased Urinary Excretion of Electrolytes
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Electrolyte disturbances are common in critically ill humans and animals. In septic horses, low serum ionized calcium (Ca^{2+}) and magnesium (Mg^{2+}) concentrations are frequent findings. High serum Ca^{2+} is common in horses with chronic renal failure, malignancies, hyperparathyroidism and vitamin D intoxication. There is evidence that the renal reabsorption of Ca^{2+} is also regulated by PTH-independent mechanisms, primarily by the calcium-sensing receptor (CaR) that affects the transepithelial transport of Ca^{2+} and Mg^{2+}. Because Ca^{2+} can potentially affect other electrolytes, we evaluated the effects of hypercalcemia on serum electrolytes and their urinary excretion in healthy horses. We speculated that by a Ca^{2+}-mediated effect, hypercalcemia will result in hypomagnesemia and increased urinary excretion of electrolytes.
Hypercalcemia was induced in twelve mares; six were infused with 23% calcium gluconate (G) and six were infused with 10% calcium chloride ($CaCl_2$) for 120 min. Blood was collected to measure serum electrolytes, PTH and insulin concentrations, and urine was collected to determine the fractional excretions of Ca^{2+} (FCa), Mg^{2+} (FMg), Na^+ (FNa), phosphate (FP), K^+ (FK) and Cl^- (FCl). In hypercalcemic mares, serum Ca^{2+} increased 6.6 ± 0.1 to 9.7 ± 0.3 (G) and 6.4 ± 0.1 to 10.2 ± 0.10 mg/dL ($CaCl_2$); Mg^{2+} decreased from 0.52 ± 0.02 to 0.33 ± 0.02 (G) and 0.51 ± 0.02 to 0.32 ± 0.02 mmol/L ($CaCl_2$); K^+ decreased from 4.3 ± 0.1 to 3.4 ± 0.1 (G) and 4.2 ± 0.2 to 3.6 ± 0.2 mEq/L ($CaCl_2$) and Pi increased from 3.4 ± 0.2 to 4.8 ± 0.3 (G) and 2.9 ± 0.2 to 4.1 ± 0.4 mg/dL ($CaCl_2$) (all $P < 0.05$). PTH decreased to very low concentrations. No changes in insulin were detected. FCa increased from 5.4 ± 1.0 to 56.2 ± 6.9 (G) and 5.4 ± 1.1 to 47.7 ± 6.4% ($CaCl_2$); FMg from 23.5 ± 2.4 to 55.0 ± 4.5 (G) and 28.5 ± 4.3 to 54.4 ± 7.3% ($CaCl_2$); FNa from 0.09 ± 0.04 to 4.3 ± 0.8 (G) and 0.03 ± 0.003 to 4.8 ± 0.8% ($CaCl_2$); FK from 45.4 ± 7.6 to 116.6 ± 19 (G) and 38.4 ± 1.5 to 89 ± 8.3% ($CaCl_2$); FCl from 0.65 ± 0.1 to 5.6 ± 1.5 (G) and 0.7 ± 0.1 to 9.3 ± 1.6% ($CaCl_2$), and FP from 0.04 ± 0.02 to 0.5 ± 0.2 (G) and 0.14 ± 0.06 to 0.81 ± 0.3% ($CaCl_2$). Urine-specific gravity and osmolality decreased and urine output increased ($P < 0.05$).
In conclusion, hypercalcemia resulted in hypomagnesemia, hypokalemia and hyperphosphatemia, increased the urinary excretion of Ca^{2+}, Mg^{2+}, K^+, Na^+, Pi and Cl^-, and induced diuresis. This study has clinical implications since excessive administration of Ca^{2+} salts can increase the waste of electrolytes, in particular Mg^{2+}.

P152-Mo
Cinacalcet and Hypocalciuric Hypercalcemia (HH)
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HH is characterized by hypercalcemia with normal or low urinary calcium (uCa). Although parathyroid hormone (PTH) levels may be elevated, the hypocalciuria is not dependent on PTH. Cinacalcet is a calcimimetic agent used to treat patients with secondary hyperparathyroidism, concurrently decreasing PTH, serum Ca (sCa), serum phosphate

(sPO4) and Ca × P. Here we report the case of a patient with HH who was treated with cinacalcet.

A 16-year-old, 185-cm, 78-kg male presented with acute pancreatitis and mild hypercalcemia at Perugia Regional Hospital. The patient had a cholecystectomy and initiated ursodesossicolic acid. After a second acute pancreatitis episode, the patient was referred to our centre. The patient's basal mineral metabolism parameters were assessed over 4 months using 4 different determinations. sCa increased from 11 to 12.3 mg/dL, sPO4 ranged from 2.8 to 3.3 mg/dL and intact PTH (iPTH) levels were normal except for 1 measurement of 85.5 pg/mL. The patient's 24 h uCa was consistently low (120 to 210 mg; except for one determination of 460 mg) compared with sCa, and the Ca/creatinine clearance (CrCl) ratio was always lower than 0.01 (0.0081 to 0.0095). High-resolution ultrasonographs and a sestamibi scan showed no evidence of parathyroid abnormality. The patient's first-degree relatives did not demonstrate abnormal sCa or iPTH levels. Multiple endocrine neoplasia syndromes types 1 and 2A were excluded. 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 (60 mg/day). The patient's mineral metabolism parameters before and during treatment with cinacalcet are shown below. After initiation of cinacalcet the patient experienced a reduction in sCa and concurrent increase in 24 h uCa. This encouraging case shows the need to evaluate cinacalcet as a potential therapy for HH.

Table
Patient mineral metabolism parameters

	Reference range	Mean basal values	Cinacalcet (30 mg/day × 15 days)	Cinacalcet (60 mg/day × 14 days)
sCa (mg/dL)	8.5–10.2	11.5	10	9.3
iPTH 1–84 (pg/mL)	10–78	73.9	109.0	74.1
sPO4 (mg/dL)	2.7–4.5	2.8	4.2	3.9
Ca/CrCl ratio	>0.01	0.0085	0.021	0.013
24 h uCa (mg)	50–400	263.3	390	/

P153-Tu

The Role of the Chemical Shift Magnetic Resonance Imaging in the Investigation of the Bone Marrow Affected by Monoclonal Gammopathies

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It is difficult to separate from each other in many cases the multiple myeloma and the monoclonal gammopathy of unknown significance (MGUS) by routine clinical and pathological examination. Because of this diagnostic disturbance, the appropriate therapy can be delayed. We investigated the potential role of chemical shift magnetic resonance imaging in the characterization of the bone marrow tissue in patients with multiple myeloma and MGUS, in order to assess a new diagnostic tool in the differential diagnosis of myeloma and MGUS. Twenty patients with monoclonal gammopathy were enrolled in this preliminary study. Conventional T1- to T2-weighted, Dixon in phase-out of phase magnetic resonance imaging and magnetic resonance spectroscopy was performed on the lumbar spine of the patients. There was not signal intensity change between the in phase-out of phase MRI in patients with myeloma. The patients with MGUS have difference in signal intensity between the two phases. We found significantly higher water/lipid ratios in myeloma group than the MGUS group in the MR spectroscopy study. Our preliminary findings suggest that the chemical shift imaging could be useful to differentiate the myeloma from MGUS, therefore can help to decide the initiation of the appropriate therapy.

P154-Su

Zoledronic Acid Suppresses Lung Metastases and Extends Overall Survival of Osteosarcoma-Bearing Mice

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Although there is no doubt that Bisphosphonates (BPs), specific inhibitors of osteoclasts, are beneficial for the treatment of bone metastases, their effects on visceral metastases are unclear. In the present study, we studied the effect of zoledronic acid (ZOL), an N-BP of the third generation, on the progression of lung metastases induced by i.v. inoculation of POS-1 osteosarcoma cells in C3H/He mice. Lung metastasis was determined at the time of autopsy. Mechanisms of action of ZOL were also assessed in vitro on osteosarcoma POS-1 cell proliferation, on caspase-1 and-3 activation and cell cycle progression.

ZOL-treated mice (0.1 mg/kg, five times a week) significantly extends the animal survival rate. While all control mice died 23 days post-metastasis induction, respectively, 83% and 66% of ZOL-treated animals survived 24 and 45 days after POS-1 cell injection. Overall survival rate of 5 independent experiments (two series treated with 0.1 mg/kg twice a week, and three series with 0.1 mg/kg five times a week) shows a significant increase of the actuarial survival: 0.422 ± 0.07 in ZOL treated-animals versus 0.167 ± 0.07 in controls ($P = 0.036$). Histological analyses demonstrate that all ZOL-treated mice exhibit no lung metastasis. In vitro, a 48-h incubation with 10 μM ZOL inhibits POS-1 cell line proliferation with a half-maximal inhibitory effect at 25 μM, associated with a cell cycle arrest in S-phase: the number of

cells in S-phase increases from 14% in control cells to 19% or 25%, respectively, in the presence of 1 and 10 μM ZOL. This observation is concomitant with a decrease of cells of G0/G1 phase: 55% and 53%, respectively for 1 and 10 μM ZOL (48 h of incubation) vs. 63% in the controls untreated POS-1 cells. No modification of nuclear morphology that is characteristic of apoptosis can be observed in ZOL-treated POS-1 cells after Hoechst staining. Results show that ZOL does not induce any activation of caspase-1 in POS-1 cells but induces an increase in caspase-3 activity at the concentration of 10 μM (72 h).
We demonstrated that ZOL exerts a direct antitumor effect on POS-1 cells in vitro, significantly diminished osteosarcoma-induced lung metastasis in vivo thereby extending the survival of POS-1 inoculated animals.

P155-Mo
Osteoclastic Migration and Matrix-Degradation in Osteolytic Metastasis
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Purpose: Osteoclasts are responsible for bone resorption during osteolytic metastasis. However, involvement of osteoclasts in the degradation of extracellular matrices is still an ongoing discussion. We therefore have examined osteoclastic migration and matrix-degradation during osteolytic metastasis.
Materials and methods: Lung cancer cells (SBC-5) were injected into the left cardiac ventricle of 6 week-old nude mice under anesthesia. After 1 month, the mice were perfused with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The metastasized femora and tibiae were extracted for decalcification with EDTA-2Na solution and were dehydrated through an ascending ethanol series prior to paraffin or epoxy resin embedding. We examined immunolocalization of alkaline phosphatase (ALP), osteopontin, CD44, cathepsin K and matrix metalloproteinase 9 (MMP-9), as well as tartrate resistant acid phosphates (TRAP) activity in the bone metastasis.
Results and discussion: Metastasized tumor nests were formed in the subcartilaginous region of the growth plate. TRAP-positive osteoclasts were found mainly in the ALP-positive osteoblastic layer covering bone surface, but were also localized in the ALP-negative stromal cell layer of the tumor nests. TRAP-positive osteoclasts showed immunoreactivity for CD44 that can bind to osteopontin. Osteopontin was distributed in the stromal tissue of tumor nests. Therefore, osteoclasts may be able to migrate into stromal tissue of tumor nests, by attaching to osteopontin. Next, we examined the localization of cathepsin K and MMP-9 in osteoclasts. Osteoclasts adjacent to the bone surfaces were positive for cathepsin K and MMP-9, whereas the osteoclasts in the stromal tissue of the tumor nests showed only MMP-9 immunoreactivity. Immunoelectron microscopy localized MMP-9 in the Golgi apparatus and vesicular structures in the baso-lateral cytoplasmic region of the osteoclasts in stromal tissue. In addition, vesicular structures positive for MMP-9 included fragmented extracellular fibrils. Therefore, it is likely that osteoclasts in stromal tissue of tumor nests synthesize MMP-9 and digest internalized extracellular fibrils.
Conclusion: Osteoclasts appeared to be capable of migrating into stromal tissue of tumor nests by mediating the attachment to osteopontin, and are involved in matrix degradation therein by secreting proteolytic enzymes such as MMP-9.

P156-Tu
The Intraosteal Tumours and the Material "LITAR"
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In the course of the intraosteal tumour localization its removal is associated with the defect formation, and this fact leads to the bone mechanical strength reduction at the defect site. Previously [1,2] it was informed about the use of the collagen-apatite material "LitAr" for restoring the big tubular bone defects. This material is distinguished from other semisynthetic implants by its maximum biotransformation rate: it has been morphologically proved that by the 15th–20th day it was fully substituted for the connective tissue with the subsequent ossification [3]. The reparative bone regeneration in the defect zone was checked radiology. In the present work our results of the composite material "LitAr" use in the course of treating the intraosteal hand bone tumours are shown. The patients' age ranged from 13 to 45 years. The defect plasty after performing the edge resection and excochleation was performed on the metacarpal bone, the proximal and meddle phalanges. Within the very near period we could observe an aseptic inflammation for 7–8 days. For its diluting we used antibiotics with a broad action spectrum. Healing the operative wound was caused by first intention. This phenomenon was observed in the course of a year. The finger function was restored in a very short space of time. The advantages of the material "LitAr" are simplicity of its preoperative preparation bone defect substitution complete filling the defect cavity owing to self-widening lack of the necessity of storing allografts. Its disadvantage is the lack of the splintage ability of the material because of its low mechanical strength.
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P157-Su

Evaluation of the Tartrate-Resistant Acid Phosphatase 5b as Serum Marker of Bone Resorption in Radiotherapy of Breast Cancer Patients with Bone Metastases

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Introduction: The active isoform 5b of TRACP has been considered a specific serum marker of osteoclast activity and a useful marker of bone resorption. The aim of our study was to evaluate if the determination of this marker provides the possibility to monitor the effect of local radiotherapy (RT) in bone metastases and if TRACP 5b will have a predictive value regarding further osseous progress.
Materials and methods: In 48 breast cancer patients with bone metastases requiring radiotherapy (30–40 Gy), patients' characteristics (pain score using a visual analogue scale, analgesic drugs), diagnostic imaging and laboratory investigation (X-ray imaging, bone scan, TRACP 5b), tumor and therapy-related parameters were registered in the beginning (T1) and the end of RT (T2) as well as 6 (T3) and 12 (T4) weeks afterwards. Most of the patients suffered from osteolytic and multiple bone metastases. The TRACP 5b activity was measured using a solid phase immunofixed enzyme activity assay with the highly characterized, specific monoclonal antibody O1A.
Results: As expected, in most of the patients we observed a pain reduction and a recalcification in irradiated lesions (85%). During follow-up, in 31 patients (65%) a progression in another part of the skeleton was diagnosed whereas in 17 patients (35%) bone scan did not show new metastases. We found a significant decrease of TRACP 5b in patients without progression in not irradiated regions (median value at T1 and T4: 5.1 U/l to 3.0 U/l) whereas in progressive disease TRACP 5b remained stable with a slightly increasing tendency (median value at T1 and T4: 6.0 U/l to 6.4 U/l) ($P < 0.05$). In patients with single or <4 metastases all TRACP 5b values were significantly lower than values of those with multiple metastases ($P = 0.01$). Using the ROC-analysis, the AUCs ("area under curve") were significantly different between visits ($P = 0.02$). The AUC of TRACP 5b values at the time of the last examination or progression (0.82) was significantly larger than AUC at baseline (0.64) ($P = 0.049$) and tendentiously larger than AUC at the end of RT (0.74) ($P = 0.095$).
Conclusions: In patients without further osseous progression TRACP 5b is able to monitor the effectiveness of local RT. The estimation of sensitivity and specificity based on each TRACP 5b value demonstrates that the ability to discriminate between those patients with or without osseous progression increases during time and therefore has a predictive value.

P158-Mo

Abstract Withdrawn

P159-Tu

Zoledronic Acid Inhibits Osteosarcoma Cell Proliferation Via Cell Cycle Arrest and Inhibition of Focal Adhesion Kinase Phosphorylation

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Osteosarcoma is the most frequent primary bone tumor and despite recent improvements in surgery and the development of different regimens of multidrug chemotherapy, survival remains around 55% to 70% after 5 years. This poor prognostic warrants new therapeutic strategies to improve the overall rate of survival. Zoledronic acid (ZOL) is a nitrogen-containing bisphosphonate (N-BP) of the third generation used as an osteoclast inhibitor in several pathologies involving bone resorption, from tumoral origin or not. However, ZOL molecular mechanisms remain unclear. In the present study, the in vitro effects of ZOL were analyzed on several osteosarcoma cell lines compared to primary rat osteoblasts, in terms of cell proliferation, cell cycle analysis (flow cytometry analysis) and apoptosis (caspase 1, 3 and 8 analysis, Hoechst staining and time lapse microscopy).
ZOL inhibits osteosarcoma cell proliferation with a half-maximal inhibitory effect observed at 0.2 μM after 72 h of treatment, whereas osteoblasts are less affected. The ZOL-induced inhibition of cell proliferation is due to cell cycle arrest in S-phase. Indeed, the number of osteosarcoma cells in S-phase increases from 10% in control conditions to 32% in 10 μM ZOL-treated cells (48 h), whereas no effect is observed in osteoblasts. No alteration of nuclear morphology can be observed in ZOL-treated osteosarcoma cell lines after Hoechst staining. In addition, ZOL does not induce any caspase-1, 3 or 8 activation. However, trypan blue staining and time lapse microscopy demonstrate that 10 μM ZOL selectively induces osteosarcoma cell death and migration inhibition. Although the main target of ZOL described in the literature is an enzyme of the mevalonate pathway, the farnesyl diphosphate synthase, another pathway can be

suggested. Indeed, Western blot analyses show that a 48-h treatment with 10 μM ZOL inhibits the phosphorylation of focal adhesion kinase, which are involved in cell survival, proliferation and migration.
These results demonstrate selective anti-tumor effects of ZOL on several osteosarcoma cell lines, thus allowing to consider these molecules as potential therapeutic agents in clinical trials of tumoral bone pathologies. Future experiments will be performed to better characterize the mechanisms involved in ZOL-induced cell death and its influence on cell adherence and integrin expression.

P160-Su
Potential Capability of Adoptive Tumor-Infiltrating Lymphocytes Therapy in Patients with Osteosarcoma: Preliminary Report
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Background: Osteosarcoma is the most common type of primary bone tumor. The use of aggressive chemotherapy has drastically improved the prognosis of the patients with non-metastatic osteosarcomas; however the prognosis of the patients with metastasis is still very poor. Then, new and more effective treatments for curing osteosarcoma, such as immunotherapy are needed. Tumor-infiltrating lymphocytes (TIL) have been characterized to be more specific in their immunological reactivity to tumor cells and their reactivity to tumors is restricted to MHC class-I molecules. TIL represent a fascinating therapeutic approach in melanoma; however there is no report concerning skeletal tumors including osteosarcoma.
Materials and methods: All of samples of the patients with musculoskeletal tumors were obtained from Orthopaedic Department of Nantes University Hospital: 4 osteosarcomas, 2 Ewing's sarcomas, 2 plasmocytomas, 4 giant cell tumors and others. TIL and peripheral blood lymphocytes (PBL) were extracted from tumor specimens and peripheral bloods, respectively. The character of both TIL and PBL were determined by flow cytometric analysis. Their cytotoxic activity was also determined by cytotoxic assay.
Results: TIL and PBL were able to extract from the specimens with skeletal tumors like other solid tumors. The CD4+/CD8+ ratio between TIL and PBL was different depend on the histology. Significantly high cytotoxic activity of TIL compared to PBL was confirmed in patients with osteosarcoma ($P < 0.002$).
Discussion: Despite the successful report of clinical phase-II trials in melanoma patients using TIL, there is no report of TIL therapy for osteosarcoma. This is the first preliminary report concerning TIL therapy in skeletal tumors. We can demonstrate that TIL and PBL was able to extract from both benign and malignant human skeletal tumors including osteosarcoma. In this report, most underlined was significantly high cytotoxic activity of TIL against tumor cells compared to PBL in case of osteosarcoma. These results indicated that TIL therapy is hopeful and fascinating strategy for osteosarcoma. TIL therapy alone or combined with other strategies might contribute to the establishment of new approach for osteosarcoma.
Conclusions: Adoptive TIL therapy are thought to have high impact on further immunotherapy for osteosarcoma.

P161-Mo
Sart3 Expression in Caucasian Patients with Musculoskeletal Tumor
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Background: Osteosarcoma is the most common type of primary malignant bone tumor. The use of aggressive chemotherapy has drastically improved the prognosis of the patients with non-metastatic osteosarcomas; however the prognosis of the patients with metastasis is still very poor. Then, new and more effective treatments for osteosarcoma, such as immunotherapy are needed. The identification of genes encoding tumor-rejection antigens that are recognized by HLA class I-restricted and tumor-specific cytotoxic T lymphocytes (CTL) have open new strategy in modern tumor immunology. The squamous cell carcinoma antigen recognized by T cells 3 (SART3) was identified in osteosarcoma as one of the tumor-rejection antigens; however its expression has not been analyzed in Caucasian.
Materials and methods: All of tumor specimens from the patients with musculoskeletal tumors were obtained from Orthopaedic Department of Nantes University Hospital: 7 osteosarcomas, 3 Ewing's sarcomas, 1 chondrosarcoma, 3 plasmocytomas. Expression of SART3 at the both RNA and protein level were analyzed by reverse transcript-polymerase chain reaction (RT-PCR) and Western blot analyses, respectively.
Results: Positive SART3 expression at the both RNA and protein level were confirmed in some patients with osteosarcoma, chondrosarcoma and Ewing's sarcoma. Contrary, some osteosarcomas, Ewing's sarcomas and plasmocytomas demonstrated negative expression of SART3 at the RNA level.
Discussion: This is the first report concerning SART3 expression in Caucasian. It has been reported that SART3 peptides were capable of inducing HLA-A 24-restricted and tumor-specific CTL in osteosarcoma and SART3 expression was ubiquitous at the mRNA level. However we could not detect SART3 RNA in some cases which can be explained by splicing effects. It has been reported that SART3 is

involved in the regulation of mRNA splicing; however there is no know report that SART3 RNA was involved some splicing. Then, RT-PCR using another primer that targets other region of SART3 RNA might answer this question. Other likely explanation is population difference. Also, the ability of SART3 inducing SART3-specific CTL recognizing SART3-positive tumor cells should be tested in Caucasian.

Conclusions: Some cases of negative expression of SART3 RNA were demonstrated. Continuous study to confirm SART3 expression in Caucasian is needed.

P162-Tu

Osteoporosis and Rate of Bone Loss Among Postmenopausal Survivors of Breast Cancer—Results from a Subgroup in the Women's Health Initiative Observational Study

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Background: Breast cancer diagnosis and treatment may potentially put women at a high risk for low bone density and osteoporosis in their later life. However, there is very limited direct and/or longitudinal data for understanding the magnitude and trend of change in risk for osteoporosis among survivors of breast cancer.

Methods: In this study of a subgroup of participants in the Women's Health Initiative Observational Study, we tested the hypothesis that postmenopausal survivors of breast cancer are at an increased risk for low bone density and osteoporosis. We compared BMD, the rate of changes in BMD, and the undiagnosed rate for osteoporosis between breast cancer survivors ($n = 209$) and a non-cancer reference group ($n = 5759$).

Results: In comparison to the reference group, breast cancer survivors had a significantly lower mean total body BMD value (0.989 vs. 1.013 g/cm^2, $P = 0.001$) and total hip BMD value (0.823 vs. 0.845 g/cm^2, $P = 0.02$) at the baseline measurements after adjustment for age, race/ethnicity, years since menopause and clinical center. These lower baseline BMD values were largely explained by a lower usage of hormone therapy among the survivors. The undiagnosed rate for osteoporosis among breast cancer survivors was 77.8%, which was not significantly different from the reference group (85.7%). The undiagnosed rate for osteoporosis was lower among survivors who had their breast cancer diagnosis before age 55 in comparison to survivors whose breast cancer diagnoses were at or after age 55 (66.7% vs. 88.5%, $P = 0.058$). Longitudinally, breast cancer survivors in this study did not demonstrate an accelerated rate of bone loss in comparison to the reference population.

Conclusions: Postmenopausal survivors of breast cancer are a high-risk group for osteoporosis and majority of the survivors with osteoporosis are undiagnosed.

P163-Su

Nephrotoxic Differences of Bisphosphonates for Metastatic Bone Disease

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Background: Some intravenous (i.v.) bisphosphonates have occasionally been linked to renal adverse events that may compromise outcomes and require hospital management. We investigated the nephrotoxic potential of different bisphosphonates for metastatic bone disease from breast cancer in pre-clinical single and intermittent dose studies in the rat.

Methods: In the first study, ibandronate and zoledronic acid were given 10 mg/kg, −20 mg/kg and 1-as a single i.v. dose over a range of 1, respectively. Clinical biochemistry and kidney histopathology were performed on the first and fourth day after bisphosphonate dosing. In a second study conducted over 25 weeks, minimally nephrotoxic doses of ibandronate (1 mg/kg) or zoledronic acid (1 or 3 mg/kg) were given intermittently every 3 weeks.

Results: Tubular degeneration was observed on the fourth day after 1–20 mg/kg i.v. ibandronate or 3–10 mg/kg i.v. zoledronic acid. Although proximal convoluted tubule degeneration and single cell necrosis were found with both bisphosphonates, there were qualitative differences in localization and type of lesion. The ratio between the lowest lethal dose and the minimally nephrotoxic dose was 25:1 for ibandronate but only 3.3:1 for zoledronic acid. Sub-clinical renal changes after a single dose accumulated to clinically relevant kidney damage when minimally nephrotoxic doses of zoledronic acid (1 or 3 mg/kg), but not ibandronate (1 mg/kg), were injected every 3 weeks.

Conclusions: Pre-clinical evidence indicates that bisphosphonates vary in their nephrotoxic potential. The risk of cumulative renal damage is related to the amount of bisphosphonate remaining in the kidney from previous doses. The absence of toxic accumulation with ibandronate compared to zoledronic acid may be explained by its shorter terminal tissue half-life (24 days versus 150–200 days). This allows sufficient time for repair of possible sub-clinical renal damage with ibandronate infused every 3–4 weeks, the

recommended dosing interval for i.v. bisphosphonates in patients with breast cancer and bone metastases.

P164-Mo

Results of a Large Multinational, Multidisciplinary Survey on the Management of Bone Metastases

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The GEMO launched in June 2004 a large (4706 physicians taking care of cancer patients), multinational (French-speaking Europe), multidisciplinary survey to better apprehend the diagnostic and therapeutic approaches in patients with bone metastases (BM). TNS Sofres contacted physicians by mail and phone. 726 (16%) sent their questionnaires back of which 692 could be analyzed. The most represented specialties were medical oncologists (19%), radiation oncologists (18%), urologists (17%) and general internists (14%). Physicians were first required to answer a "general" four-part questionnaire (diagnostic and follow-up, radiotherapy, surgery and medical treatments). They were also asked to fill a "specialized" questionnaire. We report the results to a few representative questions of the "general" part. For the diagnosis of BM, CT-scans and tumor markers are most often requested (>5 times/month by 64% and 61% of the respondents; corresponding figures are 38% for bone scan, 26% for bone formation markers and 7% for bone resorption markers). For the follow-up of BM, tumor markers are requested systematically or frequently by 73%, bone scan by 62% and markers of bone turnover by 14%. Only 35% of the respondents ever request these markers, essentially to monitor the efficacy of antineoplastic (20%) or of bisphosphonates (BP) therapy (17%). For BP prescription in breast cancer, 85% have a systematic attitude: two thirds of the respondents start BP as soon as the diagnosis of BM is proven, whatever the presence of symptoms. Only 12% wait until a first event occurs. In prostate cancer, 45% prescribe BP at the diagnosis of BM, 23% wait until the presence of symptoms, and only 12% wait that the cancer has also become hormone refractory. DEXA is requested by two thirds of the respondents in a woman who has had breast cancer surgery. Concerning cancer treatment-induced bone loss, only 50% of the respondents try to prevent bone loss after adjuvant chemotherapy-induced menopause. For this purpose, 35% of them use Ca+ Vit D+ exercise and 34% prescribe a BP if osteoporosis has been demonstrated by DEXA. In conclusion, this large survey provides an extensive overview of the use of diagnostic techniques, of the prescription of BPs according to the tumor type and if physicians take into account cancer treatment-induced bone loss. More education appears to be needed as well as a clearer transmission of evidence based medicine results.

P165-Tu

Hypercalcemia in Cancer Patients: A Review of More Than 1000 Patients

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Introduction: Hypercalcemia is not a rare complication of cancer since it classically occurs in 10 to 15 percent of the cases. It can be observed in any type of cancer but breast and lung tumors are the most frequent causal neoplasms. Other causes of hypercalcemia than cancer are often ignored by the physician in charge of cancer patients.
Objective: To review the causes of hypercalcemia in a large series of cancer patients.
Methods: We have retrospectively studied in a Cancer Center all hypercalcemic (Ca > 10.5 mg/dl) patients from January 1996 to June 2004. We present here our interim analysis of 1079 patients. Clinical information was gathered about the type of cancer, its histology, the activity of the disease and the presence of bone metastases. There were 747 patients with an active cancer and 332 in complete remission. Biological determinations included serum Ca, Pi, PTH (in 464 patients), PTHrP (in 182 patients), 25 OH- and 1,25 (OH2)-vitamin D, TSH/T4.
Results: By order of decreasing frequency, the apparent causes were dehydration (n = 352), bone metastases (n = 251), primary hyperparathyroidism (n = 208), humoral hypercalcemia of malignancy (HHM, n = 206), hyperthyroidism (n = 32), immobilization (n = 12), vitamin D intoxication (n = 5), sarcoidosis (n = 3) and Addison disease (n = 1); in 9 cases, the cause of hypercalcemia could not be determined. The number of HHM cases could be higher since PTHrP was only rarely measured in patients with bone metastases.
Conclusions: Hypercalcemia occurring in patients who have had a cancer or who suffer from an active cancer is frequently not due to the tumor. Without taking into account the patients whose hypercalcemia was attributed to dehydration, 36% (261/718) of the causes of hypercalcemia were apparently not due to cancer. Primary hyperparathyroidism was the leading other cause. In that perspective, serum PTH determination is essential in the approach of hypercalcemic cancer patients.

P166-Su

Safety of Intravenous (i.v.) and Oral Ibandronate for up to 4 Years in Patients with Breast Cancer and Bone Metastases

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Background: Safety of long-term bisphosphonate therapy is important as many patients may receive extended treatment

over several years. In a placebo-controlled phase III clinical trials, i.v. ibandronate 6 mg infused every 3–4 weeks and oral ibandronate 50 mg once daily for 2 years were very well tolerated in patients with osseous lesions from breast cancer while effectively reducing skeletal event rate.

Methods: Non-controlled extension studies were conducted to assess the safety of ibandronate for up to 4 years of treatment. On completion of the 2-year trials, patients in all treatment assignments were offered active treatment for a further 2 years (oral ibandronate 50 mg/day [n = 115] or i.v. ibandronate 6 mg every 3–4 weeks [n = 62]). Adverse events (AEs) and laboratory parameters were recorded.

Results: As expected with advanced malignant disease, 18% of patients receiving oral ibandronate did not complete the 2-year follow-up period due to AEs. Malignancy progression was the most commonly reported AE. Hypocalcemia, dyspepsia and esophagitis were the only AEs considered possibly related to oral ibandronate; none were serious or led to withdrawal. In the i.v. trial, 10% of patients receiving ibandronate 6 mg did not complete the 2-year follow-up period due to AEs, and the majority of patients (77%) experienced at least one AE. Disease progression accounted for 44% of all reported AEs. The most common treatment-related AE was gastroenteritis, affecting two patients. There were no renal AEs or laboratory/vital sign abnormalities associated with ibandronate.

Conclusions: Oral and i.v. ibandronate are well tolerated for up to 4 years of treatment in patients with metastatic breast cancer. The renal safety of ibandronate would allow its use in patients receiving nephrotoxic drugs and in those with severe renal function impairment, where the use of intravenous zoledronic acid and pamidronate is not recommended. Oral ibandronate is suitable for convenient once-daily dosing at home, without the need for close safety monitoring.

P167-Mo

Improving the Management of Metastatic Bone Pain: Phase III Trials of Ibandronate in Patients with Breast Cancer and Bone Metastases

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Background: The management of metastatic bone pain remains a challenge. In a survey of 518 patients with bone metastases, one-third of patients with moderate-to-severe pain were undertreated. Two-thirds of patients with metastatic bone disease experience significant pain and debility. Phase III clinical trials have assessed the impact of the aminobisphosphonate ibandronate on bone pain in patients with metastatic bone disease from breast cancer.

Methods: Intravenous ibandronate 6 mg (infused over 1–2 h every 3–4 weeks) and oral ibandronate 50 mg (once daily) were administered over 96 weeks in a blinded placebo-controlled trial. Bone pain scores were assessed on a 5-point scale from 0 = none to 4 = intolerable.

Results: Intravenous ibandronate 6 mg significantly reduced mean baseline bone pain scores compared with placebo (−0.28 vs. + 0.21, $P < 0.001$). A trend to lower analgesic use was seen in the ibandronate group ($P = 0.08$). In clinical trials (n = 846) of oral ibandronate 50 mg over 96 weeks, mean baseline bone pain scores were reduced at the end of the trial by −0.10 with ibandronate 50 mg, compared with an increase in score of +0.20 with placebo ($P = 0.001$). Analgesic use was significantly lower with oral ibandronate 50 mg compared with placebo ($P = 0.019$).

Conclusions: These results demonstrate that long-term treatment with intravenous ibandronate 6 mg and oral ibandronate 50 mg have a marked analgesic effect in patients with metastatic bone disease from breast cancer. Unlike other bisphosphonates, pain reduction with ibandronate is sustained below baseline levels over 2 years of treatment. Ibandronate use coupled with regular monitoring of pain via a patient diary may help improve the management of metastatic bone pain while still helping to prevent bone events.

P168-Tu

Intravenous (i.v.) and Oral Ibandronate Reduce the Risk of Skeletal-Related Events (SRES) in Patients with Breast Cancer and Bone Metastases

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Background: Ibandronate is an aminobisphosphonate approved in Europe for the treatment of metastatic bone disease due to breast cancer. Phase III clinical trials have investigated the impact of i.v. and oral ibandronate on the occurrence of SREs in women diagnosed with breast cancer and bone metastases.

Methods: Three multicenter, randomized, double-blind, placebo-controlled trials were conducted. In a trial of i.v. ibandronate, a 6-mg dose (n = 154) was compared with placebo (n = 158) infused over 1–2 h every 3–4 weeks. In two trials of oral ibandronate, a 50-mg daily dose (n = 287) was compared with placebo (n = 277). Data from the oral trials were pooled for analysis, as pre-specified in the study protocols. The primary efficacy endpoint was the skeletal morbidity period rate (SMPR), defined as the number of 12-week periods with new bone complications. Secondary analysis of SREs was conducted using a multivariate Poisson regression model. A post-hoc analysis using the Andersen–Gill method (time to multiple SREs) was also performed.

Results: Mean SMPR was significantly reduced with ibandronate (6 mg dose, 1.19 versus 1.45 with placebo, $P = 0.004$; 50 mg dose, 0.95 versus 1.18 with placebo, $P = 0.004$). The multivariate Poisson regression analysis demonstrated that i.v. ibandronate 6 mg led to a statistically significant 40% reduction in the risk of SREs

compared with placebo (RR 0.60, 95% CI = 0.43, 0.85; $P = 0.0033$). The effect of oral ibandronate 50 mg on the risk of SREs was similar (38% reduction versus placebo, RR 0.62, 95% CI = 0.48, 0.79; $P < 0.0001$). The Andersen–Gill analysis showed a 29% reduction in SREs for i.v. ibandronate (RR 0.71, $P = 0.018$) and a 35–42% reduction for oral ibandronate (RR 0.62, $P < 0.005$) compared with placebo.

Conclusions: In patients with breast cancer and bone metastases, i.v. ibandronate 6 mg and oral ibandronate 50 mg reduced the occurrence of SREs. Ibandronate offers flexibility with effective i.v. and oral dosing. Furthermore, oral ibandronate provides the choice of well-tolerated and convenient at-home dosing to eliminate time-consuming hospital visits for intravenous therapy.

P169-Su

Oral Ibandronate Treatment Preceded by a Single Rapid Dose of Intravenous (i.v.) Ibandronate Rapidly Decreases Serum-CTX a Marker of Bone Resorption in Patients with Metastatic Bone Disease Due to Multiple Myeloma and Breast Cancer

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Background: In phase III trials of patients with breast cancer and bone metastases both i.v. and oral ibandronate reduced metastatic bone pain and the risk of skeletal-related events. This 12-week trial determined the response of bone turnover markers to a single rapid infusion of i.v. ibandronate followed by daily oral ibandronate treatment. Here we discuss the bone turnover marker data of 34 patients.

Methods: Patients with advanced multiple myeloma or breast cancer and at least 1 confirmed lytic or mixed bone lesion received a single 15-min infusion of i.v. ibandronate 6 mg followed by oral ibandronate 50 mg once daily ($n = 34$). Eligibility criteria included the following: at least 18 years of age; provided written informed consent; life expectancy at least 6 months, WHO Performance Status of 0, 1 or 2 and adequate renal function (serum creatinine = 3.0 mg/dL). Cross-linked C-terminal telopeptide of type I collagen in serum (S-CTX), bone-specific alkaline phosphatase (S-bALP), the amino-terminal procollagen propeptides of type I collagen (P1NP) and osteocalcin (OC) were measured.

Results: Mean (CI) bone turnover marker percent changes are shown in the Table.

Conclusions: The combination of an initial infusion of ibandronate 6 mg followed by daily oral treatment results in a rapid decrease of S-CTX, a sensitive marker of bone resorption. Initial i.v. doses followed by oral maintenance treatment are undergoing further testing.

Table
Mean (CI) bone turnover marker (% change from baseline)

	S-CTX	S-bALP	P1NP	OC
Week 2	−77	1	4	−4
Week 4	−73	−4	−7	−2
Week 8	−71	−13	−27	−15
Week 12	−71	−27	−42	−24

P170-Mo

Oral Ibandronate Decreases Markers of Bone Resorption to the Same Extent as Intravenous (i.v.) Zoledronic Acid in Patients with Bone Metastases from Breast Cancer: Results from a Comparative Phase III Trial

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Background: Bone resorption markers are useful indicators of clinical outcome in metastatic bone disease. There is a correlation between suppression of resorption markers and the rate of reduction in skeletal-related events (SREs). This multicenter, randomized, open-label, parallel group study directly compared oral ibandronate with i.v. zoledronic acid with respect to biochemical markers of bone turnover.

Methods: Advanced breast cancer patients with at least one confirmed osteolytic or mixed bone lesion received oral ibandronate 50 mg/day ($n = 114$) or i.v. zoledronic acid 4 mg ($n = 110$), infused over 15 min every 4 weeks for 12 weeks. Eligibility criteria included = 18 years of age; life expectancy = 6 months, WHO Performance Status of 0, 1 or 2; adequate renal function (serum creatinine = 3.0 mg/dL). The primary endpoint was the mean percentage change in cross-linked C-terminal telopeptide of type I collagen in serum (S-CTX) at the end of the study. Bone-specific alkaline phosphatase (BAP), the amino-terminal procollagen propeptides of type I collagen (P1NP) and osteocalcin (OC) were also measured.

Results: Mean (95%CI) percent changes from baseline were as follows. S-CTX: ibandronate −77% (CI −82% to −73%) vs. zoledronic acid −75% (CI −82% to −67%); BAP: ibandronate −35% (CI −43% to −28%) vs. zoledronic acid −32% (CI −47% to −17%); P1NP: ibandronate −48% (CI −56% to −40%) vs. zoledronic acid −42% (CI −55% to −29%); OC: ibandronate −35%

(CI −40% to −30%) vs. zoledronic acid −34% (CI −45% to −22%).
Conclusion: In this head-to-head trial, oral ibandronate was statistically non-inferior to zoledronic acid for the primary endpoint of S-CTX. It also showed similar effects to that of zoledronic acid on serum BAP, P1NP and OC. This study suggests that a convenient oral ibandronate dose of 50 mg/day is as effective as i.v. zoledronic acid in suppressing tumor-induced bone resorption.

P171-Tu
Evaluation of Tartrate-Resistant Acid Phosphatase Measurements in Patients with Breast Cancer
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Tartrate-resistant acid phosphatase (TRAP) is a potentially useful marker of bone resorption. Serum levels of the active isoform 5b are specifically associated with osteoclast activity. We performed the present study to determine the interest of TRAP 5b measurement (ELISA, Medac) in patients with bone metastases from breast cancer. TRAP 5b was compared to other classical biochemical markers of bone resorption (serum CTX measured by ELISA, Nordic) and bone formation (BAP; IRMA, Hybritech).
113 female patients with breast cancer have been included and distributed to five different groups: 30 patients in complete remission, 23 patients with breast cancer before surgery, 20 patients with bone metastases before bisphosphonate (BP) therapy, 20 patients after at least 3 months of BP therapy and 20 patients with metastases in other sites than bone. Blood samples were collected in fasting conditions and stored at −80°C until analyses. Statistical testing (ANOVA) was performed on logarithmically transformed data.
TRAP serum levels (mean ± SD) were significantly higher in patients with bone metastases before BP therapy (6.5 ± 2.7 U/L) than in patients without metastases, either in complete remission (4.2 ± 1.4 U/L) or before surgery (4.3 ± 1.7 U/L). The ability of TRAP to detect bone involvement did not differ significantly from the one of BAP whereas CTX levels were not significantly different between the three groups. The elevation of markers in patients with bone metastases before BP therapy compared to patients with metastases in other sites did not reach statistical significance for any of the assays. When comparing patients with bone metastases before BP to patients under BP therapy, a decrease of all three markers was evident but the difference was significant only for TRAP ($P = 0.002$) and CTX ($P < 0.001$). In all five groups there was a significant positive correlation between the markers (Spearman's rho: 0.632 for TRAP and CTX, 0.599 for TRAP and BAP, 0.305 for CTX and BAP).
In conclusion, TRAP 5b seems to be more efficient than CTX and equivalent to BAP to detect bone metastases in patients with breast cancer, whereas BAP was less influenced by bisphosphonate therapy than the bone resorption markers.

P172-Su
Efficacy of Ibandronate for the Treatment of Skeletal Events in Patients with Bone Metastases Secondary to Colorectal Carcinoma
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Background: Patients with metastatic colorectal carcinoma (CRC) often develop metastases to bone with a high risk of complications.
Method: A randomized, placebo-controlled trial was conducted to evaluate the efficacy and safety of ibandronate in patients with bone metastases from CRC. The primary efficacy endpoint was proportion of patients with skeletal-related events (defined as pathologic fracture, spinal cord compression, radiation therapy to bone, change in antineoplastic therapy and surgery to bone). Secondary endpoints included time to first skeletal event, skeletal morbidity rate (events/year) and time to progression of bone lesions.
Results: In 30 patients with CRC, treatment with intravenous ibandronate 6 mg over a 15-min infusion significantly reduced the proportion of patients with skeletal events (39% versus 78% with placebo; $P = 0.019$) and prolonged the time to first event by at least 6 months (median > 279 versus 93 days with placebo; $P = 0.009$). Ibandronate also significantly reduced the skeletal morbidity rate (mean 2.36 versus 3.14 with placebo; $P = 0.018$) and prolonged time to progression of bone lesions (214 days versus 81 days with placebo; $P = 0.018$). Ibandronate was well tolerated with very rare grade 3 or 4 toxicity. The incidence of renal adverse events was comparable to placebo and there were no clinically relevant changes of serum creatinine.
Conclusion: Ibandronate provided significant clinical benefit in patients with bone metastases secondary to CRC. This study suggests a role for ibandronate in metastatic bone disease from CRC. Larger confirmatory studies are required.

P173-Mo
Renal Safety of Intravenous Ibandronate in Breast Cancer Patients with Metastatic Bone Disease
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Background: Renal adverse events are a troublesome complication of bisphosphonate therapy. This placebo-controlled study investigated the effect of intravenous ibandronate treatment on renal function.
Methods: Breast cancer patients with metastatic bone disease (n = 28) received 1-h infusions of ibandronate 6 mg every 3–4 weeks for 96 weeks. Serum creatinine and urinary excretion measurements were performed before, during and after treatment. These included total protein, albumin, a1-microglobulin, *N*-acetyl-β-D-glucosaminidase, hematuria.
Results: Ibandronate 6 mg was not associated with renal function impairment. Assessments of proteinuria, hematuria, enzymuria and serum creatinine indicated that there were no statistically significant changes between patients receiving ibandronate 6 mg and placebo. Urine parameters varied during treatment in the same range with approximately similar frequency in the ibandronate and placebo groups.
Conclusion: These results suggest that intravenous administration of ibandronate 6 mg does not impair renal function in breast cancer patients with metastatic bone disease. Because tolerability profiles vary between bisphosphonates, the lack of renal toxicity with intravenous ibandronate makes this formulation an attractive treatment option for metastatic bone disease. Therefore, ibandronate can be safely administered to patients with bone metastases, including those with compromised renal function.

P174-Tu

A Comparative Study of Ibandronate and Pamidronate in Patients with Bone Metastases from Breast or Lung Cancer

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Objective: To estimate the difference in the effectiveness of ibandronate and pamidronate in patients with bone metastases from breast or lung cancer.
Patients and methods: Groups A (ibandronate) and B (pamidronate) included 8 and 7 patients, respectively, with breast cancer and 5 patients each with lung cancer. Ibandronate and pamidronate were administered intravenously at doses of 6 mg and 90 mg, respectively, every 4 weeks for 6 months. All patients had bone metastases confirmed by scans. To evaluate the levels of pain, motility and quality of life, the linear analog scale LASA was used, grading patients from 0 to 10. For pain, 0 was the lowest possible level, for motility and quality of life, 10 was the best grade. The effect of ibandronate and pamidronate on bone resorption indices was estimated on the basis of the calcium/creatinine ratio.
Results: In group A, an impressive response was observed in pain alleviation (grades 0–1). Motility was significantly improved (grades 8–10) as well as the overall quality of life (grades 8–10). Compared with group B, the reduced use of analgesics and the reduction of bone resorption indices, reflected in the decrease of blood calcium levels, were statistically significant. In Group B, pain was graded as 3–4, motility as 6–8 and the overall quality of life as 6–9.
Conclusions: Ibandronate appears to be superior to pamidronate in alleviating pain, improving motility and the overall quality of life and reducing bone resorption indices in patients with bone metastases from breast or lung cancer.

P175-Su

Microcomputed Tomography and Texture Analysis of Radiographs in Rat Bone Metastases

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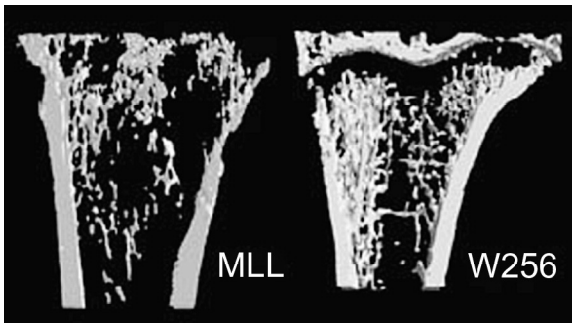
Bone metastases can be predominantly osteoblastic or osteolytic or a combination of both. We used rat models of osteoblastic metastases (MatLyLu MLL) and of osteolytic metastases (Walker W256) obtained by intracardiac injection (IC) of cells. The effect of metastases on bone architecture was investigated by microCT and texture analysis of radiographs. Copenhagen and Fisher rats were IC injected respectively with 3.5×10^4 MLL and 10^7 W256. Copenhagen and Fisher rats were euthanized respectively 15 and 9 days after injection. Femur and tibia X-ray images were analyzed by a texture analysis software with run lengths and fractal algorithms. Microarchitecture was analyzed by microCT to measure BV/TV, Tb.Th, Tb.N, TBPf, Tb.Sp, SMI and DA. MLL induced a decrease of trabecular bone mass on tibia and femur as evidenced by a decrease of BV/TV, Tb.N and an increase in Tb.Sp. DA was decreased and Tb.Pf, SMI, were increased confirming a marked disconnection of trabeculae and an increased conversion of plates into pillars. W256 induced on the femur a decrease of BV/TV, Tb.N, Tb.Th, DA and Tb.Pf, SMI were increased. On the tibia of W256 rats, BV/TV, Tb.N were decreased and Tb.Sp was increased. On radiographs of MLL rats, osteolytic lesions were observed as disseminated dark areas. Texture analysis evidenced bone disorganization with the variation of run length parameters (RLNU, F, GLNU, SRE and LRE) denoting a marked heterogeneity. On radiographs of W256 rats, an almost disappearance of the primary spongiosa was observed; the 2nd spongiosa seemed to be not affected. Run length and fractal analyses were dramatically altered. Both MLL and W256 cells induced osteolysis. MLL induced focalized osteolytic bone lesions in the metaphysis; W256 were associated with marked resorption of the 1′ spongiosa. Osteocondensation did not occurred with MLL due to a short time development of metastases.



P176-Mo

Limited Value of Hyperbaric Oxygen Therapy in the Treatment of Mandibular Osteoradionecrosis; A Case Report

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Oteoradionecrosis (ORN) is defined as bone death secondary to radiotherapy (Marx and Johnson, 1987; Constantino et al., 1995). Its incidence in the mandible varies from 2.6% to 22%; the range is most commonly from 5% to 15% in recent reports (Constantino et al., 1995; Epstein et al., 1997; Thorn et al., 2000). ORN is precipitated by surgery; trauma or it could develop spontaneously.

The diagnosis of osteoradionecrosis is based mainly on patient history and clinical signs such as non-healing (exposed) bone within the treatment area after completion of radiotherapy, and repeated infections.

It is a very unfortunate complication of head and neck radiotherapy that may result in loss of a significant volume of bone, its treatment is difficult and time-consuming and causes much discomfort to the patient (Vissink et al., 2003).

Treatment of ORN includes the use of antibiotics, antiseptics, hyperbaric oxygen and surgery.

Our patient, 28 years old male, developed ORN following surgery for treatment of recurrent cancer (well differentiated squamous cell carcinoma) of the floor of mouth that was previously treated with radiotherapy (6600cGy) a year before. The surgery was done without prophylactic hyperbaric oxygen.

Diagnosis was made depending on the clinical picture (draining sinus and bone sequestration) as well as panorama X-ray which showed resorption of the inferior border of the mandible.

The patient was treated with oral antibiotics and antiseptic mouthwashes, hyperbaric oxygen according to Marx protocol; 30 dives of 90 min of 100% oxygen at 2.4 absolute atmosphere then evaluation of condition, patient underwent an additional 10 dives, followed by surgical debridement, then another 10 dives.

Conclusion: The ORN process was stabilized by these measures but complete resolution was not achieved with the use of hyperbaric oxygen therapy. By reviewing literature it was concluded that patients with overt mandibular osteoradionecrosis did not benefit from hyperbaric oxygenation (William M. Mendenhall, 2004), and reconstructive surgery is still indicated.

P177-Tu

Impaired Proteasomal Capacity as the Target of Proteasome Inhibitors in Multiple Myeloma

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Multiple myeloma (MM) is an aggressive, debilitating and deadly hematological malignancy, arising from the clonal expansion of plasma cells at multiple sites in the bone marrow. Recently, MM proved sensitive to a new class of drugs, proteasome inhibitors (PI), currently in phase 3 clinical trial. PI induce apoptosis selectively in MM cells, but the molecular bases of this therapeutic effect remain undetermined.

We explored the mechanisms underlying sensitivity of MM to PI on plasma cells differentiated in vitro from the murine B lymphoma I.29 μ+ and ex vivo from primary mouse B cells. We found that during differentiation apoptosis correlates with the Ig-synthetic load. As apoptosis increases, the relative amount of proteasomal subunits and the resulting proteolytic activity dramatically decrease, thus denying the demand for a higher degradative capacity when antibody production becomes maximal. The excessive load for the reduced proteolytic capacity causes accumulation of poly-ubiquitinated proteins and stabilization of endogenous proteasomal substrates such as the UPR mediator Xbp1, the NF-kB inhibitor Ik-Balpha and the pro-apoptotic Bcl-2 relatives Bim and Bax, two proteins critical in limiting B lymphocyte lifespan and activity. Accumulation of these proteins critically exaggerates endoplasmic reticulum (ER) stress, thus predisposing plasma cells to apoptosis upon treatment with PI. A similar scenario can be reproduced in HeLa cells, a non-B tumoral line, by driving Ig-μ chain over-expression, which causes proteasomal overload, apoptotic sensitivity to proteasome inhibitors and eventually spontaneous apoptosis, thereby establishing a cause–effect relationship between the synthetic load and cell death.

Our results suggest that a developmental program allows plasma cells to count the integral of produced Ig, linking death to protein production, thus ending the humoral immune response upon accomplishment of its goal. Based on our data, we propose that the high efficacy of PI

against MM is due, to a significant extent, to overloading the cell's degradative capacity, a key component of the stress response, already challenged by misfolded chains generated as a side product of intense Ig synthesis. This model provides a framework for attempting to achieve tumour cell destruction through modulation of stress in MM.

P178-Su

Induction of Parathyroid Hormone-Related Protein Messenger RNA Expression in Human Lung Carcinoma Bone Metastases and Effects of Zoledronic Acid

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PTHrP plays an important role in bone resorption at sites of bone metastases, but its regulation is poorly understood. We hypothesized that PTHrP isoforms would be regulated differentially in bone. Human lung squamous cell carcinoma (HARA) cells (100,000) which express luciferase were injected intracardially (IC) or subcutaneously (SQ) into nude mice (18 for IC, 8 for SQ). To test the effect of zoledronic acid (ZA) on bone metastasis, nude mice with IC injection were divided into 3 groups of 10 mice: (1) no treatment, (2) prevention with ZA (5 μg SQ 2×/week starting 1 week before IC injection) and (3) treatment with ZA (10 μg SQ 2×/week starting when bone metastases were first detected). Bone metastases were imaged weekly using Xenogen IVIS bioluminescent system and confirmed by radiography and histology at termination. In addition, HARA cells were co-cultured in vitro with neonatal mouse calvaria. Real-time RT-PCR was used to measure the three isoforms of PTHrP mRNA in bone metastases, SQ tumors or cultured HARA cells. Human GAPDH or beta2-microglobulin expression was the normalization control. IC injection of HARA cells resulted in bone metastases in the femurs, tibias and humeri 6–8 weeks after inoculation. ZA significantly reduced HARA bone metastasis incidence in both the prevention and treatment groups. Bone metastases occurred in 83% (5/6) (no treatment), 14% (1/7) (prevention) and 38% (3/8) (treatment) of the mice. PTHrP-139, -141 and -173 mRNA were increased 4-, 14- and 7-fold, respectively, in bone metastases compared to SQ tumors ($P < 0.05$). In vitro co-culture of HARA cells with mouse calvaria had increased PTHrP mRNA with maximum effects at 6 h for PTHrP-139 (31-fold) and PTHrP-173 (17-fold) and 24 h for PTHrP-141 (24-fold). The effect was not observed when in cultures with non-viable calvaria or human embryonic kidney 293T cells.

Conclusions: All three isoforms of human PTHrP mRNA were induced in bone metastases in vivo and in HARA cells co-cultured with mouse calvaria. ZA reduced the incidence of bone metastases. PTHrP induction may be an important in the pathogenesis of bone metastasis.

P179-Mo

Tartrate-Resistant Acid Phosphatase (TRACP 5b) as Marker of Bone Metastases in Breast Cancer Patients

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Introduction: Tartrate-resistant acid phosphatase (TRACP 5b) is an osteoclast specific marker of bone metabolism. It has been considered as a potentially useful marker of bone resorption. We investigated whether TRACP 5b can serve as a serum marker for bone metastases in patients with breast cancer (BC). Detection of this marker in tumor patients holds much promise for early diagnostics of bone metastases and monitoring of the efficiency of bisphosphonate therapy.

Materials and methods: 255 women entered our study, 185 patients with breast cancer (49 pre- and 136 postmenopausal) as well as a control group of 70 healthy women (30 pre- and 40 postmenopausal). Serum values of TRAP 5b were evaluated with an ELISA specific for the osteoclast-produced TRACP isoform 5b. Patients were grouped as follows: BC patients without metastases, with bone metastases, with only visceral metastases and healthy control patients.

Results: Compared to the control group and to patients with BC, patients with BC and bone metastases had significantly higher TRACP 5b serum values. In general patients with BC had an increased mean TRACP 5b value. There was only a minor proportion of patients, in which only visceral metastases had been diagnosed and TRACP 5b values were also increased, which is presumably due to undiscovered bone metastases. The mean activity of TRACP 5b in women treated with bisphosphonates was lower than in patients not receiving antiresorptive therapy, although the number of untreated patients was rather small.

In conclusion TRACP 5b proved to be a valuable marker for the follow up BC patients with bone disease.

P180-Tu

Adhesion Regulon Alterations and Osteomimicry are Striking Features of the Molecular Crosstalk Between Prostate Tumor Cells and Bone Cells

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Bone metastasis is the primary cause of death in human prostate cancer accompanied by severe pain. To settle in bone and develop into metastases requires adhesion of tumor cells to inner bone surfaces, accompanied by an intense molecular crosstalk with bone cells. To examine

this critical but poorly characterized process, we have investigated the crosstalk via released factors in an in-vitro metastasis model system (1). Large-scale transcript profiling, quantitative RT-PCR, FACS analysis, cell adhesion assay, Western blotting and immunofluorescence microscopy revealed that osteoblast-released factors target genes of the adhesion regulon of the prostate tumor cells. We observe a particularly pronounced effect on the expression of genes encoding desmosomal proteins and on the adhesion properties of the cells; novel assemblies of putative desmosomal structures and aggregations of tumor cells occur. In addition, the expression of bone cell-related genes such as *alp1*, *bmp2*, *col1a1*, *cbfa1*, *oc*, *op*, *opg* and *rankl* are significantly affected in the tumor cells, i.e., osteoblasts provoke tumor cells to undergo osteomimicry, favoring the bone colonization process. On the other hand, gene expression in osteoblasts is affected by prostate tumor cells resulting in elevated expression of genes encoding proteins such as collagens and collagen-modifiers, matrix controllers and pain mediators, while genes encoding interferon-induced tumor defense proteins are repressed. As we find several links to native metastases-infiltrated bone tissue data (2), the crosstalk studies of prostate tumor cells with bone cells appear to provide novel accessibilities to diagnostic and therapeutic measures.

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P181-Su

In Vitro and In Vivo Assessment of Zoledronate on Rat Chondrosarcoma Model

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Chondrosarcomas are primary malignant cartilage forming bone tumors. As there is no promising chemotherapy and they do not respond to radiation therapy, surgery is the mainstay of curative treatment. Despite a local tumor control in 60–80% of cases, metastatic spreading or local recurrence lead to death in numerous cases. Bisphosphonates, mainly last generation, are promising therapeutic agents in malignant bone tumor control even in primary bone tumors as osteosarcoma. The present study focused on the effects of zoledronate (ZOL) on Swarm rat chondrosarcoma in vivo and on tumor's cells extracted from this tumor in vitro.

Chondrosarcoma tissues were implanted in three series of 12 male Sprague–Dawley rats: series A ($n = 6$), rats were treated by ZOL (100 μg/kg sc) twice a week from day 4 after implantation until death or euthanasia; series B ($n = 6$) and C ($n = 6$), rats were treated from day 4 before intralesional curettage of the tumor until death or euthanasia. In all series, rats injected with PBS ($n = 6$) were used as controls. Tumor growth was evaluated twice a week by measure of the tumor volume, animal's weight at the euthanasia. The effects of ZOL were also assessed in vitro on tumor cell proliferation and apoptosis (caspase-1 and -3 activation, nuclear fragmentation).

ZOL slows down tumor progression in all cases: in series A, mean tumor volume is significantly reduced in treated group at day 25 and day 27 ($P = 0.046$), while the tumor volume progression between day 19 and 32 is significantly higher in control group than in ZOL treated rats ($P = 0.046$). Probability of survival at day 40 is 0.3 for control group compared to 0.667 for treated rats. In series B and C, although ZOL treatment fails to prevent local recurrence (in 70% of treated rats), it occurs later in all cases. Mean tumor volume was smaller for treated rats in both series from day 32 to 54 and volume tumor progression between day 39 and 49 was significantly higher in control than in treated group (15 691 mm3 versus 7396 mm3, $P = 0.025$). In vitro, a 72-h incubation with 1 μM ZOL inhibits tumor cell proliferation by 40% which is totally inhibited in the presence of 10 μM ZOL. ZOL has no effect on nuclear fragmentation, it does not induce any activation of caspase-1, but increases caspase-3 activity at the concentration of 10 μM (48 and 72 h). These data demonstrate that ZOL could be a promising therapeutic agent in the treatment of chondrosarcoma.

P182-Mo

The Induction of Apoptosis in Human Breast Cancer Cells by Bisphosphonates

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Introduction: Bisphosphonates do not cross the cell membranes of other tissues and therefore their effects are limited to bone cells. However is this statement the real truth?

Design: The bisphosphonates pamidronate, alendronate and risedronate were administered to the human breast cancer cells MCF-7, estrogens receptor (ER) positive mammary gland adenocarcinoma cells, T-47D, ER positive gland ductal carcinoma cells and MDA-MB-231, ER negative poorly differentiated mammary adenocarcinoma cells in a concentration of 10-6 M for 144 h. Apoptosis was determined by using the DNA fragmentation assay, while proliferation was measured by quantification of the expression of Cyclin D1 mRNA using the RT-PCR technique. The

ratio's apoptosis/proliferation were calculated and a ratio >1 meant induction of apoptosis.
*$P < 0.05$ versus controls.
Results: The table demonstrates the ratio's apoptosis/proliferation of the three bisphosphonates in the breast cancer cell lines. All bisphosphonates induced apoptosis in the ER positive MCF-7 cells, while all three stimulated proliferation in the ER negative MDA-MB-231 cells. Inconclusive results were found in the ER positive T-47D cells.
Conclusion: The balance between apoptosis and proliferation is crucial in determining the overall cell growth. Neither factor alone can be used to predict cell growth. Bisphosphonates do have extra-skeletal effects and not all bisphosphonates are equal. The estrogen receptor might have a pivotal role in the induction of apoptosis of breast cancer cells by bisphosphonates.

Table
Ratio's apoptosis/proliferation

	MCF-7	T-47D	MDA-MB-231
Pamidronate	1.3*	1.0	0.6*
Alendronate	1.6*	1.3*	0.8*
Risedronate	1.3*	0.9*	0.9*

P183-Tu
Bone Mass Density in Patients with Breast Cancer After Radiotherapy
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Bone mass density in patients with breast cancer after radiotherapy.
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Objectives: Breast cancer is a common disease in Russian Federation. The incidence of breast cancer within all types of cancers is 30 women per 100000 inhabitants. The objective of this study was to investigate the effect of radiotherapy on bone mass density (BMD) in the region of projection of the breast cancer.
Materials and methods: 40 patient (mean age 43 + 8 years) with breast cancer. All patients underwent Tc99m bone scintigraphy for possible detection of bone metastasis. No bone metastasis were reported in all 40 patients. All cases of breast cancer were confirmed by ultrasound and tumor biopsy. Ca, P, ALKph, ER, LH-RH, FSH, ACTH were recorded in all patients in normal ranges. Radiotherapy treatment consisted of 40–45 grey per patient. BMD was measured in L1–L4 by dual-energy X-ray absorptiometry (Lexxos).
Results: In all 40 patients BMD was below the normal range. 16 patients were diagnosed as osteopenic (T −1.75 + 0.63) and 24 were diagnosed as osteoporotic (T −2.62 + 0.31).
Conclusion: The preliminary results of this study suggest that radiotherapy can induce low bone mass density in the region of radiotherapy protection.

P184-Su
Sequential Combination Between Ibandronate and Tamoxifen Exhibit Synergistic Effects on the Inhibition of Breast Cancer Cell Growth
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Breast cancer cells have a remarkable propensity to develop metastases in bone. Bisphosphonates are now standard therapy for the prevention of the complications of bone metastases. Recent in vitro data clearly demonstrate that bisphosphonates can exert antitumoral effects, and that combination of bisphosphonates with other antineoplastic agents, such as paclitaxel or doxorubicin, can result in synergistic interactions in vitro. On the other hand, tamoxifen still stands as the standard first-line endocrine therapy for patients with breast carcinoma expressing estrogen receptor alpha. Hence, we have investigated whether combining bisphosphonate (ibandronate) and antiestrogen (tamoxifen) would exert synergistic effects on the inhibition of tumor cell growth in ER-positive MCF-7 breast cancer cells. We also determined if drug sequencing was of importance, since emerging data indicate that synergy between two drugs can be further enhanced by sequential, rather than simultaneous, treatment. Cancer cells were cultured in steroid-depleted medium supplemented with 10^{-10} M 17beta-estradiol to stay in line with the low but clinically relevant estrogen production in postmenopausal patients. The effects of drugs, alone or together, for 24 h were evaluated on cell growth (crystal violet staining) 5 days after treatment, while the effects of sequential combinations for 24 h with drug 1 followed by another 24 h with drug 2 were determined 4 days after treatment. Dose–response experiments were performed to choose drug concentrations (5×10^{-5} M ibandronate, 10^{-7} M tamoxifen) which barely inhibited cell growth (by less than 10%). Treatment with tamoxifen before ibandronate (11.3 ± 1.4% cell growth inhibition, mean ± SE) did not increase the inhibition of cancer cell growth obtained by addition of the effects of either drug alone. However, simultaneous incubation increased by 1.4-fold the inhibition of cell growth (16.2 ± 2.6%). Moreover, treatment with ibandronate before tamoxifen strongly and significantly ($P < 0.01$) increased the degree of inhibition (25.6 ± 3.8%), suggesting synergy between these two drugs under this sequential combination. Hence, the combination of ibandronate and tamoxifen has potential synergistic effects on the inhibition of cancer cell growth, and the sequence

of the treatment could be of importance to exhibit synergy. This study gives a rationale to fully exploit the antitumoral potential of ibandronate in combination with hormone therapy.

P185-Mo
Use of Bisphosphonate Analogs of Risedronate to Elucidate Antiangiogenic Mechanisms of Bisphosphonates In Vivo

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Beyond their use as inhibitors of osteoclast-mediated bone resorption, many nitrogen-containing bisphosphonate (N-BP) analogs are also potent inhibitors of angiogenesis in vivo. N-BPs inhibit osteoclast activity through inhibition of farnesyl diphosphate synthase (FPPS), an enzyme of the mevalonate pathway. Molecular mechanisms through which N-BPs inhibit angiogenesis are however unknown. To help address this question, we compared the antiangiogenic activity of risedronate (RIS) to BP analogs of varying biological properties. In vivo, RIS reduced the revascularization of the prostate gland of castrated rats treated with testosterone. It also inhibited vessel sprouting in cultured rat aortic rings and in the chicken egg chorioallantoic membrane assay. In contrast, NE-10790, a weak antiresorptive phosphonocarboxylate, which is known to have antitumor effects in vivo, did not inhibit angiogenesis in these different in vivo and ex vivo models, indicating that the P-C-P structure of BPs and/or Rab geranylgeranyl transferase (an enzyme of the mevalonate pathway specifically inhibited by NE-10790) were not involved in the antiangiogenic activity of N-BPs. We studied whether analogs with variation in the nitrogen containing side chain (R2) of RIS could modify its antiangiogenic activity. NE-58051 is a weak antiresorptive pyridyl BP which only differs structurally from RIS in the length of the R2 side chain. NE-58025 (antiresorptive) and NE-58086 (weak antiresorptive) both contain a bicyclic side chain to induce conformational constraints to the R2 chain, but they each orient their R2 sidechain functionality very differently. All of these compounds inhibited vessel sprouting in the rat aortic ring assay. Their relative order of potency in inhibiting angiogenesis in this model was NE-58025 > RIS = NE-58051 > NE-58086. In addition, the potency ranking of these compounds perfectly paralleled that observed on inhibition of human endothelial cell proliferation in vitro. NE-58025 was also as potent as RIS in inhibiting vessel sprouting in the chicken egg chorioallantoic membrane assay. Interestingly, NE-58025, NE-58086 and NE58051 are, respectively, 11-, 385- and 293-fold less potent than RIS at inhibiting FPPS activity despite significant inhibition of angiogenesis. Overall, our findings suggest that (as opposed to osteoclasts) N-BPs act in vivo on endothelial cells through molecular mechanisms that are dependent and independent of FPPS activity.

P186-Tu
Receptor Activator of Nuclear Factor-Kappa B Ligand, Parathyroid Hormone-Related Protein and Cell Proliferation Discriminate Poorly and Well-Differentiated Prostate Carcinoma

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Characterization of factors associated with prostate cancer (PCa) aggressiveness and evolution is important to establish tumor prognosis. We investigated whether some bone cytokines produced by PCa can be used as diagnostic tumor markers. Immunohistochemistry was used to evaluate parathyroid hormone (PTH)-related protein (PTHrP), the PTH1 receptor (PTH1R), osteoprotegerin (OPG) and receptor activator of NF-kappa B ligand (RANKL), as well as cell proliferation (Ki67) and vascularization (CD34) in archival paraffin-embedded prostate samples from patients with well [= 4(2 + 2)] (n = 26) or poorly [= 7(4 + 3)] (n = 25) differentiated PCa according to Gleason grade. Immunostaining was performed with three rabbit polyclonal antisera to PTHrP, C6 and C7 (with C-terminal specificity) and C13 (recognizing the N-terminal region); affinity-purified rabbit polyclonal antibodies to PTH1R, OPG and RANKL; and affinity-purified mouse monoclonal antibodies to Ki67 (clone MIB1) and CD34. Ki67 positivity was expressed as percentage of stained nuclei of total nuclei. Positivity for the other proteins was scored from 0 (negative) to 3 (most dense). We found that staining for PTHrP, the PTH1R, OPG and RANKL was increased in both cytoplasm of acinar epithelial cells and the surrounding stromal tissue of PCa, compared to that in the corresponding areas in adjacent nontumoral tissue. Positivity for these factors, as well as for Ki67 and CD34, was significantly more intense in the poorly differentiated tumors. CD34, PTHrP (with C6), OPG and RANKL staining significantly correlated with Ki67 in CaP. Receiver operating characteristics (ROC) curve analysis was performed to assess the diagnostic value of these factors to differentiate poorly and well-differentiated tumors. The area under the curve ± SE for Ki67 and RANKL had the highest values (0.815 ± 0.73 and 0.877 ± 0.65, respectively). Using the 2nd quartile as cutoff, the best sensitivity and specificity values were, respectively, 0.76 and 0.77

(Ki67); 1 and 0.38, [PTHrP (C6)]; 0.69 and 0.92 (RANKL); 0.88 and 0.96 (Ki67-to-RANKL ratio); and 0.72 and 0.96 [PTHrP (C6)-to-RANKL ratio].
In summary, PTHrP/PTH1R, OPG/RANKL, Ki67 and CD34 are increased in PCa, mainly in the poorly differentiated tumors. Our findings suggest that combined determination of PTHrP, RANKL and Ki67 immunoreactivity might be a useful approach to discriminate poorly and well-differentiated PCa.

P187-Su

Effects of Soybean Phytoestrogens on Cell Viability and Some Bone-Related Cytokines in Human Prostate Cancer Cells

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Epidemiologic studies suggest that soybean phytoestrogens have beneficial effects on prostate carcinoma (PCa), but their mechanisms of action remain controversial. In the present in vitro study, we determined the effects of the phytoestrogens genistein and daidzein, which are abundant in soybean, on cell proliferation/apoptosis, as well as on several bone-related cytokines produced by PCa. We used PC3 and LNCaP human cell lines, two well-characterized cellular models of human PCa, grown in RPMI with 10% fetal bovine serum. Cell viability and apoptosis were analyzed by trypan blue exclusion and flow cytometry. Protein expression of parathyroid hormone (PTH)-related protein (PTHrP), the PTH1 receptor (PTH1R) and osteoprotegerin (OPG) was evaluated by Western analysis. We found that the continuous presence of each phytoestrogen dose dependently (10–50 μM) inhibited cell viability in growing PC3 and LNCaP cells. This effect induced by genistein appeared to be dependent on the exposure time to the agonist, and it showed more potency and efficacy than that of daidzein in both cell lines. Moreover, this response to the former phytoestrogen was greater in LNCaP cells than in PC-3 cells (maximal, 80% and 50% over basal, respectively). These results were confirmed by assessing cell apoptosis – using flow cytometry – induced by each phytoestrogen in these cells lines. We next analyzed the possible effects of these phytoestrogens on the PTHrP/PTH1R system, associated with bone resorption activation, and OPG, an inhibitor of osteoclastogenesis, in both cell lines. Either genistein or daidzein failed to affect basal PTHrP protein expression in PC3 cells; while each phytoestrogen (1–50 μM) stimulated (5-fold over basal) this expression within 4 days in LNCaP cells. In addition, these agents, within a similar dose range, stimulated (5-fold over basal) the PTH1R protein expression at the same time period in these cells. Genistein and daidzein (50 μM) also increased (3-fold over basal) OPG protein expression in both cell lines.
In summary, our findings suggest that the phytoestrogens genistein and daidzein might influence CaP development by affecting tumor cell viability and the expression of some bone-related factors.

P188-Mo

Biochemical Markers of Bone Turnover in Patients with Localized and Metastasized Prostate Cancer

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Introduction: Bone metastases are a common feature of prostate cancer (PCA). In the assessment of bone metastases in PCA both bone formation and resorption markers have reached diagnostic efficacy. The aim of our study was to evaluate the value of several serum bone resorption and formation markers in localized PCA (lPCA), lymph node positive PCA (mPCA) and PCA with osseous metastases (osPCA).
Materials: The prospective study included $n = 35$ men (median age 64 years) with lPCA, $n = 20$ with mPCA (median age 69 years) and $n = 15$ with osPCA (median age 63 years). 30 patients (median age 67 years) with benign urological disorders and without metabolic bone disease served as control group. Blood samples were collected before treatment. In all men serum concentrations of alkaline phosphatase (AP), β-CrossLaps (β-CTX), tartrate-resistant acid phosphatase type 5b (TRACP5b) and osteocalcin (OC) were determined.
Results: The control group showed the lowest bone turnover markers. Compared to the controls the lPCA and mPCA groups revealed significantly higher levels of bone turnover markers for TRACP5b ($P < 0.001$) and AP ($P < 0.05$) but not for OC and β-CTX. Patients with osPCA had the highest concentrations of bone turnover markers. Statistical analysis between the different groups showed significant differences for AP ($P = 0.001$), OC ($P = 0.007$) and TRACP5b ($P < 0.0001$), but not for β-CTX ($P = 0.159$) (Kruskal–Wallis ANOVA). Comparison between lPCA and osPCA group showed also statistically significant differences in AP ($P = 0.001$), OC ($P = 0.002$) and TRACP5b ($P < 0.001$) but not for β-CTX ($P = 0.086$) (Mann–Whitney test). See table.
Conclusion: Our data demonstrate that both bone resorption (TRACP5b, β-CTX) and formation (AP, OC) markers play a crucial role in skeletal metastases from prostate cancer. As patients with localized prostate cancer and only nodal metastases already showed significantly elevated serum bone turnover markers in comparison to controls the adjuvant application of bisphosphonates might be helpful to prevent bone manifestations. TRACP5b appears to be the most promising serum marker for monitoring bone metabolism in the follow up of high risk patients after radical prostatectomy.

Table

Median ± SD	AP (U/l)	OC (ng/ml)	TRACP5b (U/l)	β-CTX (ng/ml)
Controls	59 ± 18.4	19 ± 8.5	1.9 ± 0.75	0.25 ± 0.28
lPCA	71 ± 15.5	24 ± 8	2.9 ± 0.91	0.38 ± 0.17
mPCA	69 ± 22.6	20 ± 9.4	3.2 ± 0.84	0.43 ± 0.32
osPCA	396 ± 474	46 ± 21.9	8.8 ± 3.26	0.67 ± 0.5

P189-Tu
Tissue-Type Specific Anti-Cancer Effects and Vitamin K-Dependent Gamma-Glutamyl Carboxylase Activity of Vitamin K Compounds

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The main function of vitamin K is to act as a co-factor for gamma-glutamyl carboxylase (GGCX). However, the anti-cancer effect of vitamin K [phylloquinone: vitamin K1, menaquinones (MKs): vitamin K2 and menadione: vitamin K3] on several cancer cells has been reported. Although the growth inhibitory and cytotoxic effects of vitamin K are well demonstrated both in vivo and in vitro, the mechanisms underlying these actions have not been fully elucidated. Vitamin K compounds share a common chemical structure consisting of a naphthoquinone nucleus capable of redox cycling. Vitamin K1 has a long phytol side-chain, whereas vitamin K2 has an unsaturated side-chain composed 1–13 isoprene unit. Vitamin K3 lacks any side-chain and only has a methyl group at 3' position of naphthoquinone. In this study, we have examined the biological effects of vitamins K1, K2 and K3 in several cancer cells in vitro and GGCX activity of vitamins K1, K2 and K3. We used the cancer cells derived from several tissues, i.e., leukemia, hepatocellular carcinoma and prostate cancer. Vitamins K1, K2 (MK-1, -2, -3, -4 and -7) and K3 were tested with HL-60, HepG2, HuH-7, LNCaP, PC-3 and DU145. Vitamin K1, MK-1, -2, -3, -4 and Vitamin K3 were found to induce growth arrest and apoptosis in all the cancer cell lines tested. Vitamin K3 exhibited strong anti-proliferative and apoptosis-inducing activities. In contrast, vitamin K1, MK-1, -2, -3 and -4 exhibited cell-specific and side-chain specific anti-cancer activity. In particular, anti-proliferative activity and cell specificity of vitamin K2 compounds were remarkably influenced by the difference of side-chain length. We also examined the co-factor activity of vitamins K1, K2 and K3 for the GGCX. Vitamin K3 was virtually inactive. Co-factor activity of vitamin K2 compounds varied with the length of the aliphatic side-chain. The concentration required for half-maximal reaction velocity (K1/2) was measured for the vitamin K2 compounds and K1/2 values were decreased with increasing the length of the side-chain. Co-factor activity of K1 and MK-7 for GGCX were almost equipotent but weaker than that of MK-4. The data suggest that inhibitory activity of vitamins K1, K2 and K3 on cancer cell growth does not correlate with their co-factor activity for GGCX. In conclusion, these results indicate that molecular targets of vitamins K1, K2 and K3 in anti-cancer activity may be tissue-specific and structure-specific for the vitamin K molecule.

P190-Su
Osteoprotegerin Treatment Inhibits the Growth of Lytic Prostate Cancer Lesions in Bone

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Prostate cancer (CaP) bone metastases, while typically osteoblastic, have a strong resorptive component. Therefore, we investigated whether osteoprotegerin (OPG) treatment inhibits tumour growth in a lytic CaP bone metastasis model. Male NOD-SCID mice aged 6–8 weeks were injected with 4×10^5 PC-3-EGFP cells into the left tibia. Serum samples and radiographs were taken at the start, middle and end of each study. Sera were analysed for mouseTRAP 5b levels. Harvested tibias were analysed by immunohistochemistry to detect human cytokeratins. Controls were age-matched non-injected and sham-injected mice. In study 1, 10 mice/group were randomised for ×3/week treatment with vehicle or 1 mg/kg OPG starting at week 0 (prophylaxis) or week 2 (delayed treatment). In study 2, vehicle or 3 mg/kg OPG treatment ×3/week began at weeks −1 (early), 0 (late) or 2 (delayed) (n = 6–7 mice/group): endpoint sera in study 2 were also analysed for osteocalcin levels. Osteolytic tumours were radiographically apparent in all vehicle-treated mice at week 6. In study 1, OPG-treated mice had smaller osteolytic tumours and decreased serum mouseTRAP 5b levels in the prophylaxis group vs. vehicle-treated mice. In the delayed treatment arm, OPG treatment partially inhibited tumour growth and reduced serum mouseTRAP 5b levels when compared with vehicle control at week 6. In study 2, early OPG mice had little evidence of tumour and had extended calcified growth plates; late OPG mice displayed little tumour growth; and delayed OPG mice had small discrete tumours that did not breach the cortex. When compared with their respective vehicle controls, bone resorption was inhibited in early OPG mice (week −1, $P < 0.0001$; week 2, $P = 0.0137$; week 6, $P < 0.0001$), late OPG mice (week 2, $P < 0.0001$; week 6, $P = 0.0002$) and delayed OPG mice (week 2, $P = 0.0210$; week 6, $P < 0.0001$). Serum osteocalcin was significantly decreased in late and delayed OPG mice ($P < 0.0001$ and $P = 0.0017$, respectively) when compared with respective vehicle controls. All tumours were positive for anti-human pan-cytokeratin antibody. In conclusion, OPG treatment reduced bone resorption from the time treatment was initiated. Study 1 results suggested that low resorption levels were sufficient for PC-3-EGFP cells to establish and grow in bone, and that a higher OPG dose was required to inhibit PC-3-EGFP tumour growth in bone. Although low levels of bone resorption were

still detected in study 2, 3 mg/kg OPG effectively inhibited prostate cancer induced osteolysis and tumour growth.

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Intravenous Zolendronic Acid Effectively Decreases Serum Calcium in Primary Hyperparathyroidism

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In this report we describe an effect of the most potent bisphosphonate, zolendronic acid, on serum calcium level in 6 patients: 5 women and 1 man, with symptomatic hypercalcemia due to primary hyperparathyroidism. Patients received a single intravenous infusion of 4 mg of zolendronic acid. Serum calcium levels were measured daily for the first week after infusion, and next twice a week. Serum PTH-intact levels were measured before and weekly after an infusion.
In all cases we observed a rapid decrease in serum calcium level, sustained up to 4 weeks of observation (Fig. 1). Serum PTH levels remained stable, we did not observed increase in PTH levels concomitant to serum calcium decrease. In conclusion, intravenous zolendronic acid produce rapid and sustained decrease in serum calcium level in patients with primary hyperparathyroidism.

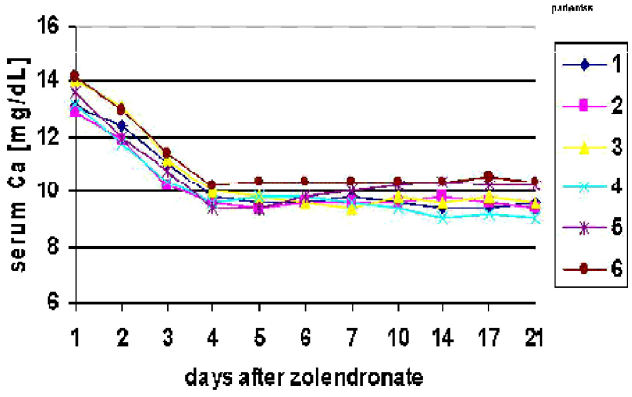

Fig. 1. Serum calcium levels after intravenous zoledronic acid treatment in 6 patients with primary hyperparathyroidism.

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Do Serum Measurements of RANKL and OPG Reflect the Increased Bone Metabolism in Primary Hyperparathyroidism?

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We have previously shown that the RANKL/OPG mRNA ratio inside bone is increased in primary hyperparathyroidism. Therefore, we have proceeded to examine if the levels of these two cytokines in serum also reflected the amount of bone resorption induced by elevated PTH.
Methods: The study comprised 24 patients (females/males = 21/3), aged 52 to 75 years (median 60) with PHPT confirmed by elevation of plasma PTH (range 7.7–44 pmol/l; median 16) and elevated serum ionized calcium (range 1.33–1.82 mmol/l; median 1.49). Patients were investigated before and one year after successful PTX. Serum RANKL, serum OPG, bone markers, BMD and a Bordier bone biopsy was done before surgery ($n = 24$) and 12 months after PTX ($n = 21$) for RT-PCR. Wherever possible ($n = 18$ before PTX, 12 after) a bone biopsy was also obtained for histomorphometry.
Results: Neither serum RANKL, OPG nor the serum RANKL/OPG ratio changed after surgical cure (Wilcoxon: $P = 0.82$, 0.79 and 0.31, respectively).
In the pre-operative, hyperparathyroid state, serum RANKL exhibited a positive correlation with erosion depth (table), but not with any other resorptive or any of the formative parameters. Histomorphometric parameters showed no association with serum OPG. There was no correlation between serum RANKL or OPG and PTH, ionized calcium, bone markers or BMD at any site.
In the cured state, A positive correlation existed between serum OPG and P1NP (table). No other correlations were seen between serum levels of the cytokines and PTH, ionized calcium, BMD, bone markers or histomorphometric parameters.
Conclusions: Our study shows that serum levels of RANKL and OPG are poor predictors of bone metabolism in primary hyperparathyroidism. For RANKL, we observed a moderate correlation with erosion depth but no association with bone turnover markers. As opposed to the skeletal mRNA findings, serum levels of RANKL and OPG are unlikely to mediate the bone resorptive effects of PTH in PHPT as levels did not follow the severity of disease or change upon successful surgical cure.

Table

Rho (p)	Before PTX RANKL	Before PTX OPG	After PTX RANKL	After PTX OPG
PTH	−0.25 (0.23)	0.04 (0.86)	−0.26 (0.25)	−0.22 (0.35)
Ac.f	−0.03 (0.92)	0.30 (0.30)	−0.37 (0.29)	−0.23 (0.53)
Erosion depth	0.52 (0.03)	0.11 (0.70)	0.12 (0.72)	−0.08 (0.82)
Osteocalcin	−0.06 (0.80)	−0.23 (0.33)	0.04 (0.87)	0.39 (0.09)
P1NP	0.15 (0.47)	−0.18 (0.44)	−0.02 (0.94)	0.58 (0.01)
Cross-links	0.14 (0.50)	0.15 (0.82)	0.23 (0.33)	0.26 (0.27)

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Sisters with Familial Isolated Hyperparathyroidism

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Familial hyperparathyroidism is classified into several disease entities such as multiple endocrine neoplasia (MEN) type I and type IIA, hyperparathyroidism-jaw tumor syndrome (HPT-JT) and familial isolated hyperparathyroidism (FIHP). Majority of HPT-JT and parathyroid carcinoma are related to the inactivation mutations of HRPT2. However, it is still not clear whether FIHP is an independent disease entity defined by mutations in other causative genes or mixture of other diseases with incomplete manifestations. HRPT2 may, therefore, also be involved in some kindreds of FIHP, but its relationship to FIHP is still not established. Here, we report two siblings affected by hyperparathyroidism without any manifestations of MEN or HPT-JT. The proband, 37 years old female, was referred to our hospital because of hypercalcemia and hypercalciuria with elevated serum parathyroid hormone (PTH). Serum calcium (Ca) was around 16 mg/dl, and the PTH was beyond upper limit of the assay. The patient developed marked dehydration and loss of consciousness, suggesting hypercalcemic crisis, and was treated as such. The localization study revealed her right lower parathyroid was markedly enlarged, but not cystic. She did not show any evidence of jaw tumor and kidney complications or abnormality in other endocrine organs. Her right lower parathyroid was excised but was found to be invasive to surrounding tissues such as trachea and thyroid. The pathological study revealed parathyroid carcinoma, and later on, the tumor recurred, and serum Ca and PTH level were increased again, confirming the malignant character of the tumor. The second patient, the younger sister of the proband, developed osteoporosis at age of 32, and, two years later, also referred to our hospital because of hypercalcemia, 12.9 mg/dl, and elevated whole PTH level, 672 pg/ml. Her right lower parathyroid gland was enlarged, which is supposed to be operated in the very near future. She also did not develop any jaw or kidney lesions or other endocrine neoplasmas, and her parathyroid looks to be lobulated but not cystic. From these clinical characteristics, we diagnosed their disease as FIHP. The analysis of germline HPRT2 gene of these patients is now under way, which is expected to provide some insights into the genetics of this kindred with FIHP.

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Genetic, Cellular and Clinical Analysis of Forty-Two Patients Affected by Osteopetrosis

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Osteopetrosis is a genetic bone disease characterized by osteoclast failure. Three types of human forms have been described: infantile malignant autosomal recessive osteopetrosis (ARO), associated with mutations of ATP6i (~50%), ClCn7 (~10%) and Gl genes (2 patients so far); intermediate autosomal recessive osteopetrosis (IRO), associated with CAII and ClCn7 gene mutations; autosomal dominant osteopetrosis (ADO) types I and II, caused by LRP5 and ClCn7 gene mutations, respectively. Patients with unknown gene mutations are frequent, suggesting additional forms yet to be classified. Among 42 patients admitted to this study, 15 were diagnosed with ARO, 1 with IRO and 26 with ADOII. Herein, we provide insights into their genetic, clinical and cellular features. Five out of 9 patients with ARO had ATP6i gene mutations previously described, 1 harbored a novel mutation (deletion exon 12, RNA position 1497), and 3 were negative. Most patients presented with increased osteoclast number, except one who did not show any osteoclast in bone biopsy. According to skin alteration and disorders in cell adhesion noted in our IRO patient, we investigated MITF, alphaV and ATP6i genes, but failed to find any mutation. Fourteen out of 20 ADOII patients bore a heterozygous mutation of the ClCn7 gene and 6 were negative, suggesting other genes associated also with this form. In 7 ADOII patients examined clinically, we found alterations of bone alkaline phosphatase isoenzyme (BALP) and osteocalcin (OSCA). BALP was enhanced in 3 patients but reduced in 1; OSCA increased in 4, but decreased in 2 patients affected by the most severe form. Increased BALP was also observed in ARO patients who, consistently, had elevated osteoblast number and activity in bone biopsies. Osteoclasts from patients were characterized in vitro and generally showed no significant changes in formation rate, morphology and intracellular acidification. In the IRO patient we observed alteration of adhesion to substrate and podosome formation, and in one ADOII patient high motility. As expected, the ADOII and ARO osteoclasts degraded the bone matrix significantly less than controls. Interestingly, pit formation was enhanced by extracellular acidification, by >2-fold in controls, ~2-fold in ADOII and ~3-fold in ARO, indicating osteoclast responsiveness to exogenous stimuli. This suggests that impaired osteoclasts are reactive to stimulating conditions which is promising for assessment of future therapies.

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Risedronate Pharmacokinetics in Children with Osteogenesis Imperfecta

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A single oral dose, randomized, parallel group study was conducted in 28 children with osteogenesis imperfecta (OI) to assess risedronate pharmacokinetic/safety. Patients weighing 10–30 kg were randomized to receive 2.5 mg or 5 mg of risedronate (n = 16) and patients weighing more than 30 kg

were randomized to receive 5 mg or 10 mg risedronate (n = 12). Blood and urine samples were collected for 24 h and 28 days following drug administration, respectively, and were analyzed for risedronate using an ELISA assay.

All 28 patients enrolled completed the study. Overall, risedronate was shown to be well tolerated at the doses studied. Adverse events were reported by 13 of the 28 enrolled patients. Most commonly reported adverse events were nausea and diarrhea. Pharmacokinetic results obtained from this study and data previously obtained from adults are summarized in Table 1.

The results obtained in this study indicate that the variability associated with risedronate is high and similar to that previously observed in adults. Dose-dependent pharmacokinetic parameters generally increased with dose in children. In addition, no age or gender related differences were observed. C_{max}, AUC and t1/2,z observed in children are within the ranges previously observed for adults.

Overall, these results indicate that risedronate was shown to be well tolerated at the doses studied, and that the range of risedronate systemic exposure (AUC and C_{max}) and t1/2,z observed in children with osteogenesis imperfecta was similar to that previously observed in adults.

Table

PK parameter	Children OI*(n = 28)	Adults Normal and PMO*(5 mg; n = 71)
C_{max} (ng/mL)	0.25–3.68	0.32–4.13
AUC (ng × h/mL)	3.49–19.18	1.11–20.44
t 1/2,z (h)	78–745	36–955

* Data as ranges. PMO = postmenopausal osteoporosis.

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A LRP5 Missense Mutation in an Extended Family Results in Autosomal Dominant Osteosclerosis in Association with Craniosynostosis and Mild Developmental Delay

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During the last few years, large efforts have been made to find genes involved in disorders with abnormal bone density. Two efforts resulted in the identification of the low-density lipoprotein receptor-related protein 5 (LRP5) gene as an important regulator of bone mass: loss-of-function mutations in LRP5 have been found in osteoporosis pseudoglioma syndrome, while the opposite gain-of-function is associated with high bone density disorders. The latter conditions share features of an increased thickness of the skull and of the cortices of the long bones. Additionally, variable expression of additional clinical symptoms is observed, including torus palatinus, jaw enlargement and neurological complications caused by overgrowth of the facial bones and calvarium.

We report here on an extended four-generation family with thirteen affected individuals in which an autosomal dominant type of osteosclerosis segregates. The osteosclerosis phenotype is most pronounced in the cranial base and calvarium. Interestingly, craniosynostosis at early age was reported in four affected family members and mild developmental delay was observed in three patients. Three important genes known to be involved in the pathogenesis of craniosynostosis, TWIST and FGFR2/3, were excluded as disease-causing, however, a molecular analysis of the LRP5 gene revealed the presence of the previously described A214T heterozygous missense mutation.

In conclusion, this study provides evidence that craniosynostosis and mild developmental delay can be added to the list of secondary clinical complications in LRP5-associated high bone density disorders, but clearly with a reduced penetrance.

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Chaperone-Procollagen Interactions Differ with Mutation Location in Osteogenesis Imperfecta

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Osteogenesis imperfecta (OI), an autosomal dominant disorder characterized by fragile bones, is caused by mutations in type I procollagen. The heterotrimeric procollagen molecule consists of a central triple helical domain flanked by C and N-terminal propeptides. The secretion of procollagen with mutations in the helical or propeptide regions has been shown to result in extracellular matrix deficiencies in OI patients. As both fibroblasts and osteoblasts express and secrete mutant collagen, the bone-specific pathophysiology of OI has not yet been delineated. However, in OI cases with mutations in the helical region of collagen, osteoblasts secrete a greater proportion of the mutant collagen forms than fibroblasts. As interactions with ER chaperones can direct the fate of proteins between secretion and intracellular degradation, we hypothesized that differential interactions of the mutant procollagens with these chaperones in osteoblasts and fibroblasts may be responsible for the 'permissiveness' of osteoblasts to mutant collagen survival.

The first step toward testing this hypothesis was to understand the interactions of mutant collagen with ER chaperones in fibroblasts. Using confocal microscopy and antibodies specific to the chaperones, BiP/Grp78, protein disulfide isomerase (PDI), calnexin as well as type I collagen, we compared the intracellular localization of mutant procollagens and chaperones in control cells versus OI fibroblasts expressing collagen with mutations in the C-propeptide or helical region. Normal procollagen and procollagen with a

helical mutation displayed a distinct reticular pattern of immunofluorescence in the ER that significantly overlapped with calnexin, but not with Hsp-47, PDI and BiP. In contrast, procollagens with C-propeptide mutations displayed a diffuse pattern of ER localization that significantly co-localized with Hsp-47, PDI and BiP, but not with calnexin. Preliminary data also indicate that the chaperone interactions described above are maintained in normal and OI osteoblasts. Our results demonstrate a clear correlation between the presence and type of mutation and the subcellular localization pattern of procollagen. Although preliminary data indicate no significant differences in chaperone interactions between osteoblasts and fibroblasts, the location of the mutation along the procollagen chain seems to determine the nature of ER chaperone interactions in both cell types.

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Disruption of Intramembranous and Endochondral Bone Development in TR Alpha 2 Null Mice

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T3 is essential for skeletal development and its actions are mediated by two nuclear receptors (TRs), with TR alpha (TRa) being functionally predominant in bone. The TRa1 isoform binds T3 with high affinity and activates target gene expression in response to hormone. TRa2, however, does not bind T3 or regulate transcription in response to hormone. Intriguingly, TRa2 is expressed at high levels from early in development in all tissues and is conserved in all mammals, although its physiological role is unknown. To investigate the function of TRa2, we characterized the skeleton in TRa2-null mice (TRa2−/−). A gross delay in frontal, parietal and interparietal bone formation was evident from embryonic day 17.5. This was associated with severely delayed closure of the cranial sutures leading to brachycephaly (cephalic index (skull width/length) 83% vs. 79% vs. 73%; TRa2−/− vs. TRa2+/− vs. wild-type) at 2 weeks. Dysplasia of the clavicles was also observed as early as E17.5, but was maximal at birth with an increased clavicular angle of 13 degrees in TRa2−/− compared with TRa2+/− and wild-type littermates ($P = 0.02$). These findings are typical features of the human cleidocranial dysplasias and indicate a defect in intramembranous ossification. TRa2−/− mice also exhibited features of abnormal endochondral bone formation. Metacarpophalangeal and metatarsophalangeal ossification was delayed as early as E17.5. Growth retardation was evident from birth and persisted until 4 weeks of age with the maximal difference seen at 3 weeks (TRa2−/−: −11%, $P = 0.001$; TRa2+/−: −3%, $P = 0.055$ versus wild-type). Histological analysis of tibial growth plate architecture and regional organization revealed a delay in hypertrophic chondrocyte differentiation of approximately 2 weeks. Such abnormalities of endochondral ossification are also well-recognized features of the cleidocranial dysplasias. These studies indicate that the correct ratio of TRa1 to TRa2 expression is essential for normal skeletal development.

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The ROSI Study (Risedronate in Adults with Osteogenesis Imperfecta Type 1)

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Osteogenesis imperfecta (OI) is an inherited disease of bone fragility, usually due to mutation in type 1 collagen. High dose IV bisphosphonates are widely used in children with severe OI. OI type 1 (_mild_ OI) results from decreased synthesis of type 1 collagen; patients also have increased bone turnover. Patients with OI type 1 are often prescribed oral bisphosphonates although to date there is no data regarding the effects in this patient group or the required dose. Patients with OI type 1 were recruited from the metabolic bone and skeletal dysplasia clinics of the Nuffield Orthopaedic Centre, Oxford, UK; from local bone physicians; and from self referral. Entry criteria included age over 18 years, a clinical history of OI type 1, no other conditions contributing to low BMD (assessed by history and biochemical tests), and active contraception in fertile women. Patients were prescribed risedronate (either 5 mg daily or 35 mg weekly) for 24 months. BMD was assessed at lumbar spine (LS) and total hip using Hologic Discovery DXA scanning at time 0 and 24 months. Bone turnover markers (serum P1NP and bone-specific ALP) were assessed at time 0, 6, 12 and 24 months. BMD results were analysed using the paired t test; bone marker results by repeat measurements ANOVA. To date, 18 patients have completed the study (8 men, 10 women, mean age 39 years with range 18 to 76 at entry). At baseline, mean BMD at LS was 0.820 g/cm^2 (t score = −2.23, z score = 2.02). At 24 months, BMD significantly improved to 0.850 g/cm^2, an increase of 3.7% ($P = 0.008$) (mean t score = 1.94 with mean z score = 1.93). At total hip, mean BMD at baseline was 0.873 g/cm^2 (t score = 0.77, z score = 0.56) with no significant change seen at 24 months ($P = 0.81$). Bone

turnover markers showed a significant drop in P1NP (P1NP = 33.6 ng/mL at baseline; P1NP = 18.0 ng/mL at 24 months; $P = 0.008$; a fall of 47% of baseline value) which was evident by 6 months (P1NP = 23.2 ng/mL; $P = 0.002$ compared with baseline). There was no significant change in BAP ($P = 0.1$). This study demonstrates that patients with OI type 1 can respond to oral bisphosphonates at standard doses for idiopathic osteoporosis with significant gain in BMD and decrease in bone turnover, surrogate measures for improved bone strength in these fragile bones. This study was supported by an unrestricted educational grant from Procter and Gamble Ltd.

P200-Mo

Failure of a Successful Bone Marrow Transplant to Rescue Osteoclast Deficient Autosomal Recessive Osteopetrosis

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Infantile onset autosomal recessive osteopetrosis (ARO) is a rare bone disorder characterised in most cases by a failure of mature osteoclasts to resorb bone, resulting in dense fragile bones. Genes known to cause ARO include *CA2* (carbonic anhydrase 2), *TCIRG1* (a component of the osteoclast V-ATPase), *CLCN7* (a chloride channel) and *OSTM1* (a human ortholog of the murine "grey lethal" gene). Occasionally, cases of ARO result from a failure of osteoclast formation, with an absence of mature osteoclasts in bone. To date, no causative gene mutations have been identified for osteoclast-deficient ARO in humans, despite numerous candidate genes from spontaneous and experimental animal models. While no specific treatments are available for patients with ARO, bone marrow transplant (BMT) offers a possibility of rescuing osteoclastic resorption. Although success rates of >70% have been achieved with well-matched donor marrow, HLA-mismatched transplants have a poor survival rate. Here we report a case of osteoclast-deficient ARO, in whom a successful BMT has failed to rescue osteoclast formation. The child was diagnosed at age 6 months during investigation for poor growth. At age 8 months, the child received a BMT from a related donor, with successful engraftment resulting in 100% of circulating blood cells deriving from the donor. 18 months later, there was no evidence of skeletal improvement on X-rays. An iliac crest biopsy showed a complete absence of osteoclasts in bone, by conventional morphology or by immunohistochemistry for TRAP or CD45. At 20 months post-transplant, peripheral blood mononuclear cells were cultured in vitro over 3 weeks with recombinant MCSF and RANK ligand. Large numbers of multinucleated osteoclasts formed which produced resorption trails and lacunae on dentine slices. This case resembles one reported recently by Nicholls BM et al. (J Bone Miner Res 2004 19: 1034) where a successful engraftment of cord blood from an unrelated donor failed to rescue osteoclast-poor osteopetrosis. The failure to generate osteoclasts in vivo from the successful graft may result from a failure of osteoclast progenitor cells to enter a poor bone environment, or may be due to the presence of a suppressor factor or the absence of a stimulatory factor in the bone interfering with osteoclast development.

P201-Tu

Alendronate 70 mg on Patients Affected with Thalassemia Major: An Observational Study

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Aim of this study was to verify the efficacy of weekly dosage of alendronate 70 mg (AL 70), in thalassemia major (TM) patients.

Several studies show that young TM patients undergo an early and fast loss of bone mineral density (BMD) because the iron overload and the chelant therapy.

We studied 45 patients, attending in our Unit for clinical evaluation and bone densitometry by DEXA (LUNAR DPX-PLUS). After 24 months these patients were recalled for a follow up. 22 of them (group A) were on AL 70 while 23 refused the suggested treatment and were considered as control group (group 2).

Group A showed an increase of BMD after two years of treatment although this difference was not significant. At the opposite, group B had a decrease of BMD.

This study confirms previous data: young TM patients undergo an early and drastic bone loss, if are not treated with an antireabsorbitive drug. A calcium-Vit D supplementation should be always considered. These data are important because the rising of the middle age of these patients.

Table

Clinical features	Group A	Group B
% Variation		
BMD L2–L4	+1.8%	−7.9%
Z Score L2–L4	+5.5%	−20%
BMD Fem Neck	−1.27%	−9.8%
Femoral Neck BMD	+1.86%	−21.9%

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Bone Pathologies in Free-Flying Captive Bats

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A colony of the Rodriguez Flying Fox, Pteropus Rodricencis, are maintained at Chester Zoo, UK, as part of their commitment to preserving and breeding species which are endangered in the wild. In 2001 the colony experienced decreased reproduction rates (from 69% in 2000 to 21% in 2001), increased mortality rates (adults increased from 8% in 2000 to 17% in 2001), weakness, weight loss and fractured long bones. Most of the deaths were female bats and no juveniles survived that were born in 2001. Metabolic bone disease is one of the most common nutritional diseases seen in captive mammals. It is caused by dietary and/or husbandry mismanagement and leads to metabolic defects affecting the morphology and functioning of bone. As fractures were evident, this study examined the histology of these animals' bones. Fourteen frozen cadavers were obtained and the femoral and humeral bones were removed for histology. Bones were fixed in neutral-buffered formalin and processed undecalcified in LR White resin. 10 μm sections were stained with Toluidine Blue. Samples from eight females and five males were examined for evidence of bone pathology. Generally speaking, all females demonstrated a normal cortex, fibrous marrow and an absence of osteoblastic activity. With one exception, there was no osteoclastic activity on the bone surfaces. Three specimens exhibited osteomalacia and two, osteoclasis. The males showed a trabecularized cortex and 3/5 had no osteoblastic activity. Although no osteopaenia was seen, the males had worse osteomalacia (2/5) than the females. One male specimen, obtained more recently, after a major change in the animals' diet, had a normal bone histology. It is of interest that the majority of deaths were adult females or juveniles and that many specimens (7/14) showed evidence of old, healed fractures. The variation in pathology may be age-related, as the majority of specimens were juveniles. This suggests that the high demands on the skeleton which occur during growth or pregnancy overwhelmed the ability of the skeleton to adapt. These data suggest that the increased morbidity and mortality seen in these animals was related to severe bone abnormalities and efforts are now being made to correlate these with diet.

P203-Mo

Hypercalcaemia: A Hospital-Based Survey

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Hypercalcaemia is a common metabolic disorder. The number of subjects diagnosed with hypercalcaemia increased with advanced laboratory automation.

Aim: To determine the prevalence and causes of hypercalcaemia and to assess the pattern of bone profile among hypercalcaemic subjects in a teaching hospital.

Results: Between April 2003–April 2004, 118286 calcium requests were received from 39360 patients. On average 3 requests/patient/year. Out of 118286 requests, proportion with hypercalcaemia (adjusted calcium > 2.60 mmol/L) was 10% (11702). Of this 11702, 34% (3973) had CRF, 12.5% (1464) RTX, 6.8% (800) no diagnosis, 5.5% (650) osteoporosis, 2.8% (329) malignancy and 1.5% (178) 1o HPT. The proportions of requests with Ca >3.5 and between 3.0 and 3.5 mmol/L were 0.5% (69) and 4% (479), respectively, 90% were due to RF and RTX.

A significantly higher mean Cl, Cl/PO4 ratio and lower anion-gap were seen in subjects with 1o HPT and RTX compared to the other groups. Negative correlations between Cl and PO4 ($r = 0.34$, $P < 0.0001$), Cl and anion-gap ($r = 0.50$, $P < 0.0001$), and positive correlation between PO4 and anion gap ($r = 0.51$, $P < 0.0001$) were detected.

PTH was significantly higher in CRF and RTX compared to the other groups (30 ± 38 and 20 ± 16 vs. 10 ± 6 pmol/L, $P < 0.0001$). CTX highest in Paget's disease (0.73 ± 0.95 vs. 0.37 ± 0.32 ng/ml, $P < 0.0001$) followed by 1o HPT (0.48 ± 0.33 vs. 0.32 ± 0.34 ng/ml, $P < 0.0001$) compared to other groups.

Conclusion: In this teaching hospital the commonest causes of hypercalcaemia were iatrogenic followed by malignancy and 1o HPT. Anion-gap and chloride reflect the physiological role of PTH and highlight their potential role in the interpretation as well as the prediction of the underlying cause of hypercalcaemia.

P204-Tu

Analysis of Bone Turnover in Anorexia Nervosa Patients

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Anorexia nervosa is associated with a decrease in bone mineral density, bone formation and an increased risk of spontaneous fractures. There are a variety of biomarkers for bone formation and resorption including amino terminal procollagen type 1N propeptide (P1NP) and urinary deoxypyridinium crosslinks (DPD), respectively. We measured bone biomarkers under standardized conditions following an overnight fast. Indices of bone turnover (BTI) and bone remodelling balance (BRBI) were calculated based on the sum or difference between a bone formation marker and a resorption marker expressed as a t value in our anoretic patients pre- and following a nutritional rehabilitation program. Nine female patients were admitted to the Hospital Psychiatric Unit, mean age = 24.30 ± 6.8 years (± SD), body mass index (BMI) = 12.92 ± 1.66 kg/m^2. On admission, P1NP = 33.26 ± 25.35 (27.2–71 μg/l), DPD = 12.63 ± 3.42 (4.1–8.1 nmol/mmol/creat). BTI (PINP + DPD) = +3.67 ± 3.09 while BRBI (PINP-DPD) = 5.80 ± 2.90 indicating high bone turnover with minimal bone formation and high bone resorption. On completion of their nutritional program, BMI

had increased to 16.4 ± 1.48 kg/m^2 (P = 0.012). Bone biomarkers demonstrated a significant increase in bone formation (PINP = 114.60 ± 68.0, $P < 0.001$) with no change in bone resorption (DPD = 11.99 ± 2.73, $P = 0.46$). There was a significant increase in BTI (8.88 ± 5.71, $P = 0.004$) and the negative BRBI on admission was corrected with bone formation and resorption now approximately equal (+0.82 ± 4.71, P = 0.006). In conclusion, anorexia nervosa is associated with a negative bone remodelling balance due to inappropriately high bone resorption. Nutritional rehabilitation significantly increases bone formation and corrects the negative bone remodelling balance however this did not translate to an increase in bone density during the study period in which although improved the BMI was subnormal.

P205-Su

Timing of Resumption of Bone Remodeling in Children Following Severe Burns

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Within 3 weeks of burn injury of at least 40% total body surface area, bone formation is markedly reduced and bone resorption as measured by urinary collagen crosslinks is also reduced, resulting in low-turnover bone loss (1). It is likely that high endogenous glucocorticoid production contributes to this condition (2). While the catabolic state resulting from burns lasts about 9 months, it is uncertain when bone remodeling resumes. The aim of our study was to determine if bone remodeling has resumed by one year post-burn. We studied 17 children, ages 7–19 years, who had iliac crest bone biopsies during acute admission for burn care and who returned at 1 year for reconstructive surgery. Ten of these children received long-term treatment with anabolic agents recombinant human growth hormone or oxandrolone. Families received the first dose of tetracycline by mail and were instructed to administer it 2 week before hospital readmission. The second dose was given 2 days before reconstructive surgery. Intra-operative iliac crest biopsies were obtained and processed for histomorphometry. Static parameters were determined in 12 of 17 biopsies due to crush artifact. Compared to acute biopsies, 67%(8 of 12) improved in bone area (P = NS), 12 of 12 (100%) improved in osteoid area ($P < 0.01$ by sign test), 9 of 12 (75%) had increased osteoid surface (P = NS), 8 of 12 (67%) had increased eroded surface (P = NS) and 11 of 12 (92%) had improved osteoid width (P = 0.01). Thirteen of 17 (76%) showed increased mineral apposition and bone formation rates (P = 0.05). There were no detectable histomorphometric differences between those given anabolic agents and placebos. Thus by one year post-burn, bone remodeling has resumed regardless of whether patients received anabolic glucocorticoid antagonists. It remains to be seen if there are similar improvements in bone mineral density. Supported by NIH GM 1P 60338.

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P206-Mo

Mycobacterium Tuberculosis HSP 60.1, but not HSP 60.2, Directly Inhibits Osteoclastic Bone Resorption by Blocking RANKL Activity

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Mycobacterium tuberculosis produces three chaperonins – 60 kDa and 10 kDa molecular chaperones also known as Hsp60/10 – which have intercellular signaling activity. Previous work had shown that Hsp10 had anti-arthritic activity. This study was aimed at comparing the abilities of the three chaperonin proteins to inhibit developing adjuvant arthritis.

Recombinant chaperonin (Hsp) 10, 60.1 and 60.2 were prepared, purified free of lipopolysaccharide and administered to rats after induction of adjuvant arthritis. Proteins were also tested in vitro to ascertain their effects on bone explants and cells.

We showed that in the chosen dosing regime, Hsp60.2 (conventionally known as Hsp65) had no effect on adjuvant arthritis. Hsp10 had a small but significant effect on joint swelling but had no effect on tissue damage. Hsp60.1, which shares 61% sequence identity with Hsp60.2, failed to inhibit joint swelling but blocked the marked osteoclastic bone destruction characteristic of adjuvant arthritis. To determine if this was a direct effect on bone cell signaling, as opposed to some modulation of lymphocyte activity, both Hsp60 proteins were tested for their ability to inhibit agonist-induced bone resorption in vitro. Hsp60.1, but not Hsp60.2, dose-dependently inhibited bone resorption induced by various agonists including the key osteoclast-inducing cytokine, RANKL. It also inhibited RANKL-driven production of osteoclasts. Hsp60.2 neither had agonist or antagonist activity in these systems. Recombinant domain mutants of Hsp60.1 have been produced and demonstrate that the osteoclast-inhibitory activity resides in the equatorial domain.

These findings reveal a novel interaction between a mycobacterial heat shock protein and a key bone regulatory cytokine network. The therapeutic potential of this finding is clear.

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P207-Tu

Effects of Thyamazol on Bone Metabolism in Hyperthyreotic Premenopausal Women

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The aim of this study was to investigate bone markers' dynamics in hyperthyreotic premenopausal women treated with thyamazol and to determine the most useful marker reflecting bone metabolism. Forty-five premenopausal hyperthyreotic women with no other cause of increased bone turnover were included in the study after obtaining the informed consent. All women were treated with thyamazol. Thyroid hormones, TSH and bone markers were determined initially, and than 6 and 18 weeks following treatment. Statistical analysis was performed by using Wilcoxon's matched pairs analysis and Spearman's rank correlation. Normal levels of thyroid hormones with suppressed TSH levels could be usually achieved after 6 weeks, and after 18 weeks the hormonal status remained unchanged. Statistically significant changes in serum bone alkaline phosphatase activity ($P < 0.01$), blood CTX ($P < 0.01$), urinary pyridinoline and urinary deoxypyridinoline ($P < 0.01$) dynamics after 6 weeks of treatment were detected. After 18 weeks of treatment bone alkaline phosphatase activity was significantly decreased in comparison to activity recorded in week 6 ($P < 0.01$), but still was significantly higher than the start value. Urinary pyridinoline, deoxipyridinoline and serum CTX levels decreased significantly after 6 weeks of treatment ($P < 0.01$), but after 18 weeks of treatment significant decrease was found only for CTX ($P < 0.05$) (Wilcoxon's matched paired analysis). Thyreosuppressive therapy markedly slowed down bone turnover in our patients, but medians of some markers were still elevated in week 18 (pyridinoline, deoxypyridinoline, CTX). Best correlation with thyroid hormones was detected in bone alkaline phosphatase activity, and pyridinoline and deoxipyridinoline concentrations (Spearman's rank correlation).

P208-Su

Risk Factors for Low Turnover Renal Osteodystrophy in Dialysis Patients

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Disturbances of bone metabolism in the course of end-stage renal disease (ESRD) require precise evaluation due to their high prevalence and important clinical significance. We estimated the non-invasive bone turnover markers in patients with ESRD before and during renal replacement therapy (RRT) with special emphasize to the relationship between different dialysis techniques and clinical types of renal osteodystrophy (ROD).

The total of 128 subjects (79 male, 49 female; age 58 ± 15 years) were enrolled in the study. Patients were studied before start of dialysotherapy, and followed up to the 3rd year of RRT. We estimated the following parameters: iPTH, Ca, P, ALP, bone formation markers (N- and C-terminal procollagen propeptides PINP and PICP) and bone resorption markers (type I collagen cross-linked telopeptide-ICTP, tartrate-resistant acid phosphatase TRAP5b). All markers were evaluated every3rd month of the study. iPTH levels were used to differentiate the following types of ROD; iPTH <100 pg/ml-for low turnover ROD, iPTH >450 pg/ml for high turnover ROD.

At the start of our study 13% (16/128) of all subjects had low turnover ROD (mean iPTH = 72 ± 30 pg/ml), whereas 23% had high-turnover ROD (mean iPTH = 725 ± 208 pg/ml.) After the first year of RRT the prevalence of low turnover ROD increased significantly up to 41% of the whole group (52/128) ($P < 0.001$). In comparison to subjects with high-turnover ROD, patients with low-turnover ROD were more frequently above 60 years (81% vs. 34%), predominantly males 66% vs. females 34%, more often treated by peritoneal dialysis (64% vs. 36%), and with diabetic nephropathy as the cause of ESRD (62% vs. 38%). We found significant correlation only between iPTH and PINP ($r = 0.7124$; $P < 0.001$) and between iPTH and TRAP ($r = 0.6872$, $P < 0.001$). In patients with low turnover ROD significantly lower values of PINP and TRAP were found in comparison with high turnover ROD (42 ± 20 μg/l vs. 283 ± 138 μg/l, $P < 0.001$, and 2.8 ± 1.4 U/l vs. 7.3 ± 1.3 U/l, $P < 0.001$, respectively).

In conclusion, the prevalence of low turnover ROD increased significantly after start of dialysotherapy. Age >60 years, male gender, diabetic nephropathy, peritoneal dialysis were the risk factors for low-turnover ROD in our patients and PINP as well as TRAP5b appeared to be the most useful markers of bone turnover.

P209-Mo

Comparison Two Assays for Fibroblast Growth Factor-23

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FGF-23 was recently shown to be involved in the development of several hypophosphatemic diseases including X-linked hypophosphatemic rickets/osteomalacia (XLH) and tumor-induced rickets/osteomalacia (TIO) as well as hyperphosphatemic disorders like tumoral calcinosis (TC) and hyperosteosis-hyperphosphatemia syndrome (HH). FGF-23 is processed between Arg^{179} and Ser^{180}, and only full-length FGF-23 was shown to have biological activity to reduce serum phosphate level. Two assays for FGF-23 were reported. One assay detects only full-length FGF-23. In contrast, C-terminal assay recognizes both full-length and processed C-terminal fragment of FGF-23. However, discrepant results concerning circulatory levels of FGF-23 in patients with TIO and XLH have been reported using these two assays. We simultaneously measured FGF-23 levels in thirteen patients with adult-onset hypophosphatemic osteomalacia and 29 patients with XLH by these two assays. Full-length assay indicated that FGF-23 was above the upper limit of the reference range in all patients with osteomalacia and 24 of 29 patients with XLH. However, C-terminal assay indicated that FGF-23 was within the reference range in 3 of 13 patients with osteomalacia and 17 of 29 patients with XLH. In addition, there was no correlation between FGF-23 levels measured by these assays in patients with XLH whose FGF-23 was within the reference range by C-terminal assay. Furthermore, full-length FGF-23 was low normal while C-terminal assay indicated extremely high level of FGF-23 (more than 1500 RU/ml) in three patients with TC and/or HH. These results indicate that FGF-23 measured by these two assays can be discrepant and FGF-23 level within reference range by C-terminal assay does not rule out increase in full-length FGF-23. Furthermore, these results also suggest that full-length FGF-23 plays important roles in the development of these diseases with deranged phosphate metabolism.

P210-Tu
Development of Bone Remodelling Balance and Turnover Indices in Patients with Metabolic Bone Disease

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Bone markers of formation and resorption provide a unique insight into the dynamic bone status of patients with metabolic bone disease, yet due to difficulty with interpretation of results, the role of bone biomarkers in clinical practice is not yet established. We have developed indices of bone remodelling balance (BRBI) and bone turnover index (BTI) to make the bone biomarkers more clinically applicable in metabolic bone disease. Markers of bone formation and resorption were measured under standardized conditions after an overnight fast in 12 patients with thyrotoxicosis, 9 with hypothyroidism, 17 with Paget's disease pre- and post-treatment. The individual biomarkers were then expressed as *T* scores by comparison to a young adult sex-matched reference range. Using the *T* scores, BRBI (bone formation − resorption marker) and BTI (bone formation + resorption marker) were calculated. The indices therefore provide a single number indicating either bone gain or bone loss and high or low bone turnover. In thyroid disease the bone formation and resorption markers procollagen type 1N propeptide and free urinary deoxypyridinoline were used to calculate the indices. In hyperthyroidism (TSH < 0.01 mU/L) at diagnosis the BRBI showed a negative bone remodelling balance (−6.79 ± 2.09) with high bone turnover (BTI = +16.46 ± 3.66), which corrected when euthyroid (TSH = 1.99 ± 0.57 mU/L, $P < 0.01$); BRBI = +2.29 ± 0.76 ($P < 0.01$) and BTI = +5.07 ± 1.82 ($P < 0.01$). In hypothyroidism (TSH = 58.02 ± 16.86 mU/L) the BRBI was equal to −0.75 ± 0.99 with low bone turnover (BTI= 0.82 ± 1.41). Treatment with L-thyroxine (TSH = 2.95 ± 0.78 mU/L) furthered bone loss (BRBI = 1.81 ± 1.15, $P = 0.14$) and increased bone turnover (BTI = +3.31 ± 1.80, $P < 0.01$). In Paget's disease the bone biomarkers osteocalcin and N-terminal telopeptide of Type 1 collagen crosslinks were used to calculate the indices. After treatment with bisphosphonates the BRBI fell from −22.34 ± 8.34 to −1.08 ± 1.02 ($P < 0.01$), while BTI fell from +36.13 ± 5.14 to +2.34 ± 2.16 ($P < 0.01$). BRBI and BTI facilitate the conceptualization of bone gain or loss and activity and the management of metabolic bone disease. Different combinations of bone formation and resorption markers are likely to be needed to derive the indices for the various metabolic bone diseases.

P211-Su
Altered mRNA Expression of Membrane Transporters in the Kidneys of X-Linked Hypophosphatemic (HYP) Mice

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X-linked hypophosphatemia is characterized by the decreased renal reabsorption of phosphate, low plasma phosphate, and rachitic and osteomalacic bone disease. No other defects in the kidney are known to exist. To explore the impact of this mutation further, kidneys from five-week-old mice, normal and Hyp (hemizygous male and heterozygous female), were collected, and RNA was extracted and hybridized to Affymetrix Mouse 430A and 430B microarrays with probe sets for over 40,000 genes. The RNA for each array was pooled from three mice. A total of 24 arrays, six for

each genotype and gender, were done. The data were transferred to an Excel spreadsheet, formulas for factorial analysis of variance were added, and the formulas were copied to all genes. GenMAPP (www.genmapp.org) was used to identify transport-related genes with a significant main effect of genotype (normal vs. Hyp). RNA levels for all phosphate transporters were significantly decreased ($P < 0.001$) in the Hyp mice. In addition, mRNA for the sulfate transporter, slc13a1, increased 2-fold ($P < 0.001$) in Hyp. mRNA for the chloride transporter, slc12a3, increased over 50% ($P < 0.001$). mRNA for organic anion transporters (OAT) also changed with a 16% decrease in OAT1 (slc22a6, $P < 0.001$), a 50% increase in OAT2 (slc22a7, $P < 0.01$), a 50% decrease in OATP1 (slco1a1, $P < 0.001$, only expressed in males) and a 33% decrease in females for slc13a3 ($P < 0.01$). The organic cation transporter, slc22-like 2 (slc22al2), also had a 33% decrease ($P < 0.001$). mRNA for the sodium/potassium transporting ATPase, alpha 2 polypeptide, atp1a2, has four probe sets on the arrays, and all increased 2 to 8-fold ($P < 0.001$), while the thiazide-sensitive NaCl transporter, slc12a3, increased 50% ($P < 0.001$). Changes in mRNA for glucose transporters were mixed with the low affinity sodium-dependent glucose transporter, slc5a2, decreasing 33% in Hyp mice ($P < 0.001$), along with smaller decreases in the facilitated glucose transporter, slc2a2 ($P < 0.001$), while mRNA for the high affinity sodium/glucose transporter, slc5a1 increased 50% ($P < 0.001$), along with a smaller increase in the insulin responsive facilitated glucose transporter slc2a4 ($P < 0.01$). In summary, changes exist in the mRNA expression of transporters in the kidney other than for phosphate. This suggests a broader metabolic effect of the Hyp mutation (Supported in part by the NIH, DK064049).

P212-Mo

Identification of Metabolic Bone Disease in Patients Undergoing Hearth Transplantation

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Heart transplantation has become fairly common and successful, and is followed by post-transplant osteoporosis in a substantial proportion of patients. Many candidates for cardiac transplantation have low bone mineral density (BMD) and there is a high frequency of fractures in the first 6 months after transplantation, mainly in patients with the lowest pretransplant BMD.

Our aim was to evaluate the pre-transplant BMD and to assess clinical characteristics and markers of bone metabolism able to identify patients with the lower bone mass and the higher risk of fracture.

We studied 216 patients (aged 52 ± 10 years, 75% males, mean BMI: 24.25 ± 3.25 kg/m^2) undergoing hearth transplantation. BMD (t score) was measured at lumbar spine and total femur (LS and TF, respectively; Hologic QDR1000). Age, sex, height, weight, functional class (NYHA) (III 65%; IV 35%), aetiology (isquemic 57%; dilated 32%; valvulopathy 6%; congenital 0.5%; misc 4.5%), cardiac output (mean ± SD; 4.04 ± 1.07 l/min), time since indication of transplant (58 ± 58 months), bone markers (BGP: 2.07 ± 2.72 ng/ml, bone ALP: 11.0 ± 4.1 μg/dl; total ALP: 189 ± 89 U/l, Ntx: 86.1 ± 45.5 nmol BCE/nM Cr, D-Pyr: 11.4 ± 5.3 nM/mM Cr) and calciotrophic hormones (PTH: 45.9 ± 30.8 pg/ml, 25 (OH) vitamin D: 15.7 ± 17.2 ng/ml) were determined. Patients were placed in three groups (osteoporosis/osteopenia/normal) according WHO criteria and comparisons across groups were performed (ANOVA, Chi2).

The mean BMD values (t score) were −1.44 ± 1.26 at the lumbar spine and −1.32 ± 0.96 at the total femur. 19% of the patients showed lumbar osteoporosis (46.8% had osteopenia and 34.1% were normal) and 9.9% had osteoporosis at the total femur (54.7% had osteopenia and 35.5% were normal). The female gender, longer waiting time for transplantation, lower weight, height, higher total and bone ALP were associated to lower BMD at the lumbar spine. The longer waiting time for transplantation, lower weight and BMI were associated to lower BMD at the total femur.

Conclusions: A reduced number of clinical and biochemical characteristics could help to identify patients undergoing hearth transplantation with higher risk of osteoporosis.

P213-Tu

Valproate has Strain-Specific Effects on Bone Mineral Content in Mice

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Long-term therapy with anti-epileptic drugs (AED) is associated strongly with increased fracture risk, but the mechanism by which AED use is associated with decreased bone mineral density and increased bone fragility is poorly understood. There are currently no established animal models of AED-induced bone disease.

Our aim was to develop a mouse model to investigate the effects of AEDs on total bone mineral content (BMC) and to better characterize the metabolic and structural bone changes associated with AED treatment. The ultimate aim of this project is to identify the mechanisms including any genetic factors underlying this effect.

Seven different inbred strains ($n = 40$ per strain, $n = 10$ per diet) of 8- to 9-week-old mice were placed on a diet mixed with 0, 2, 4 or 6 g/kg valproate (VPA) for 8 weeks. Then total BMC, fat mass and lean mass were assessed using dual energy X-ray absorptiometry (DXA). BMC was corrected

for total body weight and total lean mass to account for differences in animal size.
Statistical analysis using ANOVA identified BALB/C as being sensitive to VPA-induced bone disease showing significant differences (95% CI) of 10.4% (2.7%–18.2%) and 8.4% (0.1%–16.2%), respectively, in weight-adjusted BMC compared with control mice while on the 2 and 6 g/kg VPA diets ($P < 0.05$). 129T2 was identified as a strain resistant to the effects of VPA on BMC at all doses. Other VPA-sensitive and resistant strains also have been identified. Further investigation of these mouse strains and the identification of metabolic and genetic factors involved may help to elucidate the mechanisms underlying the effects of AED treatment on bone health. This approach will facilitate the design of therapeutic strategies for the prevention and treatment of AED-associated bone disease, and the pre-clinical testing of potential interventions.

P214-Su
25-Vitamin D and Parathyroid Hormone Levels Measured in Patients from Primary and Secondary Care Clinics
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Serum 25-vitamin D is being increasingly requested in patients for a wide variety of indications. We wanted to determine the prevalence of 25-vitamin D deficiency in patients who had this vitamin D metabolite measured and to determine the relationship between 25-vitamin D and PTH in this population.
We identified all 25 vitamin D levels that were performed by our laboratory over a 12 month period from patients referred by primary and secondary care clinicians. Only the first sample was used for patients who had multiple levels. We also determined what other biochemical parameters were requested including PTH.
Over 12 months there were 857 patients who had a 25-vitamin D level measured. The mean level was 47 (SD 29) nmol/l. Of these 85% had a 25-vitamin D <80 nmol/l, 59% a level <50 nmol/l and 35% a level <30 nmol/l. PTH was requested in 409 of the 857 patients (48%). Secondary care clinicians were more likely to request a PTH level compared to primary care (62% versus 18%, respectively; $P < 0.001$). A low 25-vitamin D level of <50 nmol/l was found in 211/409 patients who had their PTH measured. Of these patients 99/211 (47%) had secondary hyperparathyroidism with the remainder exhibiting a blunted PTH response (PTH within normal range). Compared to patients with a blunted PTH response, patients with secondary hyperparathyroidism were older (mean 61 years versus 53; $P = 0.001$), had lower 25-vitamin D levels (mean 24 nmol/l versus 33; $P < 0.001$) and had higher serum creatinine (mean 119 μmol/l versus 85; $P < 0.001$).There were no differences in serum corrected calcium or phosphate between the two groups.
Our data suggest that 25-vitamin D insufficiency is common in both primary care and secondary care patients. Only approximately half of these patients have secondary hyperparathyroidism and this is more likely to be the case if individuals are older or have a higher creatinine. Whether there are significant differences in outcome between patients who have low 25-vitamin D and blunted PTH response as opposed to secondary hyperparathyroidism remains unclear.

P215-Mo
Effect of Etanercept on Bone Resorption in Psoriatic Arthritis
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Background: Rheumatoid arthritis (RA) is characterized by both generalized and periarticular bone loss leading, respectively, to the development of osteoporosis and of joint bone erosion. Both these phenomena are known to be mediated by several cytokines, including TNFalpha, so that anti-TNFalpha treatments may slow or even arrest joint damage and probably also generalized osteoporosis in course of RA through an inhibition of osteoclast activity. Recent studies suggest that biochemical bone markers may be of clinical relevance in assessing either systemic skeletal fragility or progression of joint bone erosion and may play a role in predicting the progression of joint damage and in assessing treatment efficacy. According to this hypothesis recent findings showed a decrease in bone markers levels in RA patients treated with anti-TNFalpha drugs.
The relationship between bone metabolism and seronegative spondylarthropathies is still to be explained.
Objective: To evaluate the effect of anti-TNFalpha treatment on bone resorption in psoriatic arthritis (PsA).
Methods: We analysed beta-crosslaps-CTX (beta-CTX-ECLIA- Roche, Mannheim, D) in 7 polyarticular PsA patients (M:F = 4:3; mean age 46 ± 18; mean disease duration 6 ± 4) treated with etanercept (25 mg twice a week s.c.) for a 4-month period. We excluded patients with concomitant conditions that might influence beta-CTX levels (i.e., postmenopausal women, patients submitted to treatment with bisphosphonates, corticosteroid or other drugs affecting bone metabolism). We did not permit adjustment in DMARDs dosage during the observation period.
Results: All the patients showed raised levels of beta-CTX at baseline (mean 0.90 ± 0.58 ng/ml). After 1 month of treatment beta-CTX decreased in all patients (mean 0.58 ± 0.35 ng/ml) and the decrease maintained for all the observation period (after 4 months: mean 0.57 ± 0.34 ng/ml). A concomitant decrease of CRP levels, VAS levels and joint count was observed.
Conclusion: Our data seem to demonstrate that anti-TNFalpha treatment may influence osteoclat activity also in

PsA as well as in RA. Further studies on larger samples are needed to confirm these preliminary results and to explain whether biologic treatments may influence both periarticular and also generalized bone loss.

P216-Tu

The Analysis of Bone Mineral Density, Osteocalcin and Biochemical Index of Skeletal Fluorosis's Patient and Treatment by Adding Selenium in Guizhou Province, China

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Introduction: Guizhou province located in Chinese west-south tableland is an endemic high fluorosis region, where the skeletal fluorosis's patient occupies above 30% of its population. The investigation shows that high fluorosis (F) and low selenium (Se) in its soil and water. Many experiments have been done in order to study mechanism of skeletal fluorosis's patient. The relation of trace element F and Se are paid much attention to in prevention and treatment of skeletal fluorosis's patient. At present, the mechanism of skeletal fluorosis's disease is poorly understood.

Purpose: The experiment we performed aimed at exploring interaction between F and Se in bone metabolism of human and observing the effect of Se on skeletal fluorosis's disease.

Approaches: 103 cases of skeletal fluorosis's patients, 105 cases of skeletal fluorosis's patients treated by Na2SeO3 (oral 0.5 mg/2 day, 200 day) from endemic fluorosis region and 97 health control volunteers from normal fluorosis region were chosen. The bone mineral density (BMD) was measured by single photon absorption (SPA), the osteocalcin (OC) and AKP of serum were tested by RIA. The content of Ca, P in serum and the content of Ca, P, hydroxyproline (Hop), creatinine (Cr) in the urine were examined by biochemical method. The content of Se and F in serum, urine and hair was detected by atomic absorption spectrophotometry (AAS).

Results: The Ca, P in serum, MBD and the Ca, P in the urine were lower in skeletal fluorosis's patients than in health control volunteers ($P < 0.01–0.05$). The OC and Hop/Cr increased markedly in skeletal fluorosis's patients in comparison with health control volunteers ($P < 0.05$). F decreased and Se increased in serum, urine and hair of skeletal fluorosis's patients treated by Na2SeO3. There was no difference between skeletal fluorosis's patients and skeletal fluorosis's patients treated by Na2SeO3 in Ca, P of serum, MBD and Ca, P, Hop, Cr of the urine.

Conclusions: The previous experimental data showed that F increased the free radical and activity of lipid peroxidation enzyme. The harmful effects of F could be antagonized by adding Se. Se was an antagonist to F. But in our experiment, the content of the eostocalcin and AKP, Ca, P in serum and the content of Ca, P, Hop, Cr in the urine did not change statistically by adding Se. The theory of free radical and lipid peroxidation did not explain mechanism of skeletal fluorosis's patient.

P217-Su

The OPG/TRAIL Complex in an In Vitro Osteoclastogenesis Model Derived from Human Multiple Myeloma-Bone Disease

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The development of multiple myeloma (MM)-bone disease is mediated by increased recruitment and activity of osteoclasts (OCs). Osteoclastogenesis is regulated by a complex signaling system, that involves receptor activator of nuclear factor-kappa B (RANK), receptor activator of nuclear factor-kappa B ligand (RANKL) and osteoprotegerin (OPG), all belonging to the tumor necrosis factor (TNF) family. The aims of our study were to investigate the MM T cell involvement in osteoclastogenesis, and the expression of the major osteoclastogenic mediators. Unstimulated and unfractionated peripheral blood mononuclear cells (PBMCs) isolated from 32 MM patients with or without osteolytic bone lesions, and parallel T cell-depleted cultures were used as in vitro osteoclastogenesis models. In addition, unstimulated and unfractionated PBMC cultures from 32 controls with nonneoplastic disease without any skeletal involvement were also established. Our results showed that the OCs derived from MM-bone disease PBMCs spontaneously developed and displayed a longer survival in a T cell-dependent way. Differently in T cell-depleted MM PBMC cultures, the addition of macrophage-colony stimulating factor (M-CSF) and RANKL was necessary to promote the formation of OCs, that however did not exhibit a longer survival. MM-bone disease T cells overexpressed RANKL, OPG and TNF-related apoptosis inducing ligand (TRAIL), also detected in large amounts in the culture media. Despite high OPG levels, the persistence of osteoclastogenesis in our system can be related to the interaction between OPG and TRAIL that were co-immunoprecipitated by a monoclonal antibody (mAb) against TRAIL. The evidence that TRAIL binds to OPG blocking OPG anti-osteoclastogenic effect is also supported by the addition of different concentrations of functional anti-TRAIL mAb, significantly decreasing the OC formation. The OCs developed from MM-bone disease PBMCs expressed a T cell-modulated balance of death and decoy TRAIL receptors. In

particular, we found these OCs overexpressed TRAIL decoy receptor DcR2 in the presence of T cells, and death receptor DR4 in the T cell-depleted cultures. In conclusion, our results highlight that MM-bone disease T cells support the spontaneous OC formation with longer survival, involving the OPG/TRAIL interaction and the unbalanced OC expression of TRAIL death and decoy receptors.

P218-Mo

T Cells Support Osteoclastogenesis in an In Vitro Model Derived from Human Psoriatic Arthritis

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Psoriatic arthritis (PsA) is now recognized as a progressively destructive inflammatory arthritis that can lead to joint deformity and functional disability. Considerable evidence implicates T cells in the pathogenesis of PsA. In fact, T cells are present at sites of inflammation and exhibit an activated phenotype. Recent evidence suggests that T cells may regulate bone resorption through the critical osteoclastogenic factor, receptor activator of nuclear factor-kappaB ligand (RANKL). Using an in vitro osteoclastogenesis model consisting of unstimulated peripheral blood mononuclear cells (PBMC) from PsA patients, we show, for the first time, that osteoclasts (OCs) develop spontaneously in a T cell-dependent way. Differently, in T cell-depleted PBMC cultures, the addition of M-CSF and RANKL is necessary to OC formation. Next, we demonstrate the up-regulated production of RANKL and TNFa, at both mRNA and protein levels, by freshly isolated T cells from peripheral blood of PsA patients. The involvement of RANKL in T cell-mediated osteoclastogenesis was confirmed by the addition of RANK-Fc to the media of PBMCs from PsA patients, resulting in the inhibition of spontaneous osteoclastogenesis in a dose-dependent manner. Moreover, knowing that IL-7 induces bone from T cells, we show that loss in vivo by induction of RANKL and TNF in our system anti-IL-7 antibody inhibited osteoclastogenesis in a dose-dependent manner. Consistently, we demonstrated that IL-7 levels were significantly higher in the sera of PsA patients. Our findings indicate a T cell-regulation of OC formation in PsA patients through RANKL overexpression, possibly involving IL-7.

P219-Tu

Higher Bone Mineral Density in Reproductive Aged, Caucasian, Hirsute Patients. Positive Correlation of Testosterone Levels to Bone Mineral Density in Hirsutism

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Objective: The mechanisms leading to higher bone mineral density (BMD) levels in hirsute patients than in healthy controls have only been sparsely examined. We compared the metabolic, hormonal and bone metabolic parameters in hirsute patients and female controls and correlated BMD and bone metabolic parameters with testosterone, estradiol and metabolic parameters.

Patients: A group of 51 Caucasian, reproductive aged, hirsute patients referred to the outpatient clinic of an academic tertiary care medical center and 63 healthy, female Caucasian controls matched for season, weight and age.

Measurements: BMD (hip, neck, lumbar and total BMD), bone metabolic parameters (osteocalcin, alkaline phosphatase, PTH, ion calcium, phosphate and 25-hydroxyvitaminD (25OHD)) and endocrine profiles (androgen status, estradiol and insulin) were evaluated during follicular phase. Estradiol measurement was repeated during cycle days 8–12.

Results: Lumbar and neck BMD levels were significantly higher in hirsute patients than in controls: (mean ± SD), lumbar BMD 1.10 ± 0.12 vs. 1.06 ± 0.10 g/cm^2 and neck BMD 0.91 ± 0.11 vs. 0.87 ± 0.12 g/cm^2, $P < 0.05$. Fasting insulin and free testosterone levels were significantly higher in hirsute patients than in controls (geometric mean (± 2 SD), insulin 46 (17–126) vs. 34 (11–105) pmol/l and free testosterone 0.031 (0.011–0.086) vs. 0.021 (0.017–0.026) nmol/l, $P < 0.05$). Free testosterone levels correlated positively to hip and neck BMD levels in hirsute patients. During multiple regression analysis testosterone, estradiol and waist-hip-ratio (WHR) were found to have positive effects on BMD levels independent of body mass index (BMI).

25OHD levels were significantly lower in hirsute patients 42 (13–131) than in controls 72 (27–196) nmol/l (geometric mean ± 2 SD), $P < 0.001$.

Conclusion: Hirsute patients demonstrated significantly higher BMD levels than controls, which could be explained by hyperinsulinemia and higher testosterone levels in hirsute patients compared with controls. The pathogenesis for significantly lower 25OHD levels in hirsute patients than controls needs to be evaluated in future studies.

P220-Su

Biological and Mechanical Changes of the Bone-Graft-Cement Interface After Impaction Allografting

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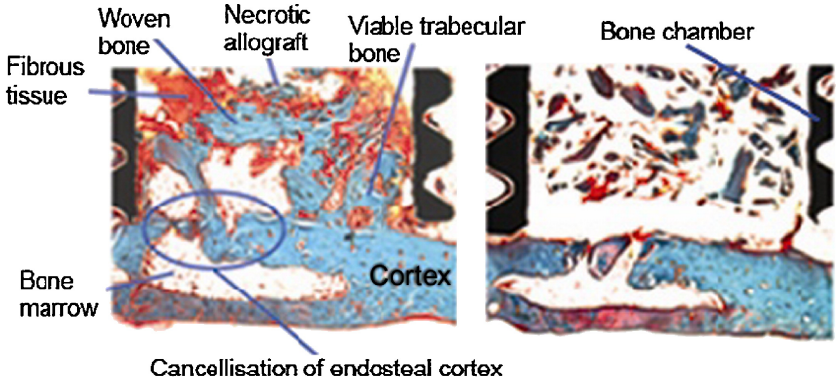
The main reason for failure of total hip replacements is osteolysis, which is characterized by destruction of the host bone due to a foreign body reaction to particulate wear debris. A technique called impaction allografting is used to

restore the host bone by impacting cancellous allograft bone into the defects. After a new femoral stem is cemented into the restored femur, the host bone interface consists of allograft alone or as a composite with cement. The purpose of this study was to investigate the temporal changes of these interfaces in a rat bone chamber model.
Bone chambers were inserted in both tibiae of 33 rats and tightened to the endosteal surface to create a microenvironment. One chamber was filled with allograft bone and the other with an allograft/cement composite. After 0, 3 and 6 weeks, the rats were euthanized, the interfaces mechanically tested and processed for histomorphometric analysis.
The composite–host bone interface strength was significantly higher at 3 weeks and was higher than the allograft construct. Extensive periosteal remodelling was observed at 3 weeks. At 6 weeks a new medullary canal was formed and the endosteal cortex was partially absorbed (Fig.).
The increased interface strength of the composite–host bone interface was due to fibrous tissue attachment rather than direct bonding of the bone particles. Cortical porosity and cancellation is known to be caused by a damaged endosteal circulation resulting in medullary canal widening and may cause clinically unstable implants.



Transverse sections of the bone chamber at 6 weeks, stained according to Goldner. Left: pure allograft. Right: allograft-cement composit.

P221-Mo

Effects of Electrogene-Transfer with Wnt3a Expression Vector on Fractured Mice

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Gene therapy presents a novel approach to the treatment of challenging bone loss problems. It has been demonstrated that in vivo administration of a viral vector, including genes encoding osteogenic proteins to promote fracture healing, represents a highly efficient method for gene transfer. However, such possible applications with virus vectors have encountered obstacles such as immunological reactions, viral infection, etc. Our purpose is to develop safe, non-viral gene therapy strategies to enhance bone repair. Wnt signaling through LRP5 has been recently identified as an important pathway regulating bone mass. Wnts in the canonical signaling pathway employ beta-catenin as a downstream effector, suggesting the importance of Wnt signaling in bone formation. We hypothesized that the secreted Wnt protein could influence bone fractures through paracrine/autocrine effects even with low amounts of secreted protein. We therefore attempted to deliver plasmid DNA containing Wnt3a gene, as a representative canonical Wnt member, to the repair process of fractured bone by electrogene-transfer, a less efficient but safer method of gene transfer when compared with viral vector. A plasmid DNA solution was administered at the fracture site 2 days after fracture in the mice femoral fracture model. The plasmid DNA was injected into the junction between the fracture site and the muscle layer. Each electrode was set up on the muscle layer to surround the fracture site, and electrical pulses were applied. By RT-PCR analysis, expression of exogenous Wnt3a mRNA was detected in the callus and the muscle at 14 days after fracture. Furthermore, expression of ALP mRNA and its activity in the callus were up-regulated by the Wnt3a expression. uCT analysis at 14 days after fracture exhibited

enlarged callus volume with Wnt3a expression when compared to the callus volume without Wnt3a expression, suggesting that Wnt3a may promote the proliferation and differentiation of the cells in the callus. Administration of LiCl at the fracture site also showed the enhancement of callus volume, suggesting that the Wnt3a effect is dependent upon the activation of endogenous beta-catenin signaling in the callus during fracture healing. Taken together, our results suggest that expressing representative Wnt3a by electrogene-transfer to activate Wnt/beta-catenin signaling pathway may be suitable for the development of safe, non-viral gene therapy for the repair of fractured bone or large bone defect.

P222-Tu

Imbalanced Expression of RANKL and Osteoprotegerin mRNA in Pannus Tissue of Rheumatoid Arthritis

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Background: Synovial inflammation in rheumatoid arthritis (RA) leads to pannus tissue invasion and destruction of cartilage/bone matrix. Synovial hyperplasia involves proliferation of synovial fibroblast-like lining cells and accumulation of macrophages and lymphocytes. The leading edge of pannus is composed of fibroblast-like cells, which produce proteolytic enzymes able to cause destruction of hyaline articular cartilage. Fibroblasts also express receptor activator of nuclear factor kappaB ligand (RANKL), an essential factor for osteoclast differentiation (1,2). Underlying bone resorption requires first demineralization of bone matrix and this is done by osteoclasts.
Objective: To investigate the expression of RANKL, its receptor RANK and their natural inhibitor osteoprotegerin (OPG) in RA.
Methods: Frozen tissue samples of pannus and synovium from advanced RA and for comparison synovium from osteoarthritic patients were used for messenger RNA (mRNA) measurement with quantitative real-time reverse transcriptase-polymerase chain reaction (RT-PCR) and for protein detection with immunohistochemical staining.
Results: RANKL mRNA expression was higher than OPG expression in pannus tissue. In contrast in OA and RA synovium RANKL expression was lower than OPG expression. Similar RANK mRNA expression was detected in all samples. RANKL staining was most intense in pannus tissue and was located near bone-soft tissue junction.
Conclusion: The extensive bone erosion in RA may be due to increased RANKL expression in pannus tissue, which is not balanced with simultaneous expression of OPG.
References:
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P223-Su

Risk Factors for Falls in Turkish Elderly

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Falling is one of the most common and serious problems facing elderly people. There are many risk factors and the risk of falling consistently increases as the number of risk factors increase. Consequently opportunities for prevention of falling are often overlooked with risks becoming evident only after injury and disability have already occurred. For this reason being aware of possible risk factors and try to eliminate these factors is very important to prevent falls. The aim of this study was to identify individual risk indicators for falls among geriatric patients. Total 2322 patients aged 65 years and over without an acute illness admitted to Division of Geriatric Medicine of Internal Medicine Department in Hacettepe University Hospital between February 2002 and December 2004 were included in this cross-sectional study. All patients had a complete comprehensive geriatric assessment and questioned for fall history in the past one year period. Risk indicators for accidental falls were analyzed by using logistic regression.
The mean age of participants was 71.8 years; 37% (848 patients) were male and 63% (1474 patients) were female. 662 patients (28.5%) were found to be fallen at least once in the past one year period. In the univariate analysis advanced age, living alone, female sex, visual and hearing problem, eyeglass usage, mobility problems, walking assist device (WAD) usage, depression, polypharmacy (using more than three drugs regularly), urinary incontinence and osteoarthritis were associated with falls, but multiple regression analysis revealed female sex [OR: 1.548, 95% CI: 1.243–1.975], living alone [OR: 1272, 95% CI: 0.997–1.623], visual problem [OR: 1.418, 95% CI: 1.163–1.727], and hearing problem[OR: 1.310 95% CI: 1.068–1.607], polypharmacy [OR: 1.205, 95% CI:0.990–1.465], WAD usage, [OR: 1.529, 95% CI: 1.117–2.092], mobility problem [OR: 1.807, 95% CI: 1.312–2.488] and depression [OR:1.261, 95% CI: 1.007–1.579] as independent predictors of falls.
The study has focused on a readily identifiable high risk group of people for falling presenting at primary health care. Study results specifically address living alone, vision,

hearing and mobility problems, depression and polypharmacy as modifiable predisposing risk factors for falls.

P224-Mo

Diagnostic Value of Micro-CT for the Assessment of Rare Human Bone Pathologies

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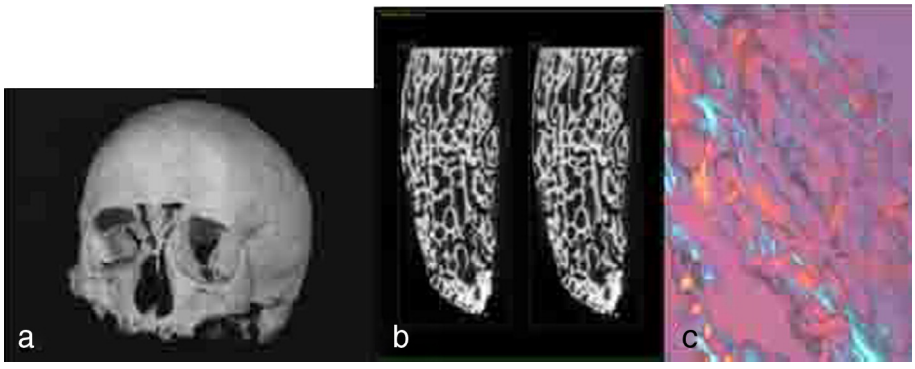
The assessment of historical human pathologies allows understanding of the remarkable microstructural alterations of various diseases involving bone. Hitherto, these days rare historical bone pathologies have been investigated by clinical CT or histology only. Here, we present the first ever application of micro-CT in the assessment of bone architecture in historical pathological samples. Fifteen cranial and postcranial macerated bone specimens from an early 20th century AD human pathology reference series (Galler collection, NHMB-Natural History Museum Basel Switzerland; Rühli et al. 2003) have been examined by micro-CT (μCT 40, Scanco Medical, Switzerland) and, as diagnostic gold-standard at the very same sample-location, by light microscopy (Schultz and Drommer 1983). The application of micro-CT has enhanced the understanding of the alterations of 2D and 3D bone architecture caused by various underlying pathologies such as osteodystrophia fibrosa generalisata, tuberculosis (Fig. 1) or syphilis. Exemplary, the re-emergence of the latter two in modern daily clinical practice accentuates the need for an improved understanding of the underlying bone remodelling. Also, the high-resolution visualization of massive bone destruction caused by osteomyelitis in a pre-antibiotic era specimen is of foremost medico-historical interest. However, the analysis of bone samples using polarized light microscopy contributes further crucial information on the orientation of collagen alignment.

Fig. 1: (a) Severe caries and focal hyperostosis of frontal, nasal, lachrymal, maxilla and zygomatic bone due to tuberculosis; corresponding micro-CT (b) and histology; (c) images of affected left zygomatic bone (Galler #2, NHMB).



P225-Tu

P1NP, B-Crosslaps and Osteocalcin as Biochemical Markers of Bone Turnover in Haemodialysis Patients

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Several biochemical markers have been proposed as markers of bone turnover during last years. The degradation fragments of the C-terminal telopeptide of type I collagen, i.e., b-CrossLaps (b-CTx) and total procollagen 1 N-propeptide (P1NP) were introduced recently as bone markers in evaluation of osteoporosis. The aim of the present study was to investigate the clinical usefulness of serum P1NP, b-CTx, osteocalcin (OC) and total alkaline phosphatase (AP) in evaluation of bone turnover in haemodialysis patients (pts).

In 30 pts (21 males and 9 females), mean age 61.7 ± 12.6 years, on hemodialysis 4.7 ± 4.3 years, serum P1NP, b-CTx, OC, AP and intact parathyroid hormone (PTH) were detected. Patients with diabetes or with parathyroidectomy were not included in the study. All pts received regular haemodialysis for 4 h, three times per week. Dialysate calcium concentration was 1.5 pmol/l, calcium carbonate in a dose of up to 4 g per day was used as phosphate binder. Calcitriol in low doses (up to 0.5 mg per day) was used in majority of pts with elevated PTH. P1NP, b-CTx, OC were determined by the Elecsys serum assay. AP was determined by standard biochemical method and PTH by radioimmunometric assay.

The mean serum P1NP concentration was 618.5 ± 742.4 ng/ml, b-CTx 2.1 ± 1.5 ng/ml, OC 182.5 ± 104.5 ng/ml,

AP 91.55 ± 40.4 U/l and PTH 397.3 ± 393.l pg/ml. There were significant positive correlations between serum P1NP and PTH ($r = 0.785$, $P < 0.01$), b-CTx and PTH ($r = 0.915$, $P < 0.01$), OC and PTH ($r = 0.815$, $P < 0.01$) and AP and PTH ($r = 0.686$, $P < 0.01$). The patients were divided in three groups according to PTH level, first with low PTH (<180 pg/ml), mild hyperparathyroidism (PTH 181–320.0 pg/ml) and severe hyperparathyroidism (PTH > 320 pg/ml). The mean level of P1NP in first group was 204.5 ± 87.4 ng/ml, b-CTx 1.14 ± 0.41 ng/ml, OC 101.02 ± 44.3 ng/ml and AP 62.1 ± 14.4 U/l, in second P1NP 401.03 ± 430.3 ng/ml, b-CTx 1.26 ± 0.5 ng/ml, OC 161.6 ± 104.4 ng/ml, AP 77.9 ± 36.8 U/l and in third group P1NP 1095.7 ± 937 ng/ml, b-CTx 3.8 ± 1.6 ng/ml, OC 300 ± 10.1 ng/ml and AP 128.7 ± 30.7 U/l. The mean values of P1NP, b-CTx and OC were significantly higher in third than in first and second group ($P < 0.01$).
P1NP, b-CTx and OC could be very useful markers in evaluation of renal bone disease but we need more data, particularly in postmenopausal women on haemodialysis, to evaluate clinically useful tool of all biochemical markers in assessing bone turnover.

P226-Su
Dental PQCT Scans of Patients with Different Mechanical and Hormonal Disturbances. Practical Examples of a New Diagnostic Tool
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Peridental bone is linked to high and frequent in situ loading impacts and is affected by regional muscles and/or systemic osteopathies. These multiple interactions modulate a sectional bone which is highly variable in quantity and quality. The pQCT technology (XCT 3000-D, Stratec-Germany) allows sectional exploration using a safe, non-invasive technique. We hereby show patients selected from daily practice with different regional abnormalities of bone structure or material properties. These abnormalities cannot be evaluated by studies performed at other skeleton sites, bone biochemical markers, or even whole section analysis. Hence, in situ exploration of bone properties is suggested. Tissue quality is classified by scales from type I (dense tissue) to type IV (severe osteopenia) according to Roldán et al. (*JBMR 2001,16(S1):S244*), and density as volumetric bone mineral density (vBMD) is calculated at cortical and medullar sites using the system software (Stratec-Germany). Healthy mean value for male/female: A_{110}: 89–72/86–92, A_{310}: 71–77/63–75, A_{430}: 58–67/50–66 and A_{900}: 23–24/22–26.
These cases show the relevance of local factors affecting bone quality and quantity, and the relevance of detecting them with peripheral tomography.

Table

Diagnosis	Patient features	A_{110}	A_{310}	A_{430}	A_{900}
Aromatase deficit	male, 33 years	89	***67***	***55***	***21***
Hypoparathyroidism	female, 25 years	86	***59***	***48***	22
Hyperparathyroidism	female, 42 years	90	***73***	***62***	25
Osteogenesis imperfecta	male, 12 years	***82***	***51***	***37***	***8***
Paget bone disease	male, 77 years	***95***	***83***	***75***	***40***
Acromegaly	female, 62 years	88	67	56	22
Marfan syndrome	female, 27 years	91	**72**	**68**	27
Hypothyroidism	female, 25 years	***65***	***43***	***39***	24

P227-Mo
Clinical Evaluation of COX2 Inhibitors vs. Indomethacin in the Prevention of Heterotopic Calcifications (HC) After Total Hip-Replacement
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Purpose: We evaluated the occurrence of HC after total hip-replacement under the treatment of COX2 inhibitors (Rofecoxib vs. Celecoxib) vs. Indometcin.
Materials and methods: 55 Patients (41 to 77 years) – 22 female and 33 male – after total hip replacement were examined. 31 were treated with COX2 inhibitor and 24 with Indometacin (duration of treatment 13.8 ± 2.93 days). For evaluation we used the Brooker classification of X-ray of the pelvis (98.5 ± 45 weeks after surgery). A chi-squared test was applied to the hypothesis that fewer patients developed HC under treatment with COX2 inhibitors compared to those treated with Indometacin.
Results: We found a significantly lower rate of HC in the patients treated with COX2 inhibitors in comparison to Indometacin. This is supported by a P value of 0.06 for the underlying hypothesis to be wrong. We found 5 HCs (4 Brooker I and 1 II) within 13.8 ± 2.93 days of treatment with Indometacin and only 2 under the therapy of COX2 inhibitors (2 Brooker I for Rofecoxib and none for Celecoxib). We could not find a difference between the two COX2-inhibitors yet.
Conclusion: COX2 inhibitors are an alternative in the treatment and prophylaxis of HC after total hip-replacement. If a significant difference between the two COX2 inhibitors can be found has to be evaluated with larger numbers of patients.

P228-Tu
Effect of Lipopolysaccharide on Migration of Osteoclast Precursors
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Periodontitis is an inflammatory disease in supporting tissue surrounding the root of tooth. Bone resorption is one of important characteristic in periodontitis. *Actinobacillus actinomycetemcomitans* (Aa) is one of representative pathogens associated with periodontitis. Although the mechanism of osteoclast formation by LPS from Aa is known, the effect of LPS from Aa on migration of osteoclast precursor is unknown. We observed the effect of LPS on the expression of matrix metalloproteinase (MMP)-9 in RAW cells, the expression of stromal cell-derived factors (SDF)-1 in osteoblasts and the migration of RAW cells in collagen-coated transwells. LPS increased the expression of MMP-9 in RAW cells and enhanced transmigration of RAW cells through collagen-coated transwells. Inhibitor of MMPs suppressed the migration of RAW cells induced by LPS. LPS also increased the expression of SDF-1 in osteoblasts. Culture supernatant (sup) of LPS-treated osteoblasts increased the migration of RAW cells and anti-SDF-1 Ab reduced the migration of RAW cells induced by culture sup. These results suggest that LPS from Aa increased the expression of MMP-9, a matrix protease expressed by osteoclast precursors, and SDF-1, a chemokine expressed by osteoblasts and that these factors are involved in migration of osteoclast precursors induced by LPS of Aa.

P229-Su

Increased Arterial Calcifications in Paget's Disease

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When examining radiographs of the pelvis, femurs or spine of patients with Paget's disease of bone (PD), we often observed calcification of the aorta and iliac or femoral arteries. The processes of extra-osseous calcification or mineralisation are very close to the mechanisms regulating mineralisation of bone tissue. The aim of our study was to quantify the number, extent (length and thickness), type (arteriosclerotic or medial arterial calcification) of vascular calcification in patients with Paget's disease and to compare them with those of controls, in order to discover whether PD is accompanied by increased calcification.

Vascular calcification was qualified and quantified on the aorta, the iliac arteries and their branches, the femoral, gluteal and pelvic arteries of 42 patients with Paget's disease and 36 control subjects.

Of the PD subjects, 52.4% had arteriosclerotic calcification versus 30.6% of controls ($P = 0.05$), and 38.6% of PD patients had medial arterial calcification versus 11.1% of controls ($P = 0.05$).The mean length of calcification was greater in PD patients: 1.93 ± 2.85 cm versus 0.84 ± 1.69 cm in controls ($P = 0.04$). The thickness of calcification was also greater in PD patients: 1.24 ± 1.30 mm versus 0.56 ± 0.94 mm ($P = 0.01$).

Patients with Paget's disease more frequently had medial arterial or arteriosclerotic calcification than controls. These calcifications were longer and thicker.

Our clinical study thus confirms that abnormal vessel wall calcification and bone remodeling in Paget's disease are probably linked, although the mechanism of this relation is as yet unexplained.

P230-Mo

Bone Changes Depend on Bone Density in Murine Osteoarthritis

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Osteoarthritis (OA) is characterized by cartilage damage and bone changes such as subchondral sclerosis and formation of bony outgrowths (osteophytosis). Although these changes increase the amount of bone in the affected joint at the end-stage of OA, it is not known whether this has negative consequences for disease progression. The cause of increased bone formation should lie in increased trabecular bone remodelling, but little is known about this process. We therefore took a dual approach to study the OA process:

(1) to establish how trabecular bone changes after induction of OA; and
(2) to discover whether bone density has a role in this process.

As models, we used the low bone density mouse strain (LBM) C57Bl/6 and the high bone density mouse strain (HBM) C3H/HeJ (male, both $n = 6$). OA was induced with an intra-articular injection of bacterial collagenase into the right knee, destabilizing the knee joint. The left knee served as a saline-injected control. After 4 weeks, the animals were sacrificed and the bone structure of the knee joints was digitized using a micro-CT scanner. We only analyzed the tibial epiphysis, calculating the following parameters: bone density (BV/TV), trabecular connectivity, the degree of plate-like structure and trabecular thickness. Next, 8–10 sections spaced 100 μm apart were histologically analyzed for cartilage damage and osteophyte formation.

The collagenase injection induced cartilage damage, which was 2.7-fold higher in HBM. Osteophytosis was found to be similar in both mouse strains. Micro-CT analysis of the saline-injected knee joints demonstrated the basal difference in bone structure between LBM and HBM. Compared to saline-injected knees, induction of OA in HBM led to a higher (+17%) trabecular connectivity and to a less plate-like structure in the epiphysis. In addition, significant decreases in bone density (−6%) and trabecular thickness (−22%) were found. In LBM the bone structure also became less plate-like, but not as pronounced as in HBM,

and decreases in bone density and trabecular thickness were not observed.

Induction of OA in murine knee joints caused trabecular bone remodelling in the tibial epiphysis in both high and low bone density mice. Bone density plays a role in this process, as the observed bone changes were more pronounced in mice with a high bone density. This may explain the more severe cartilage damage in these mice, thereby substantiating the mutual interaction of bone and cartilage in OA.

P231-Tu

Increased Bone Fracture Risk in Untreated Postmenopausal, Pollen-Allergic Women

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Our aim was to investigate whether pollen-allergy can affect bone mass and fracture risk in postmenopausal women. Forty postmenopausal pollen-allergic women (mean 61 years) were enrolled in the study. They were treated neither with H1 histamine receptor (H1R) antagonist nor with inhaled corticosteroid. Minimal duration of allergic symptoms were 5 years. Potential participants with secondary causes of osteoporosis or those on medication likely to affect skeletal metabolism were excluded from the study. Ninety-seven healthy postmenopausal subjects matched by age, body mass index (BMI) and age at menopause served as controls. 70% of allergic women were overweight ($25 \leq$ BMI $\leq$ 30kg/m^2) or obese (30 kg/m^2 < BMI). Allergic patients had lower bone density at femoral neck than healthy controls (T score -2.33 ± 0.13 vs. -1.93 ± 0.12, respectively, $P = 0.032$) and almost triple the rate of prevalent fractures (distal forearm, hip and vertebral: 34.9%) compared to healthy women (13%, χ^2 $P = 0.003$). In the allergic group the higher BMI was found to be a predictor for bone fractures (BMI in kg/m^2, odds ratio 1.278, 95% confidence intervals 1.047–1.559, $P = 0.016$). Our results indicate that pollen-allergy is a risk factor for lower bone mass and fractures. Obesity seems to predispose bone fractures in untreated pollen-allergic postmenopausal women. This study was supported by ETT 226/2003 and OTKA T038067.

P232-Su

Bone-Specific Alkaline Phosphatase in Men with Coeliac Disease

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Osteoporosis is a known complication of coeliac disease, and is ascribed mainly to malabsorption of calcium. Although this problem is more common in women, we have studied a group of male sufferers for whom we were able to select age-matched controls from another study. This also avoids the complicating factors of the menstrual cycle or the menopause. We measured bone-specific alkaline phosphatase in stored frozen serum (BALP, IDS Ltd, Boldon, UK), total serum alkaline phosphatase (TALP) and calcium/creatinine ratios (UCa/Cr) in 24-h urine collections from 45 men with coeliac disease and 45 age-matched healthy controls, who were also matched for time of year to avoid possible confounding by seasonal variation. Some of the coeliac patients had osteoporosis. We also measured serum PTH in the coeliac patients, who were treated by supplementation with calcium as well as avoidance of dietary gluten. Second samples were taken from 13 of the control men after an interval of a year, allowing estimation of within-subject variation, again avoiding seasonal factors. Inspection of the results ordered by month of sampling showed no obvious seasonal variation of any of the quantities which we measured, however. Pooled within-subject CV was 10.1%. Analytical between-run CV for the BALP assay was 8.4% at 8.6 μg/L, 2.5% at 12 μg/L and 1.2% at 49 μg/L. Within-run CV was 5.6% at 8.4 μg/L and 2.2% at 33 μg/L. The median BALP in coeliac patients was 11.8 μg/L (range 5.0–33.5) compared with 8.2 μg/L (range 5.0–14.0) in the controls ($P < 0.0001$ (Wilcoxon)). On this basis, a cutoff value of 10 μg/L would give a sensitivity of 70% at a specificity of 76%. For TALP, the equivalent results were 80.0 (range 40–153) and 57.0 (range 35– 91) IU/L ($P = 0.0002$). BALP correlated closely with TALP in coeliac patients ($r^2 = 0.80$) but much less closely in controls ($r^2 = 0.24$) (Pearson), which is consistent with the increase in TALP being mostly due to the BALP component. Median UCa/Cr was 0.17 (range 0.006–0.589) in coeliac patients and 0.24 (0.029–0.653) controls, but the difference was not significant ($P = 0.97$).

Neither BALP nor TALP fell during treatment, although PTH fell from 5.6 at 1–4 years since diagnosis to 3.8 at 5–10 years ($P = 0.04$). BALP appears to be better than TALP as a diagnostic aid, but its failure to fall during treatment requires further investigation. We recognise that comparative data on osteoporotic men without coeliac disease are also needed.

P233-Mo

Correlation of Nanomechanical Properties and Bone Organic/Inorganic Components in Osteolathyritic Treated Rat Bones

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Osteolathyrism is a generalized tissue defect, causing a defective collagen crosslink in bone and tendons. Beta-aminopropionitrile (BAPN) is the most active lathyritic agent, which irreversibly inhibits lysidyl oxidase. The basic unit of the bone material is the mineralized collagen fibril, which combines two materials at the nanometer scale: Collagen, which is a soft and tough material and carbonated apatite, which is a stiff and brittle material. The changes in mechanical properties of tendon collagen due to BAPN treatment has been reported (1). The aim of this study is to investigate the correlation of the effect of lysidyl-oxidase-cross-link-deficient collagen on mechanical properties of bone. The bone sections from vertebral bone of rats, treated with BAPN for 4 weeks, were used for this study. The measurements were performed on regions consisting of both normal bone before treatment and affected newly formed bone after treatment. The variations of local properties were investigated by two complementary methods, back-scattered electron imaging (qBEI) and scanning nanoindentation. Bone mineral density distribution (BMDD) measured by back scattered electron imaging (2) indicated a reduced mineral content within the newly formed bone matrix. The nanoindentation results suggest that the reduced indentation modulus Er and hardness of the normal bone before treatment remains unchanged, whereas the newly formed bone in treated rats have low values of Er (7 to 9 GPa) and hardness compared to untreated bone at the same mineral content. This reveals that the local mechanical properties of bone depend significantly on collagen-cross-links, in addition to the influence of mineral density and mineral particle size distribution.

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P234-Tu

Changes of Joint Forces Due to Subluxation of the Thumb Provoke Bone Adaptation in Trapezial Bone

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Background: Bone adaptation, as a reaction to altered loading conditions, is a well-accepted – however not fully understood – mechanism since the first description by Wolff. The goal of our study was to understand the microstructural changes in trapezial bone provoked by the subluxation of the thumb in trapeziometacarpal arthritis.

Methods: Fifteen osteoarthritic (OA) trapezial bones (five left-, ten right-sided) were surgically removed in female patients (65.1 ± 9.4 years) being treated for painful carpometacarpal I OA. The control group was selected from a set of 80 left-sided cadaver hands (donors 74.7 ± 9.2 years). The trapezial height and the subchondral sclerosis were measured in all trapezial bones. MicroCT scans were performed with a high-resolution microCT system (uCT 40; Scanco Medical AG). The analysis was limited to the medium 40% in dorsopalmar direction and further divided into three region of interest (ROI): radial, medium and ulnar. The structural parameters like trabecular number (Tb.N), thickness (Tb.Th) and separation (Tb.Sp) as well as bone volume fraction (BV/TV), structure model index (SMI) and connectivity density (Conn.D) were calculated for the osteoarthritic and control group. Outcome parameters were compared with an unpaired, two-sided Student's t test. Pairwise multiple comparisons between the three regions were performed using one-way ANOVA and a Tukey post hoc analysis in both osteoarthritic and control group.

Results: While the trapezial height in the osteoarthritic group was 22% less ($P = 0.0003$) the subchondral sclerosis was 50% thicker in OA compared with the control group ($P = 0.0004$). In the OA group there was a 42% higher bone density ($P = 0.0001$), an 18% increase in Tb.Th ($P = 0.006$) and a 10% greater Tb.N ($P = 0.034$) compared to the control group. Although BV/TV was significantly lower in the radial region in both groups, the radial column showed the highest relative increase in bone volume and structure compared with the control group (+67% BV/TV, +20% Tb.Th, +23% Tb.N).

Discussion: The loss of the elastic and force-spreading cartilage in carpometacarpal I OA facilitates the direct impact of forces to the subcortical bone that leads to diminished bone height and thickening of the subchondral sclerosis. The reinforcement of the bony microstructure, especially at the radial side, is a sign for bone adaptation reacting to the radially shifted joint forces. This has to be considered during the development of new treatment strategies.

P235-Su

Polyethylene Wear Influences Progression of Pelvic Peri-Prosthetic Osteolysis

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Peri-prosthetic osteolysis often limits the lifespan of total hip replacement implants. Accurate data on the extent and progression of osteolytic lesions is important for monitoring patients and planning surgical intervention. The aim of this study was to use quantitative computed tomography,

together with wear and migration analysis, to determine the location, volume and, for the first time, the rate of progression of peri-acetabular osteolysis. We also sought to examine the relationship between polyethylene wear and osteolysis. EBRA was first used to exclude cases with migrated components and the potential confounding effects of migration on osteolysis measurement. A high-resolution multislice computed tomography scanner, with metal artefact suppression protocol was used to locate and measure the volume of osteolytic lesions adjacent to the acetabular component of 42 cementless titanium hip arthroplasty prostheses. The progression of osteolysis over at least 12 months was determined for patients, who are being monitored prospectively. Polyethylene wear was determined from digitised X-rays, using Polyware. The incidence of osteolytic lesions located in each site was: ilium, 48%; medial wall, 26%; anterior column, 14%; posterior column, 9%; and ischium, 4%. In 64% of hips, lesions were located in multiple pelvic sites. Importantly, some osteolytic lesions were quiescent, while others progressed markedly. The median rate of progression of osteolytic lesions was 0.4 cm^3/12 months (range 0 to 8.1cm^3). The increase in osteolysis volume correlated strongly with volumetric polyethylene wear as well as the rate of PE wear. In hips with volumetric wear of less than 50 mm^3/year, the progression of osteolysis was small. These findings demonstrate the value of computed tomography as a sensitive technique for investigating peri-prosthetic osteolysis and accurately define a relationship between the progression of osteolysis and polyethylene wear. These studies are providing important data for patient management and a potential outcome measure for clinical trials designed to slow the progression of osteolysis.

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P236-Mo

Soft Tissue Uptake on Skeletal Scintigram

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A number of conditions may cause nonosseous uptake of skeletal radiopharmaceuticals. We report a case of 5-year-old patient previously hospitalised in another institution under the diagnosis of calcinosis extraosseous. The young girl presented numerous muscle nodules tender and painless, associated with coetaneous ulcers over them. Muscle weakens and contractures of lower limbs were developed. Laboratory findings showed elevated ERS, slightly decreased daily profile of Ca-P and tabular reabsorption of Phosphates in range lower for date. Muscle enzymes were in normal serum level. X-ray of pelvis and lower limbs showed extensive soft tissue calcifications with no connection to bones. 99m Tc-DPD scans showed multiple foci of increased tracer accumulation particularly in muscle region of hips, knees and elbows. Under the relevant criteria the diagnosis of juvenile dermatomyositis was done. Patient was treated with repeated Aredia infusion

P237-Tu

Enzyme Immunoassay for N-Terminal C-Type Natriuretic Peptide (NT-PROCNP)

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C-type natriuretic peptide (CNP) belongs to the group of natriuretic peptides that includes also ANP and BNP, which play a pivotal role as endogenous vasorelaxants. In clinical chemistry they are valuable markers of cardiac diseases. CNP, first identified in endothelial cells, is also found in chondrocytes and has been shown to be a potent stimulator of long bone growth. Most studies identify CNP as a locally acting positive regulator of endochondral ossification, which suggests potential pathophysiological and therapeutic roles in skeletal dysplasias. The proCNP sequence is highly conserved, indicating an additional so far unknown function. Thus CNP might be a valuable biomarker for studies in bone developmental biology and for diseases associated with disturbed long bone growth. Because the N-terminal forms of natriuretic peptides are more stable and present in the circulation in higher concentrations than the active peptide hormone, we chose epitopes (1–19) and (30–50) for our enzyme immunoassay. Antibodies were raised in sheep and used in a not-competitive sandwich type assay. Native human proCNP was immunoaffinity-purified from human serum, characterised by SDS–PAGE electrophoresis and used to prove the specificity of the assay. First data on assay performance and on human serum levels are presented. We have shown that Nt-proCNP is present and can be measured in human plasma. Deducing from the amino acid sequence, also determinations in mouse, rat, pig or bovine serum should be possible.

Lit:

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P238-Su

Bone Effects of Raloxifene in a Man with Estradiol Deficiency

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We present the first case of a 25-year-old man with low estradiol levels (studied for aromatase activity), who was treated with raloxifene 60 mg/day for one year. Previously, we described the favorable response to estradiol treatment of two men with aromatase deficiency, a genetic defect characterized in men by high stature, persistent linear growth, eunuchoid skeleton, unfused epiphyses, genu valgum and osteoporosis (Carani, N Engl J Med 1997; Maffei, J Clin Endocrinol Metab 2004). Clinical characteristics of the patient here presented were bone pain, tall height and persistent linear growth due to incomplete closure of growth cartilages (bone age 15.3 years), low BMD and eunuchoid proportions of the skeleton. We show the effects of raloxifene administration (60 mg/day) after 6 and 12 months of treatment [supplemented with calcium citrate 3000 mg/day (630 mg Ca+) + vitamin D 400 UI/day]. Ultrasound forearm bone mineral density (BMD) mostly improved after the first 6 months of treatment altogether with a progressive decrease of the markers of bone resorption and increase in those of formation, with no effect on bone age.
In aromatase deficient men estrogen therapy results in the induction of bone maturation, closure of growth cartilages, normalization of serum markers of bone remodeling and an increase in BMD observed during. Conversely in this patient raloxifene led only to an improvement of bone remodeling markers and to an increase of BMD. Bone biopsy before treatment showed an increased trabecular remodeling with wide bone resorption and formation surfaces, and increased osteoblastic activity; after an year of treatment bone remodeling appeared greatly reduced with a normalization of all histomorphometric parameters. These data prove that in men mechanisms of estrogen action are different in relation to the tissue involved (cartilage or bone) and the biological effects (growth or mineralization), suggesting that pathways recruited for estrogen action may be heterogeneous with respect to the activation of different receptors or post-receptorial transductive way as well as the characteristics of the estrogen compound used for treatment.

P239-Mo

Estrogen and Bone Metastases in Breast Cancer

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Bone is one of the most frequent sites for metastasis in breast cancer patients. It has been shown that patients with ER-positive tumors have had bone metastases three times more often than patients with ER-negative tumors. However, the role of estrogens (E2) in progression of breast cancer bone metastases is largely unknown partially because there are very few ER-positive models of breast cancer bone metastases. To study complex molecular mechanisms responsible for bone metastases that involve bi-directional interactions between tumor cells and bone, we have established an in vitro hormone-responsive bone metastases model, which consists of ER (a or b)-positive metastatic breast cancer cells and ER (a or b)-positive osteoblasts. Using bi-compartmental co-culture system we examined expression of E2-regulated metastasis-associated genes. We found that E2 downregulates uPA expression in both breast cancer and bone cells, however co-culturing increases expression of uPA by bone cells, which plays role in several aspects of bone resorption, including osteoclast formation, mineral dissolution and degradation of the organic matrix. In contrast, expression of IGFBPs was increased in the presence of E2 in co-cultured cancer cells. More interestingly, the levels of IGFBPs were dramatically increased in co-cultured MDA-ERb cells compared with co-cultured MDA-ERa. These results suggest that increased levels of inhibitory IGFBPs might block the activity of IGFs, which have strong mitogenic and antiapoptotic effects on breast cancer cells. Therefore, these data imply possible favorable effects of ERb in the pathogenesis of breast cancer. Experiments are in progress to determine the role of ERa/ERb in the development of osteoblastic/osteolytic bone metastases using an in vivo mouse intracardiac model.
Conclusions: Our data demonstrate that the transcriptional effects of E2 via ERa and ERb are largely distinct in co-cultured breast cancer and bone cells indicating possible unique biological roles of each isoform in the development and progression of bone metastases. Identification of critical metastasis-associated genes and respective roles of ERa and ERb in response to E2 will suggest possible new strategies of hormonal therapy in advanced metastasic disease.

P240-Tu

Free Testosterone is a Positive While Free Estradiol is a Negative Predictor of Cortical Bone Size in Young Swedish Men–The Good Study

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Introduction: Previous studies have demonstrated that free estradiol in serum is an independent predictor of areal bone mineral density (aBMD) in elderly men. The aim of the present study was to determine whether sex steroids are predictors of volumetric BMD (vBMD) and/or size of the trabecular and cortical bone compartments in young men at the age of peak bone mass.
Methods: The Gothenburg Osteoporosis and Obesity Determinants (GOOD) study consists of 1068 men, age 18.9 ± 0.6 years. Serum levels of testosterone, estradiol and SHBG were measured and free levels of testosterone and estradiol were calculated. The size of the cortical bone and the cortical and trabecular vBMDs were measured by pQCT.
Results: Regression models including age, height, weight, free estradiol and free testosterone demonstrated that free estradiol was an independent negative predictor of cortical cross-sectional area (tibia beta = 0.111, $P < 0.001$; radius beta = 0.125, $P < 0.001$), periosteal circumference and endosteal circumference while it was a positive independent predictor of cortical vBMD (tibia beta = 0.100, $P < 0.003$; radius beta = 0.115, $P = 0.001$) in both tibia and radius. Free testosterone was an independent positive predictor of cortical cross-sectional area (tibia beta = 0.071, $P = 0.013$; radius beta = 0.064, $P = 0.039$), periosteal circumference and endosteal circumference in both tibia and radius. Neither cortical nor trabecular vBMD was associated with free testosterone.
SHBG was an independent positive predictor of parameters reflecting the size of the cortical bone, including cross-sectional area (beta = 0.078, $P = 0.009$), periosteal circumference and endosteal circumference.
Conclusions: Free estradiol is a negative while free testosterone is a positive predictor of cortical bone size in young men at the age of peak bone mass. These findings support the notion that estrogens reduce while androgens increase cortical bone size, resulting in the well-known sexual dimorphism of the cortical bone geometry.

P241-Su
Chromatin Dynamics at Inflammatory, Bone-Wasting Genes Suppressed by Estrogen
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Estrogen (E)-mediated suppression of pro-inflammatory cytokines is crucial in bone homeostasis. E inhibits inflammation both downstream, by interfering with signaling cascades (AP-1, NF-kB) that transactivate pro-inflammatory cytokines, and upstream, e.g., by decreasing the expression of the MHC class II transactivator (CIITA). However, the molecular mechanisms by which E represses inflammatory genes are currently unclear. In particular, while chromatin dynamics are known to mediate activation of gene expression upon interaction of the liganded E receptor with an E responsive element (ERE), whether chromatin remodelling occurs in E-mediated gene suppression is unknown.
We investigated the regulation of CIITA as a paradigm of inflammatory genes suppressed by E. We first observed that E withdrawal in vivo enhances inducibility of CIITA in murine bone marrow macrophages (BMM), an effect mimicked by treatment with histone deacetylase inhibitors, pointing to a modulation of gene accessibility by E via chromatin remodelling. We then explored the chromatin level in vivo by chromatin immunoprecipitation on primary purified BMM from ovariectomized mice. We observed that E deficiency in vivo profoundly increases acetylation at the amino-terminal tails of histones H3 and H4 at the whole CIITA regulatory region, with a selective effect on H3 at CIITA promoter IV (pIV), the key inducible promoter. We found pIV to be the main target of E-mediated suppression, as demonstrated by the failure of E to suppress CIITA induction in BMM from mutant mice lacking selectively CIITA pIV. Within H3 E deficiency promotes acetylation of lysine 9 (K9) and methylation of K4, two modifications key in directing gene expression, while having no effect on K14, revealing a specific panel of E-dependent modifications. We then confirmed our in vivo findings in a murine macrophagic cell line treated with E in vitro. Furthermore, we attempted to generalize our findings to other bone-wasting inflammatory genes, and analyzed the effect of E on histone acetylation at the promoter regions of IL-6, IL-1beta, and TNFalpha. Interestingly, we observed that E suppresses basal histone acetylation at these cytokine promoters.
Taken together, our data describe a histone code underlying the control exerted by E on CIITA expression, and suggest that an E-dependent control of chromatin accessibility may represent a general mechanism whereby E suppresses genes encoding bone-wasting inflammatory cytokines.

P242-Mo
Investigation of Central versus Peripheral Effects of Estradiol in Ovariectomized Mice
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Estrogen deficiency results in bone loss. It is generally believed that estrogen exerts its bone sparing effects directly on the cells within the bone compartment. However, some studies indicate that the effects of estrogen on bone might be indirect and it has been suggested that the hypothalamus might be of importance for the regulation of both fat mass and bone mass. The aim of the present study was to investigate the relative contribution of peripherally versus centrally located estrogen receptors for the mediation of effects of 17β-estradiol (E2) on bone and on some other estrogen responsive tissues.

Dose–response studies of peripherally (subcutaneous, s.c.) administered E2 demonstrated that E2 treatment increased the uterine weight (ED50 = 0.9 μg/kg/day), the trabecular bone mineral density (BMD, ED50 = 12 μg/kg/day) and the cortical bone mineral content (BMC, ED50 = 0.9 μg/kg/day) while it reduced the weights of the retroperitoneal fat depot (ED50 = 1.7 μg/kg/day) and the thymus (ED50 = 1.9 μg/kg/day) in ovariectomized (ovx) mice.

We next investigated the dose response of central (intracerebroventricular) E2 administration and compared it with that of peripheral (s.c.) E2 administration in ovx mice. The dose–response curves for central and peripheral E2 administration did not differ for any of the studied estrogen-responsive tissues, indicating that these effects were mainly peripherally mediated.

In addition, to investigate the relative contribution of central versus peripheral mechanisms of E2 on estrogen responsive tissues, ovx mice were treated with E2 and/or the peripheral estrogen receptor antagonist ICI 182,780. We then calculated to which degree ICI 182,780 could attenuate the E2 response in these tissues. A high degree of attenuation would indicate that the effect on the specific tissue is peripherally mediated since ICI 182,780 does not cross the blood brain barrier. ICI 182,780 attenuated most of the estrogenic response regarding uterus weight, retroperitoneal fat weight, cortical BMC and trabecular BMD ($P < 0.05$) while the attenuation was intermediate for the thymus (not significant).

In conclusion, the sensitivity for E2 is tissue dependent and our findings indicate that primary target tissue for the mediation of the effect of E2 on bone is peripherally and not centrally located.

P243-Tu

Bone Phenotype of NERKI Mice

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Estrogens regulate bone growth and mass. The actions of estrogens are mediated mainly through estrogen receptors. NERKI mice carry a mutation that selectively eliminates classical ERa signaling. Heterozygous (+/NERKI) females are infertile. Therefore we performed and report studies on the bone phenotype of +/NERKI mice (called NERKI hereafter). In study one the bone mass, volume and femur length were determined in 5-month-old mice. Bone mass and length were also determined at 12 months of age. Global bone turnover was assessed by measuring serum levels of osteocalcin (OCN) and TRACP 5b. Whole body (WB), spine and femoral areal BMD were determined by DEXA. Volumetric distal femoral density and structure of the secondary spongiosa were determined by microCT. There were at least 5 animals in each study group except for microCT studies ($n = 3$). In a second study we determined the effect of the NERKI mutation on estradiol (E2)-mediated prevention of orchidectomy (Orx)-induced bone loss. WT and NERKI males ($n = 3$) were castrated and treated with E2 replacement for 4 weeks. WB and spine BMD at the end of the treatment period were measured and expressed as a percentage of the pre-Orx values. Normally distributed data were analyzed by Student's t test. Differences were considered statistically significant if $P < 0.05$. WB BMD was lower in 5- or 12-month-old NERKI animals of either gender compared with gender matched WT ($P \leq 0.003$). Spine and femur BMD were lower in 5 month old NERKI animals of either gender compared with gender matched WT ($P \leq 0.01$). Distal femoral trabecular bone volume and connectivity density of both genders of NERKI mice were decreased. Trabecular number was also decreased in both genders of NERKI mice. Female NERKI mice showed significantly decreased trabecular thickness. Female NERKI mice aged 5 or 12 months showed shorter femurs compared with WT ($P \leq 0.02$) whereas there was no change in the femur length of NERKI males. OCN levels were decreased in both genders of NERKI mice ($P = 0.05$). Female NERKI mice had significantly higher TRACP levels but males showed no change. Thus, there is a sexually dimorphic bone remodeling abnormality. The average percentage increase in either WB or spine BMD after Orx + E2 was higher in WT males versus NERKI males (13% vs. 2.5%; 19% vs. 7%). In summary, NERKI mice have low bone mass, sexually dimorphic defects in longitudinal growth of the appendicular skeleton and in bone remodeling.

P244-Su

Abstract Withdrawn

P245-Mo

The Methylenetetrahydrofolate Reductase (MTHFR) C677T Polymorphism and Bone Mineral Density in Postmenopausal Women in Malta

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The common variant C677T within the MTHFR gene that results in a missense substitution from alanine to valine has been associated with cardiovascular disease, neural tube defect and recently also with bone mineral density (BMD). This polymorphism was studied for any association with BMD in a group of postmenopausal Maltese women.

126 postmenopausal Maltese women (55.6; SD: 7.1 years) were recruited for this study. The C677T variant within the MTHFR gene was analysed by PCR restriction fragment length polymorphism (RFLP) while BMD at the lumbar spine, femoral neck, Ward's triangle and trochanter was measured by DEXA.

Genotype frequencies observed were 38.9% CC, 50.0% CT and 11.1% TT with the C allele found in 63.9% of the population and the T allele in 46.3%. Genotype frequencies were in Hardy–Weinberg equilibrium. The highest BMD at all anatomical sites was observed in TT homozygotes although no statistical significance was reached when comparing the three genotypes by using ANOVA ($P > 0.05$) even after adjustment for age, BMI and years since menopause. When comparing TT with CC homozygotes statistical significance was almost reached at the femoral neck (t test: $P = 0.08$). The C allele was also observed to have a dominant negative effect on trochanter BMD when testing for genetic models of dominant and recessive alleles (t test: $P = 0.03$). MTHFR C677T genotype frequencies did not differ significantly between normal postmenopausal women and those having a low BMD at either the lumbar or femoral neck (t score < -1.0) (chi-square test: $P = 0.68$). In contrasting to previous studies, the C allele was observed to have a negative effect on BMD showing allelic heterogeneity between different populations. These differences might be due to differences in environmental factors including folic acid intake together with other factors affecting gene expression, including epigenetic effects that results in differences at the phenotypic level.

P246-Tu

Impact of Multiple Candidate Genes on Bone Mineral Density in Chinese Women

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Osteoporosis is a multifactorial and polygenic disease caused by the combined effects of genetic and environmental factors. Genetic factors have been estimated by twin and family studies to be responsible for 75–80% of the variance in bone mineral density (BMD). Linkage studies in humans and mice have identified several loci that show linkage to BMD; however, the causative genes remain to be identified. So, the purpose of this study was to assess the contribution of osteoprotegerin (OPG), parathyroid hormone (PTH), calcitonin receptor (CTR), osteocalcin (BGP) and leptin receptor (LERP) gene polymorphisms to the variation of BMD in 504 Chinese women (282 pre- and 222 postmenopausal). The differences of BMD at the lumbar spine (L2–4) and femoral neck (FN) across each genotypes (OPG Lys3Asn, PTH 3244 G/A, CTR 1377 C/T, BGP 298 C/T, LEPR Gln223Arg) were tested by analysis of covariance (ANCOVA) adjusted for age and BMI.

In postmenopausal women, we found that individuals with Asn3Asn genotype of the OPG gene have significantly higher BMD at L2–4 compared to those with Lys3Lys genotype ($P = 0.007$), while women with bb genotype of the PTH gene have higher L2–4 BMD compared to those with BB genotype ($P = 0.002$). No significant association was observed between BMDs and CTR, BGP and LEPR polymorphisms. Multiple regression analysis revealed that BMI (6.8% at L2–4, 8.2% at FN) and age (4.9% at FN) accounted for the variance of BMD in premenopausal women. As for the postmenopausal women, age (9.0% at FN), BMI (5.3% at L2–4, 7.7% at FN), OPG gene (8.2% at L2–4) and PTH gene (6.9% at L2–4, 8.8% at FN) accounted for the variance of BMD. Logistic regression analysis showed that OPG Lys3Asn ($P = 0.008$) and PTH 3244G/A ($P = 0.03$) polymorphisms were independent risk factors of osteopenia/osteoporosis in postmenopausal women.

In summary, no significant association was found between BMDs and five candidate genes in premenopausal women. While Asn3Asn genotype of the OPG gene and bb genotype of the PTH gene associated with higher L2–4 BMD in postmenopausal women. In conclusion, OPG and PTH gene maybe useful genetic markers for BMD or osteoporosis in Chinese postmenopausal women.

P247-Su

Relationship Between Klotho Polymorphisms with Bone Mineral Metabolism and Cardiovascular Risk Factors in Korean Women

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A novel gene, termed klotho, has been identified that is involved in the suppression of several aging phenotypes. A defect in klotho gene expression in the mouse results in a syndrome that resembles human aging, including a short lifespan, infertility, arteriosclerosis, skin atrophy, osteoporosis and emphysema. Recent works have demonstrated the possibility that the klotho gene is a genetic risk factor for early-onset coronary artery disease, survival and osteoporosis in human populations, although it is not clear why. Thus, the aim of this study is to investigate the relationship between klotho gene polymorphisms with bone mineral metabolism and cardiovascular risk factors in Korean women. We observed 251 healthy women (mean age, 51.3 ± 6.9 years). We determined cardiovascular risk

factors. Bone turnover markers and lumbar spine and femoral neck bone mineral density (BMD) were measured by standard methods. G395A and C1818T polymorphisms of klotho gene were analyzed by allelic discrimination using the 5-nuclease polymerase chain reaction assay. Lumbar spine BMD was decreased in the G395A variant allele group as compared with wild type group (0.92 ± 0.16 g/cm^2 [GA + AA] vs. 0.98 ± 0.17 g/cm^2 [GG], $P = 0.025$). Systolic blood pressures were increased in the G395A variant allele group as compared with wild type group (128.8 ± 20.0 mm Hg [GA + AA] Vs. 123.3 ± 17.1 mm Hg [GG], $P = 0.031$). Fasting glucose levels (90.7 ± 11.8 mg/dL [CT + TT] Vs. 87.0 ± 8.4 mg/dL [CC], $P = 0.005$) and HOMA IR (1.17 ± 0.77 [CT+TT] Vs. 0.99 ± 0.54 [CC], $P = 0.035$) were elevated in the C1818T variant allele group as compared with wild type group. We observed that the klotho polymorphisms were associated with lumbar spine BMD in Korean women. Also, these data suggest that klotho polymorphism were related with some cardiovascular risk factors in Korean women. Further studies are needed to clarify this relationship.

P248-Mo

Relationship Between Osteoprotegerin Polymorphisms with Aortic Calcification and Bone Mineral Metabolism in Korean Women

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Osteoprotegerin (OPG) is a recently identified cytokine that acts as a decoy receptor for the receptor activator of NF-kB ligand (RANKL). OPG has been shown to be an important inhibitor of osteoclastogenesis and arterial calcification in animal models. Recently, OPG has been proposed as a link molecule between osteoporosis and arterial calcification, but the relationship between OPG gene and cardiovascular system in human populations is unclear. Thus, the aim of this study was to investigate the relationship between OPG gene polymorphisms and aortic calcification in Korean women. We observed 251 healthy women (mean age, 51.3 ± 6.9 years). We determined cardiovascular risk factors. Bone turnover markers and lumbar spine and femoral neck bone mineral density (BMD) were measured by standard methods. Thoracic and abdominal aortic calcifications were examined by simple radiological methods. A163G, G209A, T245G and T950C polymorphisms of OPG gene were analyzed by allelic discrimination using the 5'-nuclease polymerase chain reaction assay. The frequency of aortic calcification was increased in the variant allele group as compared with wild type group (G209A, 20.0% [AA + AG] Vs. 12.6% [GG], $P < 0.001$; T950C, 16.9% [CC + CT] Vs. 11.0% [TT], $P < 0.001$). Lumbar spine BMD of premenopausal women was marginally decreased in the in the variant allele group as compared with wild type group (A163G, 0.98 ± 0.14 g/cm^2 [GG + GA] Vs. 1.05 ± 0.15 g/cm^2 [AA], $P = 0.070$; T245G, 0.97 ± 0.13 g/cm^2 [GG + GT] Vs. 1.04 ± 0.15 g/cm^2 [TT], $P = 0.056$). We observed that the OPG polymorphisms were partly associated with aortic calcification and lumbar spine BMD in Korean women. Further studies are needed to clarify this relationship.

P249-Tu

Polymorphisms in the Promoter Region of the Estrogen Receptor Beta Gene are Associated with Higher Risks of Osteoporosis in Both Men and Women

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Introduction: Estrogen receptor beta gene (ESR2) is a candidate gene for osteoporosis and the dinucleotide CA repeats in the intronic region is associated with low bone mineral density (BMD). We hypothesized that other polymorphic sites in the promoter or exonic regions of the gene may also be associated with BMD and osteoporosis risk. This study aims to identify other polymorphic sites in the ESR2 gene and to determine their association with BMD.

Methods: Single nucleotide polymorphisms (SNPs) of the ESR2 gene in Southern Chinese were determined by sequencing the promoter and the exonic regions of the gene in 50 normal subjects. Association of 11 SNPs with BMD in both men and women was evaluated through a case-control study consisting of 1230 Southern Chinese subjects (678 female and 93 male case-control pairs). The cases were subjects with BMD Z score < -1.3 at either the spine or total hip region (equivalent to the lowest 10th percentile of the population) and the controls were subjects with BMD Z score $> +1$.

Results: Two SNPs in the promoter region (nt −1068 and nt −1285) were significantly associated with BMD at both the lumbar spine, femoral neck and total hip (all $P < 0.01$). Male subjects with SNP haplotype CCGGATCAAG was associated with a 10% reduction in BMD at both the hip and spine while females had a reduction in BMD of 6%. Furthermore, this haplotype was associated with higher risk of having osteoporosis at the lumbar spine (male: odds ratio 3.4, female: odds ratio 2.8) and at the hip (male: odds ratio 1.9, female: odds ratio 2.9).

Conclusion: Polymorphisms in the promoter region of the ESR2 gene are associated with lower BMD and higher risk of osteoporosis in both males and females. These SNPs may serve as a potential marker for assessing the risk of osteoporosis.

P250-Su

Associations Between the Novel Candidate Genes for Osteoporosis and Circulating 25-OH Vitamin D in White Postmenopausal Women

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Low-density lipoprotein receptor-related protein 5 (LRP5) gene (*C/T* polymorphism) has been recently found to be related to bone mineral density (BMD) in white human subjects. On the other hand, the *Tru*I (Uu) polymorphism in vitamin D receptor (VDR) gene has not been investigated in association with bone metabolism. We determined the distribution of the *C/T* (A1330V) polymorphism (in exon 18) and the TruI polymorphism in the cohort of 165 peri- and postmenopausal white women. Furthermore, we analyzed association between these polymorphisms and BMD at the hip or at the spine (g/cm^2) (n = 112), systemic values of parathyroid hormone (PTH) and/or serum vitamin D metabolites levels (n = 60). Single nucleotide polymorphisms (SNP) were determined by a restriction analysis of the PCR product.

Results: Distributions of the polymorphisms were as follows: LRP5-CC 73.9%, TC 23.6% and TT 2.4%; *Tru*I-UU 75.2%, Uu 23%, uu 1.8%. No relationship was observed between these polymorphisms and BMD at any investigated site of the skeleton. However, women with the *u* allele present (Uu and uu genotypes) had lower circulating 25-OH vitamin D as compared to subjects with the *u* allele absent ($P < 0.0026$, ANCOVA). Moreover, carriers of a T allele (TC and TT genotypes) had higher serum 25-OH vitamin D levels than women without this allele ($P < 0.039$). No associations were observed between both these polymorphisms and serum PTH or 1,25(OH)2 vitamin D levels.

In conclusion, this preliminary study showed that *Tru*I (VDR) and *C/T* (LRP5) polymorphisms are associated with serum 25-OH vitamin D in postmenopausal white women. Regulating importance of these polymorphisms for vitamin D homeostasis remains to be determined in larger cohorts.

P251-Mo

Functional Gene Variant Within Pai-1, but not within TGF-beta1, is Associated with Bone Mineral Density in Czech Women

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Introduction: Transforming growth factor β-1 (TGFβ-1) accelerates the steam cells differentiation to bone cells, influences the bone maturation and could probably effect the development of osteoporosis. One of the TGFβ-1 regulatory proteins is plasminogen activator inhibitor (PAI-1). Functional variants in these genes have been described (Leu10/Pro and Arg25/Pro in TGFβ-1 gene and 4G/5G variant in PAI-1 gene). Leu10/Pro variant was associated with risk of osteoporosis in Japanese females. We have evaluated if these variants determine the risk of osteoporosis development in Caucasian females.

Patients and methods: TGFβ-1 and PAI-1 polymorphisms have been analyzed using PCR and restriction analysis in 1368 females (aged 25–65 years) representatively selected from the population, 172 patients with low bone mineral density (BMD) and in 90 females with high or normal BMD (aged 45–75 years).

Results: Frequencies of the TGFβ-1 alleles or genotypes were not different among evaluated groups. The presence of the PAI-1 4G/4G homozygotes is the lowest in the group with high BMD (27%) and the highest in the group with low BMD (38%) with population having an intermediate frequency (32%, P for trend = 0.05).

Conclusion: TGFβ-1 polymorphisms (Leu10/Pro and Arg25/Pro) are not associated with BMD in Czech Caucasian females. PAI-1 gene 4G/4G homozygotes could be under higher risk of osteoporosis development.

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P252-Tu

Vitamin D Receptor Gene Haplotype is Associated with Body Height

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The vitamin D endocrine system is pleiotropic and plays a central role in calcium and phosphate homeostasis and bone metabolism. Some polymorphisms of the vitamin D receptor (VDR) gene were previously found to be associated with height and bone size in children and young adults. Recently, we resolved the linkage disequilibrium (LD) structure of the complete VDR gene. We here investigated the relationship between VDR haplotype alleles and body height and bone geometry parameters in 6137 elderly Caucasians from the Rotterdam study. We found that haplo1 in the 3′-end LD block of the VDR gene predicted decreased body height in the elderly at baseline with evidence for an allele-dose effect. The height decreased 0.3 cm per haplotype1 allele ($P = 0.007$ for trend). The relationship was independent of age, gender and presence of vertebral fractures. We also observed a trend towards decreased lumbar spine vertebral area, but this was

not significant (P = 0.21). No association between the haplotypes in any LD blocks and femoral narrow neck width was found. We also observed the Pvu–Xba haplo1 of the estrogen receptor alpha (ER-alpha) gene to be associated with decreased body height in the same population (0.35 cm/allele; P = 0.005, adjusted for age and gender). An additive effect was found for haplotype alleles of VDR and ER-alpha gene on body height with individuals who carry both homozygous genotypes having 1.7 cm decreased height compared to non-carriers (P = 0.02). To investigate the underlying mechanism of the association between VDR haplotype and height, we transfected constructs with haplotype 1 and 2 of VDR 3′-UTR into MG63 (an osteoblast cell line). The normalized VDR mRNA level of haplotype1 transcript is 25% decreased compared to haplotype2 (P = 0.001), and the decay rate of haplotype1 is 30% increased compared to haplo2 (P = 0.02) as measured 24 h after inhibiting transcription. In conclusion, haplotype1 at the 3′-end LD block of the VDR gene is associated with decreased body height in the elderly, independent of age, gender and presence of vertebral fracture. This genotype effect seems to be present at childhood and is maintained throughout life. The underlying mechanism might involve lower copy numbers of VDR protein (in carriers of this risk haplotype) in cells important for determining bone size (osteoblasts and/or chondrocytes).

P253-Su

Analysis of the ppar-Gamma3 Promoter Polymorphism (681 C/G Transversion) in Spanish Postmenopausal Women

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The ppar-gamma gene encodes a nuclear receptor involved in a wide variety of physiological processes, with particular relevance in the differentiation of adipocytes from precursor cells. It has been suggested that the induction or repression of the ppar-gamma gene could be involved on the regulation of osteoblastogenesis. Thus, this gene has been proposed as a candidate in the quest for genetic polymorphisms associated to osteoporosis. So far, several polymorphisms of the ppar-gamma gene have been described, being associated with diabetes, obesity and osteoporosis. Recently, a polymorphism (SNPS) has been reported on the promoter of the ppar-gamma3 gene, involving a C/G transversion located in the binding site of the transcription factor STAT5B. The presence of this allele prevents the union of STAT5B to the promoter region, and thus precludes the transactivation of the ppar-gamma3 promoter. Furthermore, the G allele has been associated with an increase of cholesterol LDL.

We have carried out a pilot study with a population of 225 postmenopausal women of Córdoba (Spain) to evaluate the putative association between the latter polymorphism and osteoporosis or low bone density. According to the World Health Organization (WHO) diagnostic criteria, we considered osteoporosis when T score was equal or below −2.5. This work was carried out using an allele-specific PCR genotyping procedure on single tube that we have setup in our laboratory. This methodology tells apart the alleles by means of the different melting temperature of the corresponding amplicons.

The studied population showed allelic frequencies of 0.82 (C) and 0.18 (G). The most abundant genotype was CC (65.9%), followed by CG (32.7%) and GG (1.4%). Following the WHO criterion, the allelic frequencies and genotypic percentages of both groups were, respectively: C (0.83), G (0.17); CC (67.6%), CG (31.7%) and GG (0.7%) for normal and osteopenic women vs. C (0.80), G (0.20); CC (63.1%), CG (34.5%) and GG (2.4%) for osteoporotic women. Our study points out that this polymorphism probably has a subtle, yet clinically relevant effect involving the impact of aging as well as different pharmaceutical drugs on bone mass. Therefore, we strongly propose that a large-scale multicentric study should be carried out to establish the possible genetic effects of this polymorphism on osteoporosis.

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P254-Mo

Polymorphism of Vitamin D Receptor Gene is Associated to the Phenotype and the Bone Mineral Density in Patients with Turner Syndrome

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The aim of this study was to determine the vitamin D receptor [VDR] and the estrogen receptor [ER] genotypes in patients with Turner syndrome [TS] in relation with their karyotypes, clinical phenotypes (minor, mild and severe forms) and different variables associated to the calcium and phosphorus metabolism.

Patients and methods: DNA was isolated from blood in 51 TS patients (mean age 15.82 ± 6.33 years old, range 6.4–31 years). The genotypes were determined by using *Bsm*I (VDR), and *Xba*I (ER) and *Pvu*II (ER) as restriction

enzymes. Serum calcium and phosphorus, PTH-molecule intact, beta crosslaps, 25-hidroxyvitamin D and osteocalcin were measured by standard procedures. Karyotypes were determined by chromosome banding techniques. Bone mineral density [BMD] of the lumbar spine (L2–L4) and the left proximal femur (femoral neck) was measured using a dual-energy X-ray absorptiometry (Nordland XR36 Quick Scan). Analysis of variance, covariance, Bonferroni post hoc test, Student's *t* test and Chi square were employed as statistical methods. All $P < 0.05$ were considered statistically different.

Results: The karyotype 45, Xwas present in 36 patients (70.6%) while 15 patients presented different variants of mosaicisms. The distribution of VDR and ER genotypes was BB 21.6%, Bb 66.7% and bb 11.8% (*Bsm*I); PP 13.7%, Pp 54.9% and pp 31.4% (*Pvu*II) and XX 15.7%, Xx 39.2%, and xx 45.1% (*Xba*I). Genotype bb was significantly higher in patients with severe phenotype versus those patients with mild forms (27.8% vs. 3.0%, χ^2 $P < 0.01$). The femoral neck and the lumbar spine BMD *Z* score presented a significant association with the VDR genotypes. TS patients with genotype bb had the lowest femoral and lumbar spine BMD (Test de Bonferroni, $P < 0.01$ for both skeletal sites as compared to the other genotypes). Serum biochemical values were all normal and they did not show any difference in relation with the genotypes and karyotypes. VDR and ER genotypes were not associated with the karyotypes.

Conclusions: The data suggest that TS patients with genotype bb present a significant association with severe phenotype and lower femoral and lumbar spine BMD.

P255-Tu

Estrogen Receptors Polymorphisms: Relation with Bone Mineral Density and Lipid Profile and Response to Hormone Replacement Therapy in Postmenopausal Women

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Estrogen receptor alpha (ER\alpha) genes polymorphisms has been widely studied in the evaluation of several chronic disorders, such as breast cancer, Alzheimer dementia, cardiovascular disease (CVD), and osteoporosis. However, results are often conflicting. Similarly, ER\alpha genotype appeared to segregate with lipid profile in CVD, with CVD risk factors or with CVD events but with conflicting results. Controversely limited information is available on the relation of ER\beta genotype with osteoporosis, with the body mass index and with ovarian disfunction. The effect of ER\beta polymorphism on lipid profile and lipid and bone response to hormone replacement therapy (HRT) has never been investigated.

With these premises, the purpose of our study was to evaluate the influence of ER\alpha and ER\beta genes polymorphisms on both the lipid profile and BMD in a population of healthy postmenopausal women. Additionally in a subpopulation who completed 1 year treatment with HRT, the potential influence of both ERs genotypes on the response to HRT was evaluated.

Aim: Segregation analysis of two polymorphisms in the ER\alpha gene (*Pvu*II and *Xba*I) and one polymorphism in the ER\beta gene (*Alu*I) with bone mineral density at the lumbar spine and forearm and with lipid profile was performed in 1098 women. Additionally, rate of bone loss and incidence of spinal fractures during a mean follow-up time of 11.4 years was compared to genotype in a subpopulation of 443 women, who did not receive any treatment. In another 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 genotype.

Results: Baseline BMD, rate of bone loss, incidence of spinal fractures and response to HRT did not significantly relate to ER\alpha gene polymorphisms, while a borderline difference in baseline vertebral BMD with the ER\beta gene polymorphism was found ($P = 0.07$).

Additionally, the ER\beta gene polymorphism was significantly associated with bone loss in the forearm and with the response in total cholesterol during treatment with HRT after 1 year ($P = 0.05$).

Conclusions: In a Caucasian population of postmenopausal women, no association with ER\alpha gene polymorphisms was found with bone mineral density and lipid profile at baseline and after hormone replacement therapy. Conversely, the ER\beta genotype appeared to segregate with bone loss and lipid profile in response to hormone replacement treatment.

P256-Su

The COMT val158met Polymorphism is Associated with Bone Mineral Density and Body Fat in Elderly Swedish MenMR OS Sweden

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Osteoporotic fractures are a growing health problem in the elderly. In men, this condition has so far been less studied than in women. Bone mineral density (BMD) is an important predictor of fracture risk. Genetic factors are determinants of BMD, and serum estrogen levels are positively correlated to BMD in men. Catechol-*O*-Methyltransferase (COMT) is involved in the degradation of estrogens. There is a functional polymorphism in the COMT gene (val158met), resulting in a 60–75% difference in enzyme activity between the val (high activity = H) and met (low activity = L) variants. We have previously demonstrated that this polymorphism is associated with height and estradiol levels in early pubertal girls and with BMD in young men. The aim of the present study was to investigate the associations between this polymorphism and BMD in elderly Swedish men. In the Swedish part of the MrOs study (in total 3000 Swedish subjects), 521 men (age 73.4 SD 2.2) have so far been genotyped and bone parameters have been assessed using DXA. 33.2% of the individuals were homozygous for the low activity COMT genotype ($COMT^{LL}$), 47.4% were heterozygotes ($COMT^{HL}$) and 19.4% were homozygous for the high activity COMT genotype ($COMT^{HH}$). The COMT polymorphism was associated with BMD in the total body, total hip, femur neck and left arm (one-way ANOVA $P< 0.05$), but not in the spine. Individuals with $COMT^{HH}$ had the lowest BMD, while the values for $COMT^{HL}$ and $COMT^{LL}$ were similar to one another. BMD in the total body, total hip, femur neck and left arm was 3.1%, 3.9%, 4.0% and 5.0% higher in $COMT^{HL/LL}$ than in the $COMT^{HH}$ group. Furthermore, individuals with $COMT^{HH}$ had a lower BMI and a lower percentage of body fat than $COMT^{HL/LL}$. In a regression model using COMT genotype, height, physical activity, smoking and calcium intake as covariates, COMT genotype was an independent predictor of BMD in the total body, total hip, femur trochanter and femur neck, but not in the spine. When body weight was added to the model COMT remained an independent predictor of total body BMD, but not of BMD in the total hip, femur trochanter or femur neck, suggesting that COMT genotype exerts its influence on BMD partly by affecting body weight. In conclusion, the COMT val158met polymorphism is associated with BMD and body fat in elderly Swedish men.

P257-Mo

Association of Tumour Necrosis Factor-Alpha Promoter Polymorphisms with Periprosthetic Osteolysis

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Periprosthetic osteolysis is the major cause of implant failure following total hip arthroplasty. TNF-α plays a key role in pathogenesis of periprosthetic osteolysis. The TNF-α gene promoter contains several common single nucleotide polymorphisms (SNPs) that may alter the TNF-α protein production. We investigated whether or not TNF-α promoter polymorphisms are associated with early aseptic loosening of total hip replacement. 184 patients who had undergone THA due to OA have been recruited in to the study: 100 had confirmed periprosthetic osteolysis, whereas 84 had no evidence of osteolysis. A history of inflammatory arthropathy or secondary arthritis, metabolic bone disease, endocrine pathology, chronic renal failure, early follow up (<3 years) or prolonged use of corticosteroids or bisphosphonates (>6 months) served as exclusion criteria. Subjects were also excluded if they were under 55 years at the time of primary THA, had BMI > 28, or if there was a clinical suspicion of implant infection. Patients' DNA was extracted from peripheral venous blood, and a 816 base pairs long TNF-α promoter fragment (−901 to −85) was amplified by PCR and sequenced using BigDye v 3.1 Cycle Sequencing Kit by MRC-Geneservice (Babraham, UK). Sequence traces were analyzed using Staden software package v.5.1, and statistical analysis was carried out using software R version 1.9 (Centre for Applied Medical Statistics, Cambridge, UK). The following SNPs were represented (case/control—odds ratio): −238 (10 cases/8 controls—OR 1.03), −308 (38 cases/22 controls—OR 1.67), −857 (16 cases/6 controls—OR 2.41), −863 (29 cases/25 controls—OR 0.93). Logistic regression models adjusting for prosthesis, sex and side suggest that there is no statistically significant association between any of the polymorphisms and periprosthetic osteolysis. There is, however, a strong association between periprosthetic osteolysis and implant type: 16% (6/38) of those with Exeter prosthesis were cases compared to 61% (67/109) of those with Charnley prosthesis (OR 8.51, 95% CI [3.28, 22.08] for Charnley relative to Exeter). We conclude that TNF-α promoter SNPs cannot be used as markers for periprosthetic osteolysis after THA. This work was supported by Gates Cambridge Trust, Universities UK and Smith and Nephew, Inc.

P258-Tu

The Low Activity 158met Variant of the COMT Gene is Associated with Increased Fracture Risk in Elderly Men

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Sex steroids play an important role in bone development, therefore, it is interesting to search for candidate genes for osteoporosis within both the estrogen synthesis and metabolism pathway. The second step in estrogen metabolism is

metabolism of the estradiol metabolites 2-hydroxyestradiol and 4-hydroxyestradiol. The gene of the estrogen-degrading enzyme catechol-*O*-methyltransferase (COMT) contains a functional G to A polymorphism, resulting in a Valine to Methionine substitution at codon 158. The Methionine variant has a 3- to 4-fold lower enzyme activity than the Valine variant and has recently been associated with lower peak bone mineral density (BMD) in young adolescent Swedish men (Lorentzon et al. 2004). The aim of our study was to determine if this polymorphism is associated with bone parameters and fracture risk in elderly subjects.

In 2032 men and 2526 women from the Rotterdam Study, a population-based cohort study of individuals 55 years and older, COMT genotypes were determined using Taqman allelic discrimination assay and associations with BMD were analysed using ANOVA, while fracture risks were analysed using Cox' proportional hazard regression analysis. We recorded 722 incident osteoporotic and 294 fragility (hip, pelvis, upper humerus) fractures during 8.6 years mean follow-up. All analyses were adjusted for age, height and weight.

Allele frequency of the Met allele in this Caucasian population was 55% and genotype distribution was in Hardy Weinberg equilibrium ($P = 0.81$). The Methionine allele was significantly associated with higher risk of both osteoporotic and fragility fractures in men, but not in women, with evidence for a dominant effect. We, therefore, combined heterozygotes and homozygotes into Met-carriers. Carriers of the Met allele had a 1.9 increased risk for osteoporotic fractures (HR = 1.9, 95% CI: 1.1–3.3) and a 2.6 increased risk for fragility fractures (HR = 2.6, 95% CI: 1.1–5.9). Adjustments for age, height, weight and BMD did not change the risk estimates. No significant associations with BMD and estrogen levels were found for both men and women.

The lower enzymatic activity of the Methionine variant could result in a higher level of estrogen metabolites, such as 2-hydroxyestradiol and 4-hydroxyestradiol These metabolites could result in reduced bone quality ultimately leading to increased fracture risk. However, the mechanism underlying this association is not clear and needs additional study.

P259-Su

Osteoporosis and Genetic Disposition for Primary Adult Lactose Intolerance in Men

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Background: Lactose intolerance is an autosomal recessive condition, characterized by intestinal lactase deficiency and concomitant lactose malabsorption. This hypolactasia results in subjective dislike of milk and decreased calcium supply by milk and dairy products. In postmenopausal women, this condition resulted in an association with decreased bone mineral density and increased bone fractures (Obermayer-Pietsch et al., JBMR 2004).

In men, clinical symptoms of lactose malabsorption are much less frequently reported by the patients and seem to be less important than in women, despite a similar genetic disposition. Therefore, we studied the association of primary adult lactose intolerance as defined by the LCT(-13910) polymorphism and cofactors of bone and mineral metabolism in men.

Materials and methods: Out of a population-based study, 280 men at the age of 58 ± 10 years were genotyped for the LCT(-13910) polymorphism and documented for medical and nutritional characteristics, lumbar and femoral bone mineral density (BMD, Hologic 4500 plus), biochemical parameters of bone and hormonal metabolism, H2 breath tests, and lactose as well as strontium resorption tests. Furthermore, 75 men with mild hypogonadism were compared to the eugonad controls.

Results: Lactose intolerance influenced deeply nutritional calcium supply by dairy products even in the absence of any knowledge of this condition. In hypogonad men with testosterone levels below 2,4 μg/dl, we found a significant association with BMD according to LCT genotypes, which was more pronounced than in eugonad controls. Lactose intolerant CC genotypes had significantly less body weight and height. Subjective symptoms of lactose malabsorption were frequently denied by the probands, but clinically proven by H2 and lactose absorption tests. Lactase-free strontium resorption tests per se were not influenced by the LCT genotypes.

Discussion: The LCT(-13910) polymorphism is a strong genetic test for primary adult lactose intolerance. Together with other risk factors for osteoporosis, it may be used as an important component for the assessment of calcium supply, anthropomorphic characteristics, bone mineral density, and concomitant bone fractures even in absence of clinical symptoms of lactose intolerance.

P260-Mo

Large-Scale Population Based Study Shows no Evidence of Association Between Common Polymorphism of the VDR Gene and BMD in British Women

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The Vitamin D receptor (VDR) is one of the most extensively studied candidate genes for susceptibility to osteoporosis. Whilst data from individual studies of VDR

alleles in relation to BMD have been conflicting, previous meta-analyses have suggested that BB homozygotes at the *Bsm*1 polymorphism of VDR have lower BMD values than other genotype groups at this site by approximately 0.02–0.03 g/cm^2 in postmenopausal women. Here, we conducted an association study between BMD, bone loss, biochemical markers of bone turnover and fracture in relation to five common single nucleotide polymorphisms (SNP) of VDR, in a population-based study of 3100 British women, of average age 54.8 ± 2.2 years. The SNP's studied were the Cdx-2 site in the VDR promoter; the exon 2 start codon polymorphism recognised by the restriction enzyme *Fok*I, and polymorphisms in the 3 prime flank of VDR recognised by the restriction enzymes *Bsm*I, *Apa*I and *Taq*I. There was no significant difference in BMD values at the lumbar spine (LS-BMD) or femoral neck (FN-BMD) for any of the individual polymorphisms or haplotypes defined by these polymorphisms. We observed lower rates of femoral neck bone loss over a 6.3-year follow up period in homozygotes for the Cdx-2 A allele (GG-0.77+ 1.06; GA-0.71+ 1.14; AA-0.51+ 1.20; $P = 0.01$). There was no difference in levels of PINP, Dpd/Creatinine, 25(OH)D or PTH between the genotype groups except for the Cdx2 site where AA homozygotes had slightly lower PTH values than the other groups (GG 3.28 + 2.06; GA 3.44 + 1.99; AA 2.89 + 1.63; $P = 0.02$). There was no significant interaction between genotype and calcium intake or vitamin D status except for CDX AA homozygotes ($n = 118$), where there was a negative correlation between calcium intake and FN BMD (Baseline $r = 0.197$ $P = 0.033$; follow-up $r = 0.172$ $P = 0.062$). None of the genotypes or haplotypes were associated with fracture, although we did not have adequate power to detect such an association since the number of subjects with confirmed osteoporotic fractures was less than 3%. In conclusion, we have excluded a major effect of VDR alleles on BMD in this population, but found evidence of a possible interaction between the Cdx2 polymorphism, calcium intake and FN-BMD.

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P261-Tu

Relationships Between PvuiI and Xbai Polymorphisms of ER Alpha Gene and Ultrasound Bone Parameters in Polish PopulationThe Epolos Study

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Introduction: Osteoporosis is common disease characterized by decreased values of bone mineral density (BMD) and increased risk of fractures. Genetic factors play important role in pathogenesis of osteoporosis and many studies demonstrated relationships between genes and bone status parameters. Estrogen receptor a is necessary to effective acting of estrogen hormones on bone. ERa gene, coding estrogen receptor a is one of candidate genes, attending bone status. On the same examinations focused on relationships between its polymorphisms and ultrasound parameters were not analyzed in details before.

Objective: The aim of this study was to verify if polymorphisms of ERa gene are accompanied by changes in ultrasound parameters.

Methods: The study group comprised 1426 subjects, adult males and females, in age range 20–80 years, randomly selected 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: *Pvu*II and *Xba*I simultaneously. Ultrasound parameters are represented by Stiffness values, measured for heel bone with Achilles GE Lunar.

Results: The frequency of ERa genotypes was as follows: [px,px]25,1%, [px,PX]36,7%, [PX,PX]14,5%, [PX,Px]8,3%, [Px,px]13,8%, [Px,Px]1,6%, and haplotypes was as follows: px = 50.4%, PX = 36.9%, Px = 12.7%, pX haplotype was not found in the population. Highest Stiffness values were observed for [PX,Px] genotype, and lowest Stiffness values were noticed for [px,px] genotype. Difference between those two values is 4,34% in Stiffness ($P < 0.0759$, normalized for age and sex). Analysis performed for haplotypes shown us that PX haplotype is related to significantly higher Stiffness values, comparing with others haplotypes ($P < 0.0479$, normalized for age and sex). PX haplotype represents none of nucleotide substitution in DNA chain, carried by 36,9% of Polish society. This haplotype constitute genotype [PX,PX], which has been found as related to highest Stiffness values.

Conclusions: Presented results shows that PX haplotype carriers have significantly higher Stiffness values, comparing to others haplotypes carriers.
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P262-Su

The Effect of Low Intensity Pulsed Ultrasound on Expression of Basic FGF During Lengthening of Distraction Osteogenesis

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Distraction osteogenesis is a process of bony tissue regeneration under an external tension stress. Low-intensity pulsed ultrasound (LIPUS) was found to enhance bone formation during distraction. The mechanism of LIPUS effect was still unknown. The bFGF that is one of the growth factors plays important role in process of distraction osteogenesis. Thee aim of the study was to investigate the effect of LIPUS on expression of basic fibroblast growth factor (bFGF) during lengthening of distraction osteogenesis. Open osteotomy was performed in the mid-diaphyseal region of rabbit right tibia, which was stabilized by an external lengthening device. The osteotomised tibia was distracted with 1 mm/day for 2 weeks after 7-day latency. LIPUS was applied during distraction stage. The expression of bFGF was assessed by immunohistochemistry using and avidin–biotin complex method. The result showed that bFGF was expressed in osteoblast lining on surface of newly formed trabeculae and some chondrocytes adjacent to hypertrophic chondrocytes. Under LIPUS treatment, the expression of bFGF was showed stronger than control in newly formed bone during 2-week distraction. During distraction, the bone regeneration was enhanced by LIPUS to give larger size and greater bone mineral content in distraction callus in our previous study. The LIPUS was reported to induce the expression of bFGF in osteoblast culture. The bFGF also stimulated the bone formation and angiogenesis in fracture healing. In conclusion, LIPUS might enhance the bone formation during distraction through the regulation of bFGF expression.
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P263-Mo

RANKL Directly Induces Bone Morphogenetic Protein-2 Expression in Rank-Expressing POS-1 Osteosarcoma Cells

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Animal models and corresponding cell lines are great tools to elucidate the molecular and cellular mechanisms involved in the development of primary tumors. When injected to mice, osteosarcoma POS-1 cells represent one of these models which allows to study primary bone tumour and associated lung metastasis. This cell line is derived from an osteosarcoma tumor which develops spontaneously in C3H mice and whose clinical data parallel to humans.
The characterization of the POS-1 cell line was studied in vitro by mineralization capacity and expression of bone markers by semi-quantitative RT-PCR, compared to primary osteoblasts and bone marrow cells. POS-1 cells show no mineralization capacity and exhibit an undifferentiated phenotype, as they express both osteoblastic and osteoclastic markers. Indeed, semi-quantitative RT-PCR analysis show that POS-1 cells do not express several osteoblast markers such as BMP-2, OC, BSP, Coll I and RANKL. However, the expression of Cbfa1 and OPG transcripts is observed. As osteosarcoma may be heterogeneous in its cell origin and composition, the expression of other markers was analysed, including osteoclastic ones: Cathepsin K, TRAP, Calcitonin Receptor and RANK. Interestingly, POS-1 cells express Cathepsin K, TRAP and RANK, but not Calcitonin, that represents one of the most specific osteoclastic marker. Thereby, experiments were performed to determine whether RANK was functional, by studying biological activity of human RANKL through the receptor RANK expressed on POS-1 cells. Results revealed a RANKL-induced increase in ERK phosphorylation, as well as an induction of BMP-2 at the mRNA and protein levels, and a decrease of POS-1 cell proliferation in the presence of 10 ng/ml RANKL. BMP-2 mRNA induction is dependent on the ERK 1/2 signal transduction pathway, as its expression is abolished in the presence of UO126, a specific synthetic inhibitor of the ERK 1/2 pathway. Moreover, a 2-fold molar excess of soluble RANK blocks the RANKL-induced BMP-2 expression, demonstrating that the biological effects of RANKL observed in POS-1 cells are mediated by RANK. This is the first report describing a functional RANK expressed on osteosarcoma cells, as shown by its ability to induce signal transduction pathways and biological activity when stimulated by RANKL.

P264-Tu

Statin Induces Mineralized Tissue Formation in Cultured Rat Dental Pulp Cells

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Objective: Dental pulp cells include multi-potential cells which may differentiate to lineages producing mineralized tissues. The newly-formed mineralized tissue is thought to protect the teeth from dental caries and trauma. Statins, therapeutic agents for hypercholestemia, have been recently reported to stimulate bone formation in vitro and in vivo. In this study, we investigated the effects of simvastatin on mineralized tissue formation in cultured rat dental pulp cells.
Materials and methods: Dental pulp cells were prepared from maxillary incisors of 8-week-old Wister male rats. Dental pulp was minced and trypsinized, and collected cells were plated in Eagle's MEM containing 10% FBS supplemented with 0.2 mM ascorbic acid, 5 mM β-glycerophosphate, and 0, 1, 2 and 5 μM simvastatin (Wako Pure Chemical Industries, Osaka, Japan). Alkaline phosphatase (ALP) activity was measured on day 7 and mineralized-nodule (MN) formed on day 14 was determined by staining with von Kossa. Total RNAs of 2 μM simvastatin-treated cells and the control were extracted on day 9. Then, bone morphogenic protein-2 (BMP-2) and osteocalcin mRNA expressions were analyzed by RT-PCR.
Results: Simvastatin stimulated ALP activity on day 7, showing 2- to 3-fold increase compared to the control. MN was observed both in simvastatin-treated cells and the control on day 14. The area of MN stained by von Kossa was dose-dependently increased in simvastatin-treated cells. PCR analysis revealed that 2 μM simvastatin increase gene expressions of BMP-2 and osteocalcin on day 9.
Conclusion: These results indicate that simvastatin could increase mineralized tissue formation in cultured rat dental pulp cells and that this effect might be associated with the increase of BMP-2 and osteoclacin expressions.

P265-Su
Bone Phenotype of IL-6 Transgenic Mice Mimicking Human Chronic Inflammatory Diseases in Children
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Osteoporosis and stunted growth are complications of systemic juvenile idiopathic arthritis and other chronic inflammatory diseases characterized by IL-6 over-production. We previously demonstrated that the NSE/hIL6-transgenic mice, expressing high circulating levels of IL-6 since birth, have a decrease in growth rate, with normal serum GH and low IGF-I, the latter due to reduced IGFBP-3 and subsequent accelerated IGF-I catabolism. Adult mice were 50–70% the size of wild-types and the defect was prevented by IL-6 neutralization. Herein, we investigated the bone phenotype and confirmed significant reduction of tibia and femur lengths by X-ray analysis. Histomorphometry of tibias showed decreased trabecular volume and number in the proximal secondary spongiosa of 5- to 20-day old transgenic mice, with increased osteoclast- and decreased osteoblast-number and-surface. Remarkable reduction of cortical thickness was observed and dynamic measurements by calcein/alizarin red showed no endosteal and 50% reduced periosteal apposition in the NSE/hIL-6 mice compared to controls. Consistently, increased endosteal osteoclast number and bone resorption was observed in the transgenic mice suggesting that endosteal erosion and reduced periosteal deposition contributed to cortical thinness. Clear-cut reduction of growth plate hyperthrophic zone thickness was obvious and, in young mice, secondary ossification centers were delayed, showing hyperthrophic chondrocytes, but not mineralized trabeculae, nor medullary tissues containing blood vessels, osteoclasts or osteoblasts. In vitro studies demonstrated an increased osteoclast formation rate in the transgenic bone marrow cultures, and reduced calvarial osteoblast proliferation, alkaline phosphatase and mineralized nodules. These effects were mimicked in wild-type cell cultures by addition of 5 ng/ml IL-6, and were not further increased in NSE/hIL-6 cells by IL-6 treatment. Transgenic calvarial osteoblasts expressed high hIL-6, and exogenous IL-6 increased transcriptional expression of the endogenous cytokine in wild-types. Thus, chronic over-expression of IL-6 alone induces bone and cartilage changes, underscoring a role for IL-6 in stunted growth and bone abnormalities in chronic inflammatory diseases. We propose the NSE/hIL-6-transgenic mice as a suitable model to investigate possible therapeutic approaches for the bone destruction induced by chronic inflammation.

P266-Mo
Hyperprolactinemia Stimulated Duodenal Active Calcium Absorption in Ovariectomized Rats
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Duodenal active calcium (Ca) absorption is normally regulated by vitamin D, and possibly estrogen. We have previously demonstrated an acute stimulatory effect of prolactin (PRL) on both transcellular and paracellular components of the active Ca absorption. Thus, the present study aimed to investigate a long-term (1 month) effect of hyperprolactinemia (HyperPRL) induced by anterior pituitary transplantation on Ca transport. Influences of vitamin D and estrogen were abolished by high Ca diet and ovariectomy. Nine and 22-week-old female Sprague–Dawley rats were each divided into 5 groups, i.e., sham fed normal diet (Sham+N), ovariectomized fed normal diet (OVX+N) or high Ca diet (OVX+H), and hyperprolactinemic ovariectomized fed normal diet (AP+OVX+N) or high Ca diet (AP+OVX+H). 45Ca was used as a tracer for mucosa to

serosa transcellular (Jms-trans) and solvent drag-induced (Jms-solv) Ca flux, and serosa- to-mucosa paracellular (Jsm-para) Ca flux.

Results showed that in young adults, all three components of Ca flux were not altered by OVX or high Ca diet, indicating that estrogen and vitamin D had no direct role in the regulation of Ca transport in this age group. Compared with OVX+N, Jms-trans, Jms-solv, and Jsm-para in AP+OVX+N were increased from 7.56 ± 0.79 to 16.54 ± 2.05 ($P < 0.001$), 95.51 ± 10.64 to 163.15 ± 18.03 ($P < 0.01$), and 12.88 ± 1.45 to 27.15 ± 3.45 ($P < 0.05$) nmol h^{-1} cm^{-2}, respectively. These changes resulted in a 69% increase in net Ca flux.

In adult rats, OVX but not high Ca diet significantly decreased Jms-solv, suggesting a role of estrogen but not vitamin D in maintaining Jms-solv. HyperPRL, however, increased Jms-trans and decreased Jsm-para, thus resulting in a significant increase in net Ca flux. The effects of HyperPRL in both age groups were abolished by high Ca diet.

Conclusions: (1) Vitamin D and estrogen had little effect on Ca absorption in young adult rats, (2) estrogen regulated the solvent drag-induced Ca absorption in adult rats, and (3) hyperprolactinemia enhanced both transcellular and paracellular components of Ca absorption in young adult, but stimulated only the transcellular Ca absorption in adult rats in the presence of vitamin D.

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P267-Tu

The Effects of Extracellular Nucleotides on EGF-Induced C-FOS Gene Expression

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There is increasing evidence that extracellular nucleotides play a pivotal role in cancer growth, metastasis, and pain. Previous studies from our laboratories have shown that in both bone and breast cancer cell lines extracellular nucleotides synergise with growth factors to induce expression of the proto-oncogene c-*fos*. Synergy between extracellular nucleotides and growth factors may represent an important mechanism promoting the growth and spread of tumours. This may be particularly relevant for breast cancer metastasis to bone as bone cells constitutively release ATP, and this release is enhanced in response to mechanical strain. This could play a key role in creating an environment propitious for breast cancer cell growth after metastasis. Using the breast cancer cell lines HS578T and T47D, we found that ATP and UTP enhanced induction of the endogenous c-*fos* gene by EGF, an important growth factor associated with the development and spread of breast cancer. EGF treatment strongly activated ERK1/2 and Akt, neither of which was augmented by co-stimulation with nucleotides. In contrast, co-stimulation with EGF and nucleotides increased ERK5 phosphorylation relative to the level observed with EGF alone. Notably, extracellular nucleotides had little stimulatory effect on their own. This synergy appears to reflect nucleotide potentiation of EGF-driven EGFR activation, since phosphorylation of the EGFR was enhanced upon co-stimulation. Surprisingly, EGFR levels were diminished in cold 1% Triton lysates of cells treated with EGF and either ATP or UTP, whereas no reduction in EGFR levels were observed in lysates prepared with denaturing SDS buffer. Thus, extracellular nucleotide addition leads to EGFR inclusion into a Triton-insoluble membrane fraction, possibly lipid rafts, which may explain facilitated signalling to the c-*fos* promoter. During the course of these studies, we also generated several breast cancer c-*fos*-luciferase reporter cell lines; activation of the c-*fos*-luciferase reporter gene was also enhanced by co-stimulation with EGF and either ATP or UTP. These cell lines will prove to be invaluable tools in screening quickly and reliably the effects of various compounds on this important proto-oncogene in breast cancer cells.

P268-Su

Blood Serum RANKL in Rats Drinking Strontium Chloride Water

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RANKL (receptor activator of nuclear factor (NF)-kb ligand (also: Osteoprotegerin (OPG) ligand, OPGL) is a member of the Tumour Necrosis Factor (TNF) family. RANKL, produced by osteoblastic lineage cells and activated T lymphocytes, is presently regarded as a main stimulatory factor for osteoclastogenesis. It activates its specific receptor RANK that is located on osteoclasts and dendritic cells. System of RANKL/RANK/OPG is present in many organs and seems to be one of the main regulators of processes of bone resorption.

Aim: It seemed to be of scientific value to assess whether strontium ion, well-known uncoupling agent, affects RANKL system in the rats.

Material and method: Six weeks old male Wistar rats (n = 10) were given strontium chloride in drinking water at a concentration of 7.532 mmol/l for 6 weeks and controls (n = 10) drank ordinary tap water. Animals of both groups were fed standard diet and were kept under standard laboratory conditions. After decapitation under deep ketamine-barbital anesthesia, blood samples were collected and allowed to clot. Serum samples were deep-frozen (−70°C) for 6 weeks. After thawing at room temperature, serum-free

sRANKL concentration was assessed in duplicates for each sample with ELISA method according to the manufacturer's kit guidelines (Biomedica Medizinprodukte GmbH and Co KG, Vienna, Austria). Data were presented as means ± SEM. Statistical analysis was performed with Statistica 6.0 program and $P < 0.05$ was regarded as significant.

Results: Mean value of serum RANKL in control rats was 2.794 ± 0.385 and in the rats that were drinking strontium chloride water 2.12 ± 0.26 ($P < 0.05$).

Conclusion: Strontium applied for 6 weeks to male rats in drinking water in concentration of 7.532 mmol/l decreased serum sRANKL by approximately 25%. The authors are of the opinion that decrease in RANKL could be a putative mechanism of anabolic action of strontium ion on bone tissue metabolism in rats.

P269-Mo

Influence of Low Concentrations of Strontium Chloride in Drinking Water on Mineral Density of the Rat Skeleton

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The aim of the study was to assess influence of strontium chloride administered in drinking water on bone density of different regions of the skeleton in young male rats.

Materials and methods: Tap water with added strontium chloride was given ad libitum to three groups ($n = 18$) 6 weeks old male Wistar rats (200 ± 20 g) fed standard diet (Ca-1,1%) and kept under standard laboratory conditions. Three concentrations of strontium in water were used (group Sr1—3766 mmol/l, group Sr2—7532 mmol/l, Sr3—11,298 mmol/l). Controls (C, $n = 20$) drank ordinary tap water. After 6 months serum samples were analyzed for strontium concentration (S.c. Sr[ppm], GFAASS method) and lumbar vertebrae L1-L4 (LS), right femora (F) and both humeri (H) were harvested for a-p densitometry (area [cm^2], BMC [g], BMD [g/cm^2]) with the use of small Animal Program of the QDR 4500A. Statistical analysis (ANOVA) was performed with Statistica 5 PL computer program. Results were expressed as means and standard deviations (in brackets). *$P < 0.05$ v.C was regarded significant.

Results: S.c.
Sr:C—<0.02, Sr1—0.59(0.12), Sr2—1.68*(0.34), Sr3—2.10*(0.59).
Area—LS: C—1.5619(0.09), Sr1—1, 5661(0.12), Sr2—1.6761*(0.10), Sr3—1.6261(0.14); F: C—2.2747(0.13), Sr1—3694(0.13), Sr2—2.4375*(0.16), Sr3—2.3187(0.20); H: C—2.6329(0.19), Sr1—2.637(0.27), Sr2—2.7247(0.45), Sr3–2, 5205(0.44).BMC-LS: C—0.3268(0.034), Sr1—0.3290(0.056), Sr2—0.4024*(0.044), Sr3—0.3961*(0.063); F:C—0.6482(0.064), Sr1—0.6857(0.076), Sr2—0.7837*(0.075), Sr3—0.7229*(0.100); H: C—0.5875(0.048), Sr1—0.5907(0.054), Sr2—0.6732*(0.106), Sr3—0.6207(0.127).BMD-LS: C—0.2088(0.011), Sr1—0.2089(0.021), Sr2—0.2396*(0.016), Sr3—0.2422*(0.020); F: C—0.2844(0.015), Sr1—0.2888(0.020), Sr2—0.3211*(0.014), Sr3—0.3105*(0.020); H: C—0.2234(0.015), Sr1—0.2213(0.016), Sr2—0.2480*(0.018), Sr3—0.2454*(0.021).

Conclusions: Strontium given to male young adult rats for 6 months in drinking water significantly increased BMD of all investigated regions, the highest being for the spine and the lowest for the humeri. Only concentration of 7.532 mm/l of the strontium chloride in drinking water seems to be the most appropriate for investigating influence of strontium ion on bone metabolism in rats.

P270-Tu

Bone Development, Physical Growth and Sex Steroids in Adolescent Girls

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The aim of our study was to evaluate the possible relation between ultrasound heel measurement, serum concentration of sex hormones, pubertal stages and physical development in adolescent girls.

Subjects and methods: The research was carried out on 85 girls aged 9.8–14.6 years (the average 11.8 ± 1.0). The classification of pubertal stage in girls was related to the appearance of the breast according to Tanner's scale. Adequately to the stage of puberty, four groups were created. The 1st group consisted of 21 girls in the prepubertal stage (Ist Tanner's stage). Second group was represented by 32 girls in the IInd stage of Tanner. Third group was composed of 20 girls in IIIrd stage and 4th group of 12 girls in IVth stage of Tanner. Bone ultrasonic attenuation—BUA and speed of sound—SOS of right heel bone were measured using ultrasonic bone densitometer (Sahara, Hologic). Serum concentrations of estradiol and progesterone were assessed using RIA methods. Body weight and height measurements were taken twice in intervals of one year. Height and weight velocity was calculated for all the subjects.

Results: Mean values of anthropometric parameters and serum sex hormone concentration increased with pubertal stages reaching the highest values in girls in the IVth pubertal stage. The highest annual increments of body height in girls was noticed in the IInd Tanner's stage. The highest annual increments of body weight was observed in girls in the IIIrd stage of puberty. The lowest values of BUA, SOS and stiffness were observed in girls in the IIIrd pubertal stage and the mean values of these parameters were lower than in

girls in the Ist and IInd stages ($P < 0.05$). No statistically significant correlation was found between level of sex hormones (estradiol, progesterone), BUA and SOS in girls during puberty.

Conclusions: Ultrasound heel measurements do not increase with pubertal stages parallel to sex hormones and anthropometrical parameters. Decrease of BUA and SOS was noticed at the end of pubertal height spurt.

P271-Su

Rinsing After Impaction Does not Influence Osteoinductivity of Morselized Cancellous Bone Grafts

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Washing morsellized cancellous bone (MCB) before or after the impaction might have different effects, but are both used by clinicians. Washing MCB before impaction will enhance bone ingrowth. Washing after impaction is performed by clinicians in consideration of an expected effect on the initial stability, but might have a negative effect on bone induction if released growth factors are washed away. The objectives were to study if TGFβ and BMP's in mineralized bone matrix were released in physiological concentrations by impaction and if these released growth factors have an effect on bone formation in vivo.

MCB was obtained from five human femoral heads. Rinsed MCB was compressed using an MTS machine. Escaping fluid was gathered and centrifuged. The supernatant we named "impaction fluid" (IF). TGFβ was measured by ELISA. BMP's were measured using a BMP-selective reporter bioassay (BRE-luc). After that, hMSC's were stimulated using IF. Cells were cultured on osteogenic differentiation medium (ODM), ODM supplemented with dexamethasone, ODM supplemented with both dexamethasone and vitamin D3 and ODM supplemented with IF. After 9 days, ALP-activity was measured and a neutral red assay was performed. Furthermore, the effect of IF on bone formation in goats was studied in the bone conduction chamber. Allograft was compared with a ceramic bone substitute with and without IF.

An average amount of 17.8 ng TGFβ/ml IF was detected. No BMP's could be found.

Induction of ALP in hMSC using IF was comparable with the induction obtained by the medium alone. Medium supplemented with dexamethasone and vitamin D3 showed highest ALP induction.

No significant difference in amount of fibrous tissue ingrowth between allograft, TCP/HA and TCP/HA supplemented with impaction fluid was found.

No evidence was found for the presence of BMP's in impaction fluid. Both ELISA and culture studies show that particularly TGFβ is released from the matrix. ALP activity of the hMSC's can be stimulated by BMP-6. TGFβ suppresses this BMP-6 induced ALP-expression. We also found this phenomenon with impaction fluid. Since TGFβ is a growth factor stimulating the production of extracellular matrix rather then that it stimulates the proliferation of progenitors, it is not surprising that impaction fluid has no effect in this bone chamber study. Clinically this means that rinsing after impaction will not have a big influence on the osteoinductive properties of the graft.

P272-Mo

Modulation of Exogenous OPG Bio-Disponibility by Membranous RANKL Expressing Cells

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The members of the OPG/RANK/RANKL triad are involved in various osteolytic pathologies such as bone tumors. The receptor activator of nuclear factor-kB ligand (RANKL) enhances osteoclastogenesis via interaction with its receptor RANK, whereas Osteoprotegerin (OPG) inhibits this osteoclastogenesis by acting as a soluble RANKL receptor competitor. The equilibrium between RANKL and OPG plays a crucial role in bone physiopathology. The concentration of RANKL is observed to be highly increased during bone metastasis and primitive malignant bone tumors resulting in a disturb equilibrium of RANKL/OPG ratio in favour of RANKL. If some studies have shown the efficacy of OPG as a therapeutic agent against bone tumors, however, its bioavailability and the mechanism by which extracellular OPG disappears after an injection remains blurred. In our present work, human kidney cell line 293 which initially express neither OPG nor RANKL have been transfected with the full length of mouse trans membranous form of RANKL (293RL) to assess the becoming of OPG in the culture medium. Scatchard analysis pointed out a specific binding of OPG onto 293RL cells compared to 293 cells for which no binding is observed. When OPG is incubated with 293RL cells, the extracellular concentration of OPG is found to fall down through a time dependent manner. This event unaffected by an antibody against Syndecan-1 is however abolished by an antibody against RANKL. Since the use of proteases inhibitors did not show any inhibitory effect of this OPG disappearance thus excluding any extracellular protease degradation, an internalisation process is put forward. Our study based upon the becoming of OPG has revealed by confocal microscopy that OPG through an obligatory binding to RANKL is internalised by the cells and degraded by the proteasome and lysosome mechanism.

This internalisation process has also been observed by osteoblastes cells expressing RANKL naturally, thus, validating our mechanistic model. By the light of our result, the expression of RANKL could be a possible means of regulating extracellular OPG and the enhancement of OPG bio-availability is therefore a target to reach in an osteolytic disease therapy.

P273-Tu

The Circadian Rhythm of Osteoprotegerin and Its Association with Parathyroid Hormone Secretion in Non-Osteoporotic Elderly Postmenopausal Women

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Background: Parathyroid hormone (PTH) is normally secreted in a circadian rhythm and modulates bone turnover through the differential stimulation of receptor activator for nuclear factor kappa B ligand (RANKL) and suppression of osteoprotegerin (OPG), both of which are fundamental factors in regulating bone turnover. We have studied the relationship between PTH and OPG over a 24-h period.
Methods: Hourly peripheral venous blood samples were obtained from 6 healthy 2.1 years) with normal bone mineral ± elderly men (mean age 68.2 density. Plasma PTH (1–84) and OPG were measured on all samples. Cosinor analysis was performed to analyze circadian rhythm parameters. Cross-correlation and Pearson's analysis was used to determine the relationship between variables and whether one time series led another. Cross-correlation analysis determines the correlation between two time series of equal length that have been paired, data point by data point, and then one of the time series is shifted by one or more time points (lag time) and the correlation process is repeated.
Results: Significant circadian rhythms were observed for PTH and OPG ($P < 0.01$). Secretory patterns of PTH and OPG were out-of-phase during a 24 h period and maximal negative correlation between PTH and OPG ($r = 0.5$) was observed when PTH changes preceded OPG changes by 1 h. Pearson's correlation analysis confirmed that the diurnal rhythm of PTH correlated significantly and negatively with that of OPG ($r = 0.4$; $P < 0.05$).
Conclusion: We have demonstrated that peripheral blood concentrations of OPG demonstrate a concerted circadian rhythm in elderly non-osteoporotic postmenopausal women, which may, in part, be regulated by the circadian changes in PTH concentration that control bone turnover on a daily basis. A significant decrease in OPG in response to increasing PTH may result in increased bone resorption by osteoclasts.

P274-Su

Quantification of Different Growth Factors in Reaming Aspirate, Iliac Crest, Blood And PRP

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Introduction: Large bony defects and non-unions are still a feared complication in trauma and orthopaedic surgery. Treatment strategies include the use of allogenic cancellous bone, autogenic bony materials (iliac crest), and nowadays the stimulation with growth factors such as BMP-2 or platelet rich plasma (PRP). A further source of material with a high osteogenic potency might be the reaming debris that accumulates during reaming of the intramedullary canal of long bones. Aim of this study was the comparison of the quantity of different growth factors within iliac crest, reaming aspirate, reaming irrigation fluid, and platelet rich plasma.
Materials and methods: Iliac crest and reaming material samples from human femura were harvested during operation ($n = 6$ each). PRP was prepared from the blood of 9 volunteers. The growth factor quantity of the bony materials (iliac crest and reaming debris) and of the liquid materials (Platelet poor plasma (PPP), platelet rich plasma (PPP) and reaming irrigation) was compared. Following growth factors were quantified by ELISA: IGF-I, TGF-β1, BMP-2, BMP-4, FGFa, FGFb, PDGF (R&D-Systems, Germany).
Results: In the reaming debris more FGFa (2.1×) compared to iliac crest was found. Also enhanced levels of PDGF (2.9×), IGF-I (1.6×), TGF-β1 (3.5×) and BMP-2 (3×) were measured in the reaming debris compared to iliac crest. VEGF and FGFb were lower in the reaming debris compared to the iliac crest. Comparing PRP and PPP all detectable growth factors, except IGF-I, were enhanced in the platelet rich plasma. In the reaming irrigation were FGFa (no measurable value in the PRP) and FGFb (8.3×) enhanced and VEGF (0.1×), PDGF (0.1×), IGF-I (0.4×), TGF-β1 (0.6×) and BMP-2 (0.3×) reduced compared to PRP. BMP-4 was not measurable in any sample.
Conclusion: In conclusion, the reaming aspirate is a source of growth factors with a comparable growth factor potency than iliac crest. The irrigation fluid from the reaming contains also a high amount of growth factors. The use of autogenic reaming aspirate alone or in combination with reaming irrigation fluid might be an alternative method for defect filling. It has an osteoconductive potential due to the

bony structure and is osteoinductive because of a high quantity of growth factors and osteogenic cells.

P275-Mo

Analyzing the Growth Factor Quantity in Commercially Available Demineralized Bone Matrix (DBX)

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Introduction: Osteoconduction and osteoinduction are two principles that promote bone formation and healing. The experiments from Urist revealed that demineralized bone matrix is a source of osteoinductive factors. Despite the function as a filling material with osteoconductive properties, these bone substitutes might serve as a source for osteoinductive factors. Only a few studies analyzed the quantity of growth factors in different bone substitutes. Some publications report an osteoinductive potential of DBM in extraskeletal tissue. Aim of the present study was to analyse a commercially available DBM and to quantify the amount of 8 different growth factors related to bone formation. In addition three different sample preparation methods were used to extract the growth factors.
Materials and methods: DBX Putty(Synthes, USA)= DBM+sodium hyaluronate carrier, 10 samples from different lots. After homogenization, the materials were diluted in one of following solutions:

(a) Proteinase Inhibitor: Samples were incubated for 2 h with Proteinase inhibitor (Complete) at 4°C and the supernatant was used.
(b) Collagenase Extraction: The samples were eluted for 17 h at 37°C under agitation in Tris buffer with Collagenase type 1. Supernatant was dialyzed against water ON at 4°C.
(c) Guanidine HCL method: The sample were extracted in 4 M GuanidineHCL/0.05 M EDTA buffer and dialyzed against water for 24 h.

GF concentration in the samples was quantified using ELISA methods: BMP-2, BMP-4, FGF acidic, FGF basic, IGF-I, PDGFbb, TGF-β1, VEGF (R&D Systems, USA).
Results: The total amount of protein extracted from the DBX varied depending on the extraction method. Using the guanidine HCL method much more protein could be extracted from the samples. FGF basic was not detectable in any analyzed sample. BMP-4 was only measurable in the samples extracted with the proteinaseinhibitor method. Insulin like growth factor-I (IGF-I). Transforming growth factor-β1, Bone morphogenetic growth factor-4, BMP-2, Platelet derived growth factor-bb, FGFa and Vascular endothelial growth factor were measurable in the DBX samples.
Discussion: Commercially available demineralized bone matrix contains osteoinductive growth factors. From 8 investigated growth factors, 7 were detectable in DBX. FGFb was under the detection level of the used ELISA kits. The amount of growth factors varied depending on the extraction method. For quantification of the growth factors within the DBM, the extraction method is important.

P276-Tu

Serum FGF 23 is Elevated in the Early Phase of Bone Healing

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Introduction: Fibroblast growth factor FGF 23 was identified to be one of the phosphaturic factors in diseases involving renal phosphate wasting, including tumor-induced osteomalacia, X-linked hypophosphatemic rickets and autosomal dominant hypophosphatemia. Serum-FGF 23 levels are elevated in some cases of fibrous dysplasia especially in the context of McCune Albright Syndrome. FGF23 was shown to be secreted by osteoblast precursors. FGF 23 also downregulates 1a-hydroxylase expression and the production of 1a,25(OH)2 D3.
Materials and methods: A cohort of 60 patients with a loosening hip arthroplasty were screened pre- and postoperatively with regard to the FGF 23 secretion. In a second study, 25 patients undergoing primary hip arthroplasty were prospectively analysed on the preoperative day and on days 1, 3, 6 and 10 postoperatively with regard to FGF 23 serum levels and serum phosphate levels. FGF23 levels were measured using a commercially available Immunoassay (Immundiagnostik, Bensheim).
Results: Within the cohort of the 60 patients undergoing hip arthroplasty revision serum FGF 23 levels were already slightly elevated at baseline before operation. FGF23 levels showed a marked rise at day 3 after surgery. Patients undergoing primary hip arthroplasty showed regular FGF 23 levels at baseline. On day 1, postoperatively serum FGF23 levels were significantly elevated above the normal range and declined towards the normal range from day 3 till day 10 after surgery. Concomitantly, a slight serum phosphate decline was seen on the 1st and 3rd days after surgery, which was normalized by day 6 postoperatively.
Conclusion: FGF 23 is elevated during the first period of bone formation after implantation of hip arthroplasty. Concomitantly serum phosphate levels are decreasing during the same period after surgery. Our results indicate that FGF 23 plays a major role during the early phase of bone formation, e.g., the inflammatory phase and the early period of the granulation phase. The source of FGF23 secretion

might be proliferating osteoblast precursors. The concomitant decline of serum phosphate might indicate consecutively enhanced phosphaturia. The local role especially in the context of bone formation remains to be investigated.

P277-Su

Early CYR61 Protein Expression in Fracture Callus is Influenced by Fixation Stability

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The cysteine-rich protein 61 (CYR61) is a potent stimulator of angiogenesis and chondrogenic differentiation, both important events in fracture healing. The hypothesis of the present study was that decreased fixation stability is associated with reduced CYR61 protein expression during fracture healing. The aim of this study was to quantitatively analyze CYR61 protein expression and vascularization in the osteotomy gap with regard to the stability over the course of healing. A mid-shaft osteotomy of the tibia was performed in 2 groups of sheep (n = 32 each) and stabilized with either a rigid (group I) or semirigid (group II) external fixator. The sheep were sacrificed at 2, 3, 6, and 9 weeks post-operative. The tibiae were tested biomechanically and histological sections from the callus were analyzed immunohistochemically with regard to CYR61 protein expression and vascularization (alpha-SMA). The immunopositive area fraction (IAF) for CYR61 defined by the immunopositive CYR61 area as a percentage of the callus or tissue area was greatest during the early phase of healing and decreased with healing time. At 2 weeks, group II showed a lower IAF for CYR61 and a significantly lower vessel density (P = 0.021) than group I. The maximum cartilage callus fraction in both groups was reached at 3 weeks. However, group II showed a significantly lower CYR61 immunoreactivity in cartilage (P = 0.028) than group I at this time point. Although group II showed a slight increase in the IAF for CYR61 in cartilage from 3 to 6 weeks, the maximum value of group I was not reached. At 6 weeks, group II showed an inferior histological (less trabecular bone, P = 0.021; more cartilage, P = 0.038) and mechanical (P = 0.041) callus quality in comparison with group I. The mechanical conditions provided by two different fracture fixation stabilities influenced the early vascularization and CYR61 protein expression in the callus. Decreased fixation stability was associated with a reduced upregulation of CYR61 expression and a reduced vascularization at 2 weeks and resulted in a less optimal healing path. In particular, the CYR61 protein expression in cartilage was influenced by fixation stability, as demonstrated by a delayed and reduced protein expression in the semirigid fixator group. The results provide further evidence that CYR61 may serve as an important regulator of bone healing and may be a candidate for therapeutically stimulating bone healing by promoting angiogenesis and chondrogenesis.

P278-Mo

Pattern of Mineralization After Periodontal Tissue Engineering with Enamel Matrix Proteins

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Introduction: A derivative (EMD) of enamel matrix proteins (EMPs) is used for periodontal regeneration, because EMPs are believed to induce the formation of acellular extrinsic fiber cementum (AEFC). Other reports, however, indicate that EMPs have osteogenic potential. The aim of this study was to characterize the nature of the tissue that formed on the root surface following application of EMD.
Materials and methods: Ten human teeth affected by periodontitis and scheduled for extraction were treated with EMD. Four to 6 weeks later, they were extracted and processed for light and transmission electron microscopy. Immuno-cytochemistry with antibodies against bone sialoprotein (BSP) and osteopontin (OPN) was performed to determine the mineralization pattern.
Results: The newly formed tissues on the root were thick, collagenous, devoid of extrinsic fibers, and contained embedded cells. The formative cells were large and possessed the full armamentarium for protein synthesis and export. Discrete mineralization foci were regularly and large organic matrix patches occasionally seen, but a distinct mineralization front was absent. While labeling for BSP was always associated with mineralization foci and large matrix patches, OPN labeling was inconsistently seen.
Conclusions: Instead of the expected AEFC, tissues resembling either cellular intrinsic fiber cementum or a type of bone were observed. The mineralization pattern resembled most that found in bone, except for a few areas that exhibited a hitherto not described mineralization pattern.

P279-Tu

Osteoprotegerin in Umbilical Blood as a Marker of Bone Development in Newborns

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Osteoprotegerin (OPG) is a glycoprotein of TNF receptor superfamily that enhances bone formation by decreasing

bone resorption and inhibiting differentiation of osteoclast precursors and activation of mature cells. It is well known that bone development depends on gestational age and is directly proportional to intrauterine growth of newborns. Because the role of osteoprotegerin in skeletal formation in perinatal period is unknown we assessed the level of osteoprotegerin in cord blood of newborns according to their gestational age and birth parameters.

Materials and methods: Clinical material consisted of 88 newborns born from 25 to 42 weeks of gestation, 46 boys and 42 girls. According to their gestational age two groups were created: I: $n = 32$ preterms born before 37 gestational week; II: $n = 56$ fullterms born after 37 week of gestation. Birth weight (g), length (cm), head circumference (cm) and chest circumference (cm) were measured in all the newborns with standard methods just after the labour. The level of osteoprotegerin (pmol/l) (Biomedica Gruppe, Austria) in cord blood was assessed with ELISA method. Statistical analysis was performed by Mann–Whitney U and Spearman rank correlation test. $P < 0.05$ was regarded as significant.

Results: Statistically significant differences were found for osteoprotegerin level between the groups: group I-5.40 ± 3.27 pmol/l, group II-3.94 ± 1.25 pmol/l, I vs. II $P < 0.001$. No statistical differences were detected for cord blood osteoprotegerin in boys and girls ($P > 0.05$). The negative statistically significant correlation was confirmed for osteoprotegerin and gestational age of newborns ($R = 0.34$, $P < 0.05$). Statistically significant negative correlation were also found between the osteoprotegerin in cord blood and physical growth of newborns: birth weight ($R = 0.38$, $P < 0.001$), length ($R = 0.43$, $P < 0.001$), head circumference ($R = 0.41$, $P < 0.001$) and chest circumference ($R = 0.41$, $P < 0.001$).

Conclusion: Statistically significant correlations between the level of OPG in cord blood, gestational age and birth parameters of newborns were confirmed. The role of OPG in foetus bone development needs further investigation.

P280-Su

Liver-Derived Insulin-Like Growth Factor I is Permissive for Ovariectomy-Induced Bone Loss

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Estrogen deficiency as a result of ovariectomy (ovx) results in a pronounced trabecular bone loss. Insulin-like growth factor I (IGF-I) is involved in the regulation of bone metabolism and a major part of serum IGF-I is derived from the liver.

The aim of the present study was to investigate the role of liver-derived IGF-I for ovx-induced bone loss. Three-month-old mice with Cre-loxP induced liver-specific IGF-I inactivation (LI-IGF-I$^{-/-}$) and wild type mice (WT) were either ovx or sham operated. As expected, 5 weeks after ovx, there was a pronounced reduction in trabecular bone mineral density (BMD) in WT mice as measured using pQCT (−52%, $P < 0.001$). The decreased trabecular BMD was caused both by a reduced number (−45%, $P < 0.01$) and thickness (−13%, $P < 0.01$) of trabeculae compared with sham operated mice as measured using μCT. The trabecular bone mass in sham operated LI-IGF-I$^{-/-}$ mice was similar to that seen in sham operated WT mice. Interestingly, the trabecular bone parameters were not affected by ovx in LI-IGF-I$^{-/-}$ mice.

Recent studies indicate that skeletal homeostasis is influenced by several components of the immune system. Therefore, we measured the number of T cells in bone marrow of the femur using flow cytometric analysis. Ovx increased the number of T cells in the bone marrow of the femur in WT (193% over sham, $P < 0.001$) but not in LI-IGF-I$^{-/-}$ mice. Interleukin 7 (IL-7) has been reported to stimulate the formation and function of osteoclasts by inducing the expression of receptor activator of NF-κB ligand (RANKL) on T-cells. The expression of IL-7 was increased by ovx in WT (26% over sham, $P < 0.05$) but not in LI-IGF-I$^{-/-}$ mice as studied by real time PCR analysis. Similarly, ovx of WT mice resulted in an increase in the RANKL/osteoprotegerin ratio (52% over sham, $P < 0.05$), while this ratio was unaffected by ovx in LI-IGF-I$^{-/-}$ mice. In conclusion, liver-derived IGF-I is permissive both for ovx-induced trabecular bone loss and for the associated increase in number of T-cells in bone marrow. Our mechanistic studies indicate that the permissive effect of liver-derived IGF-I for ovx-induced trabecular bone loss involves modulation of the expression of IL-7 and a subsequent modulation of RANKL on T-cells in the bone marrow.

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Congenital Aromatase Deficiency Causes Abnormal Pubertal Growth and Growth Hormone Signaling in Mice

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Aromatase catalyzes the biosynthesis of estrogens from androgen precursors. Aromatase-deficient (ArKO) adult male mice have low serum IGF-I levels and short femurs. In this study, we followed the growth of wildtype (WT) and ArKO mice during puberty, age 21–49 days ($n = 10$ group). Nasal anal length and body weight were measured weekly. A group of ArKO male mice were treated with estradiol (E2, 20 μg/mouse 3×/week) or recombinant human IGF-I (rhIGF-I, 23.3 μg 3×/day 3×/week) to determine if femoral length could be restored to normal.

At the end of the study period, animals were sacrificed, serum harvested and mRNA prepared from liver and bone. Serum IGF-I levels were determined by RIA. Serum levels of IGFBPs were determined by ligand blot assay. Real-time PCR was used to quantitate liver mRNA levels of IGF-I, acid labile subunit (ALS), IGFBPs 2–4. The mRNA levels of IGF-I, IGFBP4–5 were determined in bone. Hepatic protein levels of SOCS-2, a suppressor of GH action, were determined by Western blot. Normally distributed data was analyzed by Student's t test. Differences were considered statistically significant if $P < 0.05$. ArKO male mice had significantly lower body weights and shorter nasal anal lengths over the study period. The femur length and mid-shaft diameter were significantly less in ArKO. These differences were corrected by rhIGF-I therapy. Surprisingly, E2 treatment caused a greater decrease in the growth of the ArKO animals. Serum IGF-I levels were lower in ArKO males. Ligand blot analysis showed decreased levels of IGFBP-3 but higher levels of BP4 and BP1,2. In the liver, mRNA levels for IGF-I and ALS were lower but interestingly BP3 mRNA levels were higher in ArKO mice. In bone of ArKO mice, mRNA levels of IGF-I and BP5 were lower. The rIGF-I therapy was associated with an increase in serum IGFBP3 on ligand blots and ALS mRNA. Hepatic expression of SOCS2 protein was much greater in ArKO mice. In summary, congenital aromatase deficiency is associated abnormal pubertal growth caused by abnormal GH/IGF-I axis action. The high levels of SOCS-2 decreasing GH signaling in the liver could explain the decreased hepatic levels of IGF-I and ALS. Since IGF-I was also decreased in bone the aromatase deficiency caused systemic (low serum IGF-I) and local changes in GH/IGF-I action.

P282-Tu

CBL-Mediated Ubiquitination of Alpha 5 Integrin Subunit Mediates Fibronectin-Dependent Osteoblast Detachment and Apoptosis Induced by Fibroblast Growth Factor Receptor 2 Activations

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Fibroblast growth factor receptor signaling is an important mechanism that controls osteoblast function. To gain insight into the regulatory role of FGF receptor-2 (FGFR2) signaling in osteoblasts, we investigated integrin-mediated attachment and cell survival in human calvarial osteoblasts expressing an activating FGFR2 mutation. FGFR2 activation reduced osteoblast attachment on fibronectin. This was associated with reduced expression of the alpha 5 integrin subunit which is expressed in human calvarial osteoblasts in vivo. Treatment with lactacystin, a potent inhibitor of proteasome, restored alpha 5 integrin levels in FGFR2 mutant osteoblasts. Immunoprecipitation analysis showed that alpha 5 integrin interacts with both the E3 ubiquitin ligase Cbl and ubiquitin. Immunocytochemistry showed that alpha 5 integrin colocalises with FGFR2 and Cbl at the leading edge in membrane ruffle regions. Transfection with 70Z-Cbl mutant, which lacks the RING domain required for Cbl-ubiquitin interaction, or with the G306E Cbl mutant, that abolishes the binding ability of Cbl phosphotyrosine-binding domain, restored alpha 5 integrin levels, showing that Cbl-mediated ubiquitination plays essential roles in alpha 5 integrin proteasome degradation induced by FGFR2 activation. The reduced alpha 5 integrin expression was associated with increased Bax/Bcl-2 ratio and caspase-9 and-3 activity in FGFR2 mutant osteoblasts. Forced expression of alpha 5 integrin rescued cell attachment and corrected both the Bax/Bcl-2 ratio and caspase-3 and caspase-9 activity in FGFR2 mutant osteoblasts. The data show that Cbl recruitment induced by FGFR2 activation triggers alpha 5 integrin proteasome degradation, which results in reduced osteoblast attachment on fibronectin and caspase-dependent apoptosis. This identifies a functional role of alpha 5 integrin subunit in the induction of apoptosis triggered by FGFR2 activation, and reveals a Cbl-dependent mechanism involved in the coordinate regulation of cell apoptosis induced by alpha 5 integrin degradation in response to fibroblast growth factor receptor 2 signaling in osteoblasts.

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Vascular Endothelial Growth Factor (VEGF) Acts as a Survival Factor in Human Osteoblastic Cells

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VEGF has been shown to play an important role during endochondral bone formation, but its role on osteoblastic growth/viability and differentiation is poorly understood. VEGF promotes survival in different tissues in part via the Bcl-2 protein family. In the present study, we assessed the effect of VEGF on osteoblastic cell viability (evaluated by trypan blue and propidium iodide staining). Subconfluent human osteoblastic cells MG-63 were treated with 20 ng/ml VEGF during 1–24 h, following by addition of 1 mM dexamethasone or 50 mM etoposide for 16 h. VEGF preincubation induced a significant inhibition (about 40%; $P < 0.05$) in cell death caused by either dexamethasone or etoposide in these cells. In addition, VEGF (between 1 and 24 h) stimulated (maximal, 2-fold; $P < 0.05$) both BclxL protein expression and the activity of Runx2, a transcription factor which appears to be related to the expression of Bcl-2 gene family in osteoblasts. Moreover, the medium conditioned by MG-63 cells in the presence of

VEGF for 1–24 h inhibited (about 30%, $P < 0.05$) the cell death triggered by the death-promoting agents in these cells. A neutralizing VEGF antibody failed to affect this action of the cell-conditioned medium in MG-63 cells. In summary, these results demonstrate that VEGF promotes the survival of human osteoblastic cells in vitro. This effect might occur by an autocrine/paracrine mechanism involving the secretion of a soluble factor induced by VEGF in these cells.

P284-Mo

Oncostatin M Sensitizes Osteosarcoma Cells to Apoptosis via the Mitochondrial Pathway

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Oncostatin M (OSM), a cytokine of the IL-6 family, reduces the growth and induces differentiation of numerous solid tumors like osteosarcomas via the transcription factor STAT3, kinases such as ERK 1/2 or PKCd and the cyclin dependent kinases inhibitor p21WAF1. OSM has been shown to protect MG63 osteosarcoma cells from apoptosis triggered by death receptors but very few data are available concerning the role of this cytokine in apoptosis. The goal of this study is to better document the effect of OSM on osteosarcoma apoptosis.

Alone, OSM did not induce cell death, but OSM-treated osteosarcoma cells were particularly more sensitive to apoptosis induced by staurosporin (STS), and not that induced by TNFa. Cell death induced by OSM+STS was associated with activation of caspase 9 and 3 and could be prevented by the caspase inhibitor Z-VAD-FMK. Interestingly, OSM alone induced activation (phosphorylation) of p53, and concomitantly enhanced expression of Bax and reduced expression of Bcl-2. Other Bcl-2 family members (Bak, Bcl-xL, Bcl-xS) were not modulated. However, p53 was not strictly necessary for the death induced by OSM+STS because this combination was also active on p53-deficient MG63 cells. On the various dominant negative STATs that were tested (dnSTAT1, 3 and 5), only dnSTAT5 was able to prevent apoptosis induced by OSM+STS. Since STS is known to inhibit numerous kinases, we asked whether specific kinase inhibitors could also induce apoptosis in association with OSM. We observed that inhibitors for PKCd, PI3K, Akt or MEK/ERK were all able to trigger apoptosis with OSM.

Altogether, our results suggest that OSM sensitizes osteosarcoma cells to apoptosis via the mitochondrial pathway. Our current hypothesis is that OSM, via STAT5 and p53, induces the Bax/Bcl-2 ratio that control cell death. However, strong anti-apoptotic signals are also activated by OSM via the PKCd, PI3K/Akt and MEK/ERK pathways. The use of kinases inhibitors in association with OSM could therefore represent new treatments for osteosarcomas and deserves further investigations.

P285-Tu

Regulation of Osteoblast Apoptosis by Cytokines

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Several members of TNF-α family, such as TNF-α, induce cell death and are critically involved in the pathogenesis of various diseases. Tweak is a recently identified member of the TNF superfamily, a transmembrane protein that can be cleaved to generate a soluble factor with biological activity. Fibroblast growth factor inducible 14 (Fn14) has been also identified as a Tweak receptor with physiological activity. We recently reported that postmenopausal osteoblasts undergo apoptosis when exposed to Fas agonist, and that this lethal effect is exacerbated in the presence of TNF-α. However, the effects and mechanisms of action of Tweak on bone cells are still not fully understood. The aim of this work was to characterize the Tweak-induced cell death in MG-63 cells, a human osteosarcoma cell line with osteoblast phenotype. MG-63 cells were cultured in DMEM with IFN-gamma (300 U/ml), or TNF-α (5000 U/ml) or Tweak (100 ng/ml) alone or with combination of these cytokines, in the presence or absence of survival factors from serum for 24, 48 and 72 h and apoptosis was quantified by flow cytometry. In other set of experiments, 5000 U/ml TNF-α, 300 U/ml IFN-gamma and 1/10/100 ng/ml of Tweak were added in order to examine apoptosis with different concentrations of Tweak. RT-PCR was used for the detection of Fn14 mRNA from MG-63 cells cultured at confluence 1 and 3 h following addition of 5000 U/ml of TNF-α and/or 300 U/ml of IFN-gamma. When MG-63 cells were cultured with IFN-gamma or TNF-α or Tweak alone, no significant increase in apoptosis was observed. The association of IFN-gamma and TNF-α increased cell death. Culture with the three cytokines further increased apoptosis both in the presence and in the absence of serum. Cell death was not apparent for the first 24 h of incubation, but increased at 48 and 72 h. Cell death was also Tweak-dose dependent from 1 to 100 ng/ml. MG-63 cells express the Tweak receptor Fn14, and IFN-gamma increases significantly Fn14 mRNA expression. In conclusion, this work demonstrates that a novel cytokine, Tweak, modulates survival of MG-63 osteoblastic cells. (National Institute of Health, Spain, FIS PI02/0292).

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Alpha-Lipoic Acid Inhibits TNF-α-Induced Apoptosis in Human Bone Marrow Stromal Cells

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Introduction: Oxidative stress is an important mediator of bone loss. TNF-α, which plays a critical role in the bone loss following menopause, has been shown to increase intracellular oxidative stress. Since oxidative stress is associated with cell death, we analyzed the apoptotic effects of TNF-α and H_2O_2 on hBMSC. We also examined the protective effects of an important biological thiol antioxidant, α-lipoic acid, against TNF-α- and H_2O_2-induced apoptosis.
Materials and methods: Using the HS-5 hBMSC cell line, we tested whether TNF-α-induced apoptosis was mediated by the generation of excessive ROS. Apoptosis was determined by MTT assay, trypan blue exclusion assay, quantitation of histone-associated DNA fragments in cytosol, and the activation of caspases. The mechanisms mediating these apoptotic effects were determined by Western blotting and enzyme immunoassay.
Results: Both TNF-α and H_2O_2 increased intracellular ROS levels, reduced total cellular glutathione levels, activated caspases-3, -9 and -8, and enhanced hBMSC apoptosis. The activation of c-jun N-terminal kinase (JNK) and NF-κB mediated these apoptotic effects. Pretreatment of cells with α-LA prevented these changes induced by TNF-α and H_2O_2.
Conclusions: Our data show that TNF-α increases intracellular ROS in hBMSC and that TNF-α and H_2O_2 induce apoptosis in hBMSC via the activation of JNK and NF-κB. Our findings also suggest that α-LA may have therapeutic applications in halting or attenuating bone loss associated with increased oxidative stress.

P287-Mo
Cyclooxygenase-2 Mediates Thrombin's Inhibition of Osteoblast Apoptosis by a PAR-1-Independent Mechanism
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We have previously shown that treatment of serum-deprived or dexamethasone-treated primary mouse calvarial osteoblasts with 100 nM thrombin significantly reduces apoptosis in these cultures, and that this effect is not mediated by any of the known thrombin-responsive protease-activated receptors (PAR-1, -3 and -4), but is mediated by a secreted inhibitor of apoptosis. In the current study, we have used inhibitors of various intracellular signaling pathways to identify the pathways involved in thrombin's inhibition of osteoblast apoptosis. As only inhibitors of cyclooxygenases prevented thrombin's effect on osteoblast apoptosis, the expression of cyclooxygenase-2 (COX-2) and synthesis of prostaglandin E_2 (PGE_2), following thrombin treatment of PAR-1 null primary osteoblasts, and the ability of PGE_2 to inhibit serum deprivation-induced osteoblast apoptosis, were studied. Six hours after thrombin treatment of PAR-1 null osteoblasts, expression of COX-2 mRNA and secretion of PGE_2 were significantly elevated. To study the potential role of either prostaglandin E_2 in the inhibition of osteoblast apoptosis, serum-deprived primary mouse osteoblasts were treated with either PGE_2 (1 μM) or the cAMP analogue 8-bromo cAMP (1 pM–1 μM). Both PGE_2 and between 1 pM and 1 nM 8-bromo cAMP significantly inhibited serum deprivation-induced osteoblast apoptosis as judged by staining for DNA strand breaks and nuclear morphology. Taken together, these results suggest that thrombin stimulates COX-2-mediated PGE_2 production by a mechanism does not require PAR-1 and that this rise in PGE_2 signals through cAMP to inhibit serum-deprivation-induced osteoblast apoptosis.

P288-Tu
The BH3-Only Protein BIM is Upregulated in Osteoblasts in Response to Dexamethasone Treatment, Growth Factor Deprivation and Anoikis
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Osteoblast lineage cells undergo apoptosis following withdrawal of growth factors, detachment from matrix and treatment with bone toxic drugs, such as glucocorticoids (GC). Signalling through kinases such as ERK and PI3-kinase plays a critical role in osteoblast survival and this is compromised during anoikis (which affects signalling downstream of integrins and FAK) and following withdrawal of mitogens. GC-induced apoptosis of osteoblasts shows features similar to both anoikis and growth factor withdrawal, and can be prevented by treatment with vanadate which activates signalling driven by tyrosine phosphorylation. This survival-promoting activity can be partially blocked by U0126 and Wortmannin, indicating involvement of both ERK and PI3-K respectively. GCs do not repress transcription of the pro-survival signalling molecules Bcl2, Bcl-XL, cIAP1, cIAP2, XIAP, cFLIPshort or cFLIPLong, and vanadate does not increase expression of these molecules at the mRNA level. Since apoptosis signalling is very closely regulated by kinase-mediated proteasomal degradation and control of sub-cellular compartmentalisation, we examined the regulation of the pro-apoptotic protein, Bim (Bcl-2 Interacting Mediator of

Cell Death). As shown in other cell types, Bim protein levels are regulated by the 26S proteasome and by ERK and PI3-K signalling in osteoblasts. The three major isoforms of Bim, BimEL, BimL and BimS are overexpressed in response to detachment in MBA15.4 (mouse), hPOB (human) osteoblasts and primary human bone marrow stromal cells (hBMSC), peaking within 24 h of detachment and remaining high for at least 72 h. During this time massive osteoblast apoptosis occurred. BimEL was upregulated following 24 h serum starvation in MBA15.4, concurrent with a 50% loss of viability (trypan blue exclusion) and a 15 fold increase in apoptosis (DAPI stain and TUNEL). In MBA15.4 osteoblasts 10−6 M dexamethasone upregulated BimEL protein in a time-dependent manner, with levels peaking at 48 h and remaining elevated at 72 h. This osteoblast cell line undergoes GC-induced apoptosis, induced three-fold after 72 h treatment with 10−6 M Dex and rising to eight-fold after 6 days (TUNEL). Basal levels of any isoform of Bim protein are hardly detectable in MBA15.4 and MG63 cell lines and in hBMSC. Establishment of its physiological roles and how damage signals relay through Bim will facilitate manipulation of pro-survival signalling in vulnerable skeletal cells in the clinical setting.

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Response of the Murine Bone Cell Lines to Ionizing Radiation

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During long-term space missions, astronauts suffer from the loss of minerals especially from weight bearing bones due to prolonged sojourn under weightlessness. Exposure to cosmic ionization radiation is another space-related factor endangering the astronauts' health. In order to elucidate changes in bone cell metabolism by ionizing radiation, survival, strand break induction and activation of the anti-apoptotic and eventually differentiation promoting transcription factor Nuclear Factor kappaB (NF-kappaB) in bone cell lines after exposure to X-rays were analyzed. The bone cell lines were classified into four differentiation stages (preosteoblast, osteoblast, mature osteoblast and osteocyte) according to their morphology, alkaline phosphatase activity, von Kossa reaction after culturing in osteogenic medium, and expression of osteocalcin and the transmembrane protein E11 as determined by Reverse Transcriptase PCR. Survival after exposure to X-rays was determined in colony forming ability tests. The resulting dose-effect relationships revealed normal radiation sensitivity (compared to human fibroblasts). The osteocyte cell line MLO-Y4 showed the lowest radiation sensitivity. The quantitative acquisition of DNA strand breaks was performed by Fluorescence Analysis of DNA-Unwinding (FADU). Results can be correlated with the survival curves of the three cell lines. The cell line with the highest differentiation level displays a lower radiation sensitivity compared to the less differentiated osteoblast cell lines. The activity of NF-κB in response to X-rays was determined in MLO-Y4 using a reporter gene assay. The cell line was stably transfected with a vector carrying the genes for the reporter proteins Enhanced Green Fluorescent Protein (EGFP) or its destabilized variant d2EGFP under control of four copies of the NF-κB response element. Treatment with the NF-κB activating tumor necrosis factor alpha gave rise to EGFP/d2EGFP expression in several stably transfected clones, measured by Fluorescent Activated Cell Scanning (FACS). Two clones, expressing EGFP or d2EGFP and showing stellate morphology, were chosen for X-irradiation. Only in the high dose range (>8 Gy), a substantial activation of NF-κB could be monitored by FACS analysis. So far, the lower sensitivity of MLO-Y4 cells to X-rays can not be explained by an activation of NF-κB, which may protect from apoptosis, in a larger part of the population.

P290-Mo

Role of the Inhibitors of Hydroxy-Methyl-Glutaryl-Coa Inhibitors (Statins) in the Osteosarcoma Cell Behavior

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Aim of study: Statins, acting on the mevalonate pathway, are vital for a variety of key cellular functions affecting membrane integrity, cell signaling, protein synthesis, and cell cycle progression. They stimulate normal bone formation in rodents both in vitro and in vivo. However, there are no enough evidences of its basic mechanism. The aim of this study is to evaluate in vitro the role of statins in osteoblasts cell biology.

Materials and methods: Rat (UMR-106) and human (HOS, SaOS-2, U2OS) osteosarcoma cell lines were grown under conventional conditions. The following parameters were studied after administration of simvastatin at different doses (vehicle, 0.1, 0.3, 1.0, 3.0 and 10 μM) and during different times (24, 48 and 72 h), with or without mevalonate, FPP, GGPP, FTI or GGTI: cell growth rate by cell counting; cell viability by Trypan blue cell exclusion assay and by MTT; morphologic changes; apoptotic response studied by DAPI, flow cytometry with PI/annexine V and by DNA laddering; cell cycle alterations were analyzed by flow cytometry with PI and protein expression by Western blot of p27Waf1/Cip1 and cyclin A; and cell motility was studied by cell wound assay. Data was

presented as mean + SEM of at least three different experiments. A rejection level of $P < 0.05$ will be considered significant.

Results: We observed that the statins induced: (1) a decrease in cell growth rate; (2) an increase in the number on non-viable cells; (3) morphological alterations characterized by cell rounding and cell detachment from the substrate; (4) cell growth arrest in G1 and G2/M phases, dependent of an increase in the p27Cip/Kip/Waf and a decrease of cyclin A protein expressions; (5) and, a decrease in cell motility evaluated by cell wound assay.

Discussion Statins, at least in vitro, are useful in the treatment of osteosarcomas. They are able to decrease cell proliferation, induce cell death by apoptosis and affect the cell motility necessary in the metastatic processes. Further studies are necessary to study how statins can affect chemotherapy response; and to correlate these biological parameters with patient outcome. At present, we are evaluating the in vivo effect of these drugs in osteosarcomas growing in nude mice.

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P291-Tu

Effect of Statins on Osteoprotegerin Production by Bone Cells

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Bone turnover normally occurs in a highly regulated manner. Cells of the osteoblast lineage deposit bone and cells of the osteoclast lineage resorb bone in a coupled way to maintain bone mass. Osteoblastic cells secrete a soluble glycoprotein named osteoprotegerin (OPG) which acts as a decoy receptor by binding to the receptor activator of nuclear factor κ-B ligand (RANKL) protein. RANKL is integrated in to the membrane of osteoblastic cells. In the absence of OPG, RANKL binds to its receptor, RANK, expressed on osteoclasts and their precursors to bring about differentiation and activation of bone resorption. Thus, OPG has a crucial role in preventing bone resorption.

Statins are specific inhibitors of HMG coenzyme A reductase which is part of the pathway leading to cholesterol, hence, the use of these drugs as treatment for hyperlipidemia. Statins have also been shown to stimulate bone formation in rodents and to inhibit osteoclast differentiation. Our aim was to see if statins influenced OPG production since such an effect might contribute to these two observations on bone.

Mevastatin (10−5 to 10−6M) stimulates the secretion of OPG into the culture medium from human (SAOS-2) cells and murine (MC3T3-E1) osteoblastic cells in the 24-h period following a 24-h incubation with the drug. The time course of the effect suggests an indirect mechanism. No effect on cell number was detected under these conditions. OPG secretion from MG63 and ST-2 osteoblastic cells was not affected by mevastatin. Mevalonate, a metabolite produced by HMG coenzyme A reductase, prevents the stimulation of OPG secretion by mevastatin in both SAOS-2 and MC3T3-E1 cells. The stimulation of OPG production by mevastatin may contribute to the effects of statins observed in bone.

P292-Su

Apoptotic Bodies from Dying Osteocytes Induce Specific and Unique Responses in Different Phagocytes

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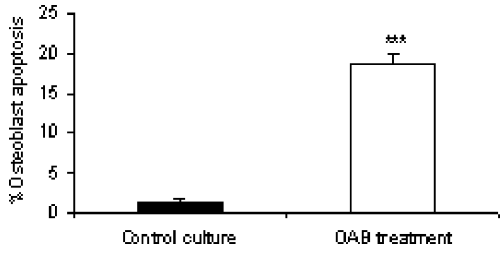
Targeted remodeling is a requirement for structural and mechanical adaptation of bone. Recent evidence points to the production of signals from the dying osteocyte that might target the activity of bone resorbing and forming cells. In a related way, it is known that apoptotic bodies elicit in phagocytic cells behavioural responses that have far reaching effects on tissue function. Here, we introduced osteocyte apoptotic bodies (OAB) to osteoblast cultures for 24 h in order to test for signals carried by apoptotic osteocytes capable of modifying osteoblast behavior. OAB increased the percentage of apoptotic osteoblasts by 14-fold ($P = 0.0001$ vs. control) (Fig. 1). By contrast, other cell lineages were not affected by introduction of OAB. Apoptotic bodies derived from non-osteocytic cell types failed to induce osteoblast apoptosis. Agents that interfered with membrane composition on the osteoblast such as glyburide and oligomycin reduced the uptake of OAB (15 ± 2% $P = 0.001$ and 10 ± 1% $P = 0.0009$, respectively, vs. 40 ± 3% in OAB treated cultures and the induction of osteoblast apoptosis (4.9 ± 0.7% $P = 0.02$ and 3 ± 0.6% $P = 0.001$ respectively, vs. 20 ± 1% in OAB treated cultures). Gene knockout animal models lacking scavenger receptor A demonstrated partial blockade of OAB ingestion (50%) but not apoptosis, while a blockade of both ingestion (up to 50%) and osteoblast apoptosis (up to 70%) occurred in cells lacking the apoptotic recognition molecule CD14. We propose that OAB deliver phenotype-specific signals to phagocytes and that CD14 is involved in the specific response of osteoblasts to OAB. Such specificity in the recognition system might confer further levels of physiological meaning to the apoptotic process in bone and other tissues. In particular, the existence of locally produced signals with high osteoblast specificity might underlie the targeted remodeling process in bone.



P293-Mo

The Effect of Temperature on Bone Cell Function

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Relatively little attention has been paid to the regulation of bone cell function by fundamental variables in the extracellular environment such as pH, oxygen tension and temperature. Here, we studied the effects of changes in ambient temperature within the pathophysiological range on the formation and activity of bone cells. OB were harvested from neonatal rat calvariae by trypsin/collagenase digestion and cultured up to 18 days in DMEM/10% FCS with 0.05 mg/ml ascorbate, 2 mM β-glycerophosphate and 10 nM dexamethasone at 34°C, 37°C (control) or 40°C. Bone nodule formation, assessed by alizarin red staining, was decreased by ~37% and ~57% respectively in OB maintained at 34°C and 40°C, compared to 37°C controls ($P < 0.001$); cell numbers were not significantly affected by temperature. OC formation from human peripheral blood mononuclear cells (PBMCs) and mouse marrow was also highly sensitive to temperature. Human PBMCs and mouse marrow cells were cultured on ivory discs for 10 days in MEM with 10–15% FCS, 10 ng/ml M-CSF and 2 ng/ml RANKL in 25 cm^2 flasks gassed with 2–5% O_2 (plus 5% CO_2; balance N_2) at 34°C, 37°C or 40°C at pH 7.3. Culture medium was acidified to pH 7.0 for the final 2 days to activate resorption by OC; PO_2, PCO_2 and pH were monitored by blood gas analyzer. In both human and murine cultures, a 4-fold decrease in TRAP-positive OC formation was observed at 40°C, compared to 37°C controls ($P < 0.001$); this reduction in OC number was accompanied by 10-fold and 2.5-fold decreases in area resorbed/osteoclast, respectively (leading to large inhibitions in resorption pit formation). At 34°C, however, OC formation increased by up to 2-fold in both species, although resorption/OC was decreased by ~30%. These surprising temperature effects on bone resorption were highly reproducible. A local temperature of 40°C could be attained in bone as a result of fever or severe inflammation. Conversely, lower bone temperatures could result from hypothermia, ageing or rigorous dieting. Our results suggest that hyperthermia itself would strongly reduce bone remodelling activity (although this action might be offset in vivo by increased osteolytic, inflammatory mediators such as IL-1, TNF-α and prostaglandins). Hypothermia, however, could shift the remodelling balance in the negative direction, and may thus deserve consideration as a possible factor in the pathogenesis of senile osteoporosis in some countries.

P294-Tu

Long Wave Ultrasound may Enhance Bone Regeneration by Altering OPG/RANKL Ratio in Human Osteoblast-Like Cells

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Therapeutic ultrasound has successfully promoted healing of mandibular osteradionecrosis, fractures, and post-distraction osteogenesis. Cytokines, TNF-α (tumor necrosis factor alpha) receptor activator of NF-κB Ligand (RANKL) and osteoprotegerin (OPG) have been shown to act directly or indirectly on osteogenic cells and their precursors to control differentiation, resorption and bone formation. RANKL and TNF-α promote osteoclast differentiation and thus bone resorption whereas OPG promotes osteoblast differentiation and also competes with RANKL for the RANK receptor present on the osteoclast. We treated MG63 cells (human osteoblast-like cells) with the long wave (45 kHz continuous, intensity −30 mW/cm^2) ultrasound machine and incubated the cells for 0, 3, 6, 12, 18 and 24 h following the treatment. The reverse transcriptase polymerase chain reaction (RT-PCR) technique was used for observing genetic expression and real time PCR for quantitative analysis of the genetic expression of RANKL and OPG along with alkaline phosphatase (ALP), an early bone marker and osteocalcin (OCN), a late marker. ELISA was performed for estimating the amount of the cytokine released into the culture media, following sonication. The MG63 cells responded to the ultrasound treatment by significantly upregulating both the OPG mRNA and protein levels. There was no RANKL mRNA expression observed in both the ultrasound and control groups and the protein levels were also very low in general and significantly low in the ultrasound group. There was also no TNF-α expression and the TNF-α protein levels were insignificant. ALP mRNA was significantly upregulated in the ultrasound group. Osteocalcin also was prominently expressed in the ultrasound group.

Ultrasound appears to upregulate OPG and may downregulate RANKL production. From these findings, we conclude that therapeutic ultrasound may increase bone

regeneration by altering the OPG/RANKL ratio in the bone micro-environment.

P295-Su

Gene Array Analysis of Shock Wave-Induced Changes of Gene Expression in Human Osteoblast Cultures from Patient with Fracture Non-Unions

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Over the last decade, extracorporeal shock wave therapy (ESWT) has been applied as a non-invasive method to enhance bone growth. Several clinical studies showed success rates of ESWT for the treatment of fracture non-unions varying between 41 and 91%. However, underlying regulatory mechanisms of the induction of cell growth and differentiation are still unclear. To address this important question, we examined the changes of gene expression in osteoblast cultures induced by ESWT under in-vitro conditions. Primary cultures of human osteoblasts were isolated from cancellous bone of five patients undergoing reconstructive surgery for fracture non-unions. Cells were treated with two different extremes of energy flux densities (0.06 and 0.5 mJ/mm^2) and cultured for 24 and 96 h in monolayer cultures. Afterwards, RNA was isolated. Control cultures were isolated from respective patients and cultured under the same conditions, but without ESWT. Expression of fourteen different genes was found to be most significantly influenced by ESWT (peptidases, transcription factors, transporters). Particularly, three novel markers were highly activated after ESWT. 1. Neprilysin (NEP), a membrane-associated peptidase, which is an important regulator of physiological processes by controlling the half-life of bioactive peptides in bone-forming cells, suggesting a relationship between NEP-expression and ESWT-induced osteoblast activation. 2. Mimecan/osteoglycin, a member of the SLRPs (small leucin rich proteins, previously identified in bone and cornea), which may play a role in cellular growth control, as described by ability of different growth factors and cytokines to modulate its expression. 3. IL13RA2 (IL13 receptor alpha2), a decoy receptor for IL13, which was previously described to inhibit cell proliferation in human osteoblasts. These significant results were confirmed by PCR. Our results indicate that already low energy ESWT changed the gene expression profile in human osteoblasts. Additionally, high-energy treatment induced expression of apoptosis related genes. We could show an enhanced expression of specific factors, which could explain the underlying osteoinductive effects of ESWT at a molecular level. Further studies will help to understand the function of these factors, and also to find the appropriate treatment needed for the clinical practice.

P296-Mo

CCAAT/Enhancer Binding Protein (C/EBP) beta is Involved in Bone Formation

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CCAAT/Enhancer Binding Proteins (C/EBP) are gene regulators involved in proliferation and differentiation in various cell types, such as in haematopoiesis and adipogenesis. Recent data obtained with cell culture experiments suggest that C/EBPbeta also plays a role in bone cells. However, the involvement of C/EBPbeta in bone formation in vivo is unclear. Here, we show that C/EBPbeta mRNA is expressed in osteoblasts and in growth plate chondrocytes in tibiae of 4-week-old mice. C/EBPbeta knock-out mice (1) showed a significant decrease in both body length (85 ± 6%, $P < 0.02$) and tibia length (86 ± 4%, $P < 0.005$). Histological analyses of the growth plate showed a decrease in the total width of the growth plate (305 ± 7 μm vs. 258 ± 15; $P < 0.05$ μm), caused by a decrease in the hypertrophic and the proliferative zones, whereas the width of the resting zone was increased. Histomorphometric analyses, furthermore, showed a decrease in bone volume in C/EBPbeta-deficient mice (30 ± 3% vs. 21 ± 2% BV/TV; $P < 0.03$). Osteoclast number per bone perimeter was similar between wild-type and C/EBPbeta-deficient mice suggesting that the decrease in bone volume is due to a malfunction of the osteoblasts.

C/EBPbeta protein is expressed as different isoforms due to alternative initiation of translation. Long and short isoforms display antagonising biological functions. Interestingly, analyses of 4-week-old mice that only express the C/EBPbeta small isoform showed an increase in bone volume (26 ± 2% vs. 48 ± 6% BV/TV; $P < 0.03$), whereas the osteoclast-covered surface remained unaltered. However, in these mice neither body and tibia length nor the growth plate morphology was affected. Taken together, our data strongly suggest that C/EBPbeta plays an important role in bone formation and that deregulation of C/EBPbeta isoform expression might be involved in bone diseases.

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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P297-Tu

Down-Regulation of Tsg101 Expression During Osteoblast Differentiation

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Osteoporosis is becoming more and more important. Bone is continuing renewal by a procedure, called remodeling. This procedure is affected by several growth factors, cytokines and hormones. The osteoblast is the key role for this procedure. Understanding the differentiation of osteoblast will be very helpful for treatment of osteoporosis.
The tumor susceptibility gene 101 (Tsg101) was first discovered in murine fibroblasts in a screen for potential tumor suppressors. Various biological functions of Tsg101 have been postulated. Several reports show that Tsg101 may influence cell cycle control and cell differentiation. Using PCR method, we have successfully cloned a Tsg101 promoter (2647 bp 5′ flanking upstream of gene coding region) with 13 different segments into Puc18 based T-vector. The resulted recombinant plasmid DNA was purified and used as template for further reporter plasmid construction. We used hFOB as a model for studying the differentiation of osteoblast because this cell line can be easily controlled the differentiation by temperature manipulation.
Firstly, we had evaluated the transcription and translation of Tsg101 in hFOB at two different conditions, proliferation stage (34°C) and mature stage (39°C). We found the Tsg101 promoter activity is over 2 folds highter in proliferative stage (34°C) of hFOB than in mature stage (39°C). We also found the Tsg101 protein production was decreased over 50% during culturing at 39°C in Western blot analysis.
When culturing with standard medium at 34°C, hFOB will become mature gradually. We cultured the hFOB cell for 2 weeks at 39°C and checked the osteoblast phenotype. The expression of Tsg101 protein was analyzed by Western blot analysis during this period. The protein production was found significantly decreasing within the first 3 days after hFOB confluence. After 3 days culturing, the protein production was in a steady state. This finding was also confirmed by RT-PCR.
Down-regulation of the Tsg101 expression is correlated to the osteoblast differentiation, especially the early stage. Our finding may imply the expression of Tsg101 play an important role in osteoblast differentiation.

P298-Su
Molecular Dissection of *Krox20* Gene Regulation in Bone: NFAT and/or Srf Dependence?
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The *Krox20* gene encodes a zinc finger transcription factor that plays a key role in regulating bone formation. We have shown that *Krox20* is expressed in a subpopulation of growth plate hypertrophic chondrocytes and in differentiating osteoblasts and that the *Krox20* conditional knockout mice develop severe osteoporosis.
To investigate the transcriptional regulation of *Krox20* in bone, we have identified a bone-specific enhancer in the 5′ flanking region of the mouse *Krox20* gene which spans 860 bp and recapitulates *Krox20* expression during bone development using transgenic mice analysis. Combining phylogenetic footprinting analyses and in vitro and in vivo experiments, we have defined three types of elements within the enhancer: Krox20 binding sites involved in a direct positive autoregulatory loop, Cbfa1 sites modulating *Krox20* expression and an A/T rich element of 13 bp long essential for *Krox20* activation in both osteogenic and chondrogenic cells. This key regulatory element contains partially overlapping canonical binding sites for two transcription factors: NFAT and Srf known to control several developmental processes. Bandshift and transactivation experiments are being performed to demonstrate that these sites are functional and to establish potential cooperative interaction between them. Furthermore, we are generating a conditional mutant for one of them to analyse possible modification in *Krox20* expression. These studies should help elucidating the molecular mechanisms of bone formation.

P299-Mo
Overexpression of Cbfa1/RUNX2 in Osteoblasts Results in Striking Reduction of SOST Expression in Bone
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Recently it was shown in vitro that the osteocyte-expressed potent negative bone formation regulator SOST is a target gene for Cbfa1—a key transcriptional regulator of osteoblast function [1]. Therefore, we studied SOST expression in bones of mice overexpressing Cbfa1 in osteoblasts under the control of a collagen type I promoter. These mice exhibit defects in osteoblast maturation and increased bone resorption resulting in osteoporosis [2, 3]. Gene expression was measured by real-time quantitative PCR in the cortex of the tibial and femoral diaphysis of 1-year-old male Cbfa1 transgenic mice and compared to values obtained for wildtype littermates (n = 6/group). As expected, these mice were osteoporotic as evaluated by DEXA. In line with earlier results, we observed increased expression of Cbfa1 (5-fold), of the extracellular matrix protein osteopontin (3-fold) and of the osteoclastogenic factor RANKL (1.6-fold), while expression of the housekeeping gene GAPDH was unchanged and osteocalcin expression as marker of terminal bone mineralization was slightly reduced (1.2-fold). Interestingly, SOST expression was dramatically reduced by 9-fold. Expression of the osteocyte-specific protease PHEX43 was down-

regulated 1.6 fold, while the gap junction protein connexin 43, which is expressed in osteocytes, was unchanged. The observed reduction of PHEX43 and SOST expression is consistent with the previously reported decrease of osteocyte number by 50% in the cortical bone of Cbfa1 overexpressing mice [3]. Osterix, which is essential for generation of mineralizing osteoblasts and displays spaciotemporal association with SOST expression during embryonic osteogenesis [4], was up-regulated (1.6-fold) in Cbfa1 overexpressing mice, suggesting differential regulation of osterix and SOST by Cbfa1. In summary, the dramatic magnitude of SOST downregulation in an animal model of defective osteoblast maturation provides further evidence for the high specificity of SOST for terminally differentiated cells of the bone forming lineage. They also support the suggestion that SOST is a target gene of Cbfa1 consistent with published results [1]. However, while in vitro Cbfa1 activated the SOST promoter, in vivo overexpression of Cbfa1 in the transgenic mice down-regulated SOST mRNA.
[1] Sevetson et al. JBC 279 2004; [2] Liu et al. JCB 155 2004; [3] Geoffroy et al. MCB 22 2002; [4] Ohyama et al. Endocrinology 145 2004.

P300-Tu

The Osteoblast Transcription Factor Osterix in Osteoblasts from Patients with Metabolic Bone Diseases Correlates to Clinical Osteoporotic Markers

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Osterix (OSX) and core binding factor alpha1 (cbfa1/RUNX 2) are transcription factors which activate osteoblast marker genes in vivo during the process of bone formation and have been shown to be essential for normal bone development. The role of these osteogenic transcription factors for the clinical assessment of patients has not been evaluated to date. The culture of primary human osteoblasts (pHOB) has been shown to be a useful tool to analyze osteoblast function and to assess bone formation in different metabolic bone diseases. With so-called in vitro-ex vivo studies pHOB from bone biopsies were successfully used to investigate patient- or disease-specific morbidity.
In this study, we obtained bone biopsies from nine patients with different metabolic bone diseases for histomorphometrical analysis. Part of the biopsy was used for the initiation of pHOB cultures after informed consent. Cells from primary cultures were used to analyze gene expression by semiquantitative PCR of OSX and cbfa1/RUNX2 in addition to cytokines interleukin 1, interleukin 6 (IL-6), TNF-α, in addition to osteoprotegerin and RANKL and compared to four normal control cultures. In addition, OSX and cbfa1 mRNA were correlated to clinical parameters, laboratory values and histomorphometrical results. There were significant differences between cultures from patients versus controls for OSX and cbfa1/RUNX 2. The mRNA levels of OSX correlated negatively with gene expression of IL-6 (r = 0.75, P = 0.019) and positively with OPG (r = 0.74, P = 0.023). OSX was highly correlated to bone density at the spine (r = 0.95, P = 0.013) with a tendency also at the femoral neck (r = 0.92, P = 0.076). Histomorphometrically, OSX correlated significantly negative with the mineral apposition rate (r = 0.85, P = 0.015). Cbfa1/RUNX 2 tended to correlate positively with TNF-α (r = 0.62, P = 0.073) but with no other cytokine nor any other clinical parameter. However, cbfa1/RUNX 2 was significantly correlated to a resorption parameter in histomorphometry (erosion depth, r = 0.68, P = 0.042).
In conclusion, the transcription factor OSX used in the analysis of in vitro-ex vivo studies is highly correlated to clinical and histomorphometrical parameters suggesting an important role also for clinical use.

P301-Su

Rapid in Vivo Monitoring of SOST Regulation in Bone

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SOST is a potent negative regulator of bone formation in murine models and in patients with the bone overgrowth disorders Sclerosteosis and Van Buchem disease. SOST expression is restricted in bone to osteocytes. Hence, it is difficult to study its regulation in a relevant in vitro context. Consequently, we have created a SOST reporter mouse to facilitate efficient studies of its regulation in bone in vivo. We integrated an IRES/Luciferase cassette into the 3′ UTR of the SOST gene to generate mice that express the luciferase protein in parallel to the SOST gene product sclerostin. Six-month-old heterozygous F1 mice were analyzed for luciferase expression following luciferin application using a Xenogen IVISTM imaging system for luminescence detection. Both sexes (n = 3/group and sex) displayed robust highly selective luciferase expression in all skeletal structures far above background bioluminescence levels in other organs and in wildtype littermate mice as evaluated in vivo in whole mice and ex vivo in dissected organs. Surprisingly, kidney, a site of robust SOST mRNA expression, showed no appreciable luciferase expression. The only additional organ displaying luciferase expression was testis. We recently discovered that SOST is a target gene for PTH action in bone [1]. Hence, we screened for luciferase signal changes following parathyroid hormone (PTH) treatment, to validate the model for studies of SOST regulation in bone. To this end, we applied subcutaneously 100 or 300 nM hPTH(1-34) or vehicle onto the calvaria of

6-month-old female SOST reporter mice as a classical model of local bone formation. These doses are bone anabolic as determined in previous experiments by histomorphometry. Luciferase expression was monitored before and 1, 4, and 26 h after treatment. A rapid dose-related decrease of luciferase signal occurred by 1 h reaching maximum after 4 h. Luciferase levels were still reduced 26 h after application but had returned to baseline, when checked 2 weeks later. A similar response could be induced after renewed PTH application 1 month later. Decrease of luciferase levels at 4 h (25–42%) correlated well with changes of SOST mRNA expression detected by real-time quantitative PCR in previous experiments with mice following intermittent application of PTH onto the calvaria [1]. Our data suggest that this mouse model is a useful in vivo reporter system to rapidly study SOST regulation in bone. [1] Keller et al., 2004, JBMR 19: 1166.

P302-Mo

Ghrelin and Bone Metabolism

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Ghrelin is a gut peptide involved in growth hormone (GH) secretion and energy homeostasis. The ghrelin receptor, GHS-R1a mRNA is found in a variety of organs and the wide distribution of this receptor indicates that ghrelin may have a variety of regulatory functions. In fact, emerging evidence indicates that ghrelin performs an array of additional biological actions: it stimulates appetite, promotes adipogenesis, decreases energy metabolism, improves cardiovascular function, and stimulates prolactin and cortisol releases. These findings suggest that ghrelin may have a role in bone metabolism, however, this has not been comprehensively investigated and the role of ghrelin in bone metabolism remains unknown. Furthermore, it has been reported that leptin which has an opposing role to that of ghrelin in energy homeostasis plays a significant role in bone metabolism. This evidence also suggests that ghrelin has a role in bone metabolism. To investigate the effect of ghrelin on bone metabolism, we examined the direct effect of ghrelin on osteoblast and osteoclast development in vitro. We measured the expression of GHS-R1a in rat osteoblasts using the RT-PCR and immunohistochemistry. The effect of ghrelin on osteoblast proliferation was examined by recording changes in cell number and the level of DNA synthesis. Osteoblast differentiation markers (Runx2, collagen type I, alkaline-phosphatase, osteocalcine) were analyzed using quantitative RT-PCR. We also examined calcium accumulation and ALP activity in osteoblasts induced by ghrelin. In addition, we assessed RANKL and OPG mRNA expressions in osteoblasts and osteoclast formation in a coculture of osteoblasts with bone marrow cells. Ghrelin and GHS-R1a were identified in osteoblasts. Ghrelin significantly increased osteoblast cell numbers and DNA synthesis (up to 1.33-fold compared with vehicle). The proliferative effects of ghrelin were suppressed by [D-Lys3]-GHRP-6, which is antagonist of GHS-R1a, in a dose-dependent manner. Furthermore, ghrelin increased the expression of osteoblast differentiation markers, ALP activity, and calcium accumulation of matrix ($P < 0.01$). In addition, ghrelin treatment also induced RANKL mRNA expression in osteoblasts ($P < 0.01$) and significantly increased osteoclast formation by the induction of RANKL in osteoblasts ($P < 0.05$). This study indicates that ghrelin which is an important peptide in the regulation of energy metabolism is a positive regulator that acts directly on osteoblasts.

P303-Tu

25 Hydroxy-Vitamin D3-1α Hydroxylase is Expressed in Cultured Human Bone Cells and is Modulated by Estradiol and Phytoestrogens

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We have previously reported that vitamin D analogs stimulate creatine kinase-specific activity (CK) and modulated the hormonal responsiveness of primary cultures of human bone cells. As vitamin D metabolites have been shown to promote differentiation and arrest growth in multiple cell types, we tested the possibility that cultured human bone cells possess an endogenous 25 hydroxy-vitamin D3-1α hydroxylase system, the final enzyme in the biosynthetic pathway of 1,25(OH)2D3. We assessed the expression and activity of 25 hydroxy vitamin D3-1α hydroxylase by real time PCR and via the conversion of 25(OH)D3 into 1,25(OH)2D3 in untreated cells and in the presence of estradiol-17beta (E2) and phytoestrogens as well as parathyroid hormone (PTH). 1α hydroxylase mRNA was identified in cultured bone cells using primers encompassing exon 4 by real time PCR. Higher expression was found in cells from pre-menopausal than in post-menopausal women. The expression of 1α hydroxylase mRNA in male cells was significantly lower than that found in pre-menopausal women, but higher than in cells from post-menopausal women. Female-derived cells from both age groups treated daily (3 days) with PTH (90 nM), E2 (30 nM)

genistein (G, 3 μM), daidzein (D, microM), biochainin A (BA, 3 μM) or 6-carboxy biochainin A (cBA, 0.3 μM), showed a significant increase in 1a hydroxylase mRNA. In contrast, the protein-conjugated hormones had no effect in either age. Male-derived bone cells treated with the vitamin D analog JKF1624F2 (JKF), cBA or dihydrotestosterone (DHT; 0.3 micromM) showed stimulation of 1α hydroxylase mRNA expression, while E2 had no effect and BA inhibited significantly. Basal production of 1,25D was 29+2 pmoles/mg protein in female derived bone cells. All hormones stimulated significantly the production of 1,25(OH)2D3 in female cells from both age groups, whereas raloxifene and DHT had no effect. In conclusion, we provide evidence for the expression of an active 25(OH) D3-1a hydroxylase system in cultured human bone cells, which can be upregulated by both natural and synthetic phytoestrogens as well as by PTH, in a sex- and age-dependent manner. Since exogenous vitamin D affects the cells and their hormonal responsiveness, the potential role of this system as an autocrine homeostatic mechanism is the subject of current investigation.

P304-Su

Vitamin D Analog JKF 1624F2-2 and Hyperglycemia Modulate Cultured Human Bone Cells Response to Estrogens by Differentially Altering ER mRNA Expression and Binding Activity of ER

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We have previously reported that treatment of cultured human bone cells (Obs) with estrogenic compounds stimulated creatine kinase-specific activity (CK) in a sex- and age-specific manner. In the present study, we assessed the effects of the less calcemic analog of vitamin D, JKF 1624F2-2 (JKF) and hyperglycemia (HG) on: (a) CK induced by the different compounds, (b) whole cell estrogen binding capacity, (c) membrane estrogen receptor binding and (d) estrogen receptor (ER) mRNA expression in pre- and post-menopausal female Obs. Pre-treatment of Obs with JKF increased CK stimulation by most of the estrogenic compounds in both age groups. Growing Obs in HG abolished CK stimulation by estradiol-17beta, genistein and daidzein, but not by raloxifene, biochainin A and quecertin in both age groups. Pre-treatment of Obs from both age groups with JKF and HG increased ERalpha mRNA expression. In contrast, pre-treatment with JKF decreased ERbeta mRNA expression, whereas HG increased ERbeta mRNA expression in Obs from both age groups. Using the membrane impermeant ligand E2-Ov linked to Eu, we developed a binding assay for specific membrane binding sites for estrogenic compounds in Obs, which is similar in both age groups. Membrane binding (ERm) was reduced by either JKF or HG except for the binding of carboxy-genisteine-ovalbumin or carboxy-daidzein-ovalbumin in both age groups. In contrast, whole cell 3[H] E2 binding, which is mainly nuclear, was inhibited by HG, but was increased by JKF. We also found that Obs from both ages express mRNA for 25 hydroxy 1α vitamin D hydroxylase, and this is upregulated by JKF and down-regulated by HG. Hence, there is no correlation between changes in membrane binding sites for estrogenic compounds with the modulation of ERalpha and ERbeta mRNA expression by hyperglycemia, whereas the changes by JKF are co-modulated in a coordinated manner.

P305-Mo

Effects of Growth Hormone on Osteoprotegerin Production in Human Osteoblast-Like Cells

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Osteoprotegerin (OPG), a glycoprotein belonging to the TNF receptor family, is an endogenous inhibitor of osteoclastogenesis, produced by cells of the osteoblast lineage. Current understanding indicates that OPG is a key cytokine involved in the regulation of osteoblast/osteoclast cross-talk and maintenance of bone mass. In a previous study, we showed that 6 months of growth hormone (GH) replacement therapy in GH-deficient patients was able to induce a significant increase of OPG in plasma (1). Since the OPG increment was inversely correlated to the changes in plasma osteocalcin and urinary deoxypyridinoline excretion, it could reflect an enhanced OPG production from bone. In order to further verify this hypothesis, we studied the effect of GH on human osteoblast-like cells (hOB) in primary culture. After detecting the presence of the mRNA for the GH receptor (GHR) by RT-PCR, we exposed hOB to increasing concentrations of GH (0.1 ng/ml–20 ng/ml) for 24 h. The results showed that GH treatment was able to stimulate OPG secretion in a dose-dependent manner. Maximum stimulation (twice the basal value) was obtained at 5 ng/ml. Upon increasing the GH concentration (10,20 ng/ml), no further increase in OPG production was observed. We also evaluated the expression of the OPG gene by RT-PCR after GH exposure and we observed a time-dependent increase in OPG mRNA levels that became significant after 24 h, indicating that the hormone has a stimulatory effect on OPG gene expression. The stimulatory

effect on OPG production was prevented by pretreating the cells with Tyrphostin AG490 (10 μM), an inhibitor of the Janus kinase 2 which is the intracellular pathway activated by the interaction of GH with its receptor. Similar results were obtained when the cells were pretreated with a receptor antagonist of GH, pegvisomant at 5×10^{-8} M.
In conclusion, our results suggest that the stimulation of OPG production induced by GH in hOB is specific and receptor mediated and further support the view that GH is able to modulate bone remodeling by directly influencing osteoblast function and interaction with osteoclast genesis and differentiation.
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P306-Tu
Responses of Osteocytes to Fluid Flow-Induced Shear Stress: Analysis of Gene Expression by Subtractive Hybridization
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The skeletal system is continuously subjected to repetitive mechanical loading due to weight bearing and muscle contraction. The osteocyte is thought to represent the most relevant mechanosensor cell in bone but so far the evidence is only circumstantial. MLO-Y4 osteocyte-like cells were used to study the effects of fluid shear stress on gene expression. These cells have functional gap junctions and their connexin-43 expression is regulated by fluid flow. A cone-plate system, in which a rotating cone generated a wide range of shear stress on a monolayer of cells, was developed, and the morphological alteration of cells, their cyclo-oxygenase expression and nitric oxide production was examined. The effect of shear stress on gene expression was analyzed by subtractive hybridization and in detail by quantitative PCR (QPCR). In our fluid flow system, shear stress induced an increase in COX-2 but not in COX-1 expression in MLO-Y4 cells. Nitric oxide in the conditioned medium was measured as nitrite, which showed no increase in non-sheared cultures but in the sheared cells a transient accumulation was observed. A list of differentially expressed genes was obtained by subtractive hybridization. These genes were mostly related to cytoskeletal structures and cellular stress. Interestingly, osteoblast-stimulating factor-1 (OSF-1, also known as HB-GAM and pleiotrophin) was up-regulated in shear stressed MLO-Y4 cells. Our previous results showed that MLO-Y4 conditioned medium stimulated mesenchymal stem cell proliferation and their osteoblastic differentiation (Heino et al. 2004). Thus, the differential expression of OSF-1 was studied in more detail by QPCR. Six to nine cycles of continuous fluid flow-induced shear stress at 2.5 Pa was applied to the cells for 2 min hourly, then RNA was isolated and cDNAs were reverse transcribed for QPCR. OSF-1 was found to be slightly up-regulated in shear stressed cells. These results support earlier conclusions that osteocytes may have a role in stimulating bone formation via secreted factors.

P307-Su
In Vitro Mechanical Stimulation Effects on Osteoblastic Release of Osteoprotegerin
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RANK ligand (RANKL) and osteoprotegerin (OPG) have been implicated in bone metabolism. Specifically, the balance of these factors in conjunction with RANK is believed to be key in determining the rate of osteoclastogenesis and the net outcome of bone formation/resorption. While it is well accepted that mechanical loading in vivo affects bone formation/resorption and alterations in the responsiveness of bone cells to mechanical load have been implicated in metabolic bone diseases, the effect of in vitro mechanical loading on osteoblastic levels of OPG and RANKL has not been extensively studied. In the current study, we mechanically stimulated osteoblastic cells seeded on polypropylene slides and quantified levels of OPG and RANKL. We hypothesized stimulation would increase the release of soluble OPG relative to RANKL favoring a bone-forming event. MG-63 cells were subjected to an oscillatory loading profile with testing conducted at 0.35% maximum strain via three-point bending for 2 Hr at 1 Hz. Following 1 Hr of incubation, cells and supernatant were collected and subjected to OPG, RANKL and M-CSF quantification. Whereas all soluble protein levels were quantified with ELISA, total and membrane-bound protein levels of RANKL were quantified with Western blotting and immunochemistry. We found that mechanical stimulation significantly increased soluble levels of OPG relative to no-load controls whereas soluble RANKL levels were low and unaffected by stimulation while membrane-bound and total cellular RANKL protein levels exhibited an increased trend, though not significant. Immunochemistry supported these findings. Increases in OPG were not significantly affected by 15, 30 or 60 min post-load incubation collection time points. Interestingly, we also found that soluble M-CSF levels were significantly reduced by mechanical stimulation suggesting an inverse relationship to OPG. Furthermore, as inflammatory macrophages develop independently of M-CSF, our results suggest that the stimulated OPG/RANKL soluble response is indicative of bone formation/resorption. These data demonstrate that osteoblastic cells respond to mechanical stimulation with increased production of soluble OPG relative to RANKL. The relative shift in abundance of soluble OPG over RANKL associated with applied

mechanical stimulation suggests the OPG/RANKL ratio may be important in the load-induced coupling mechanisms of bone cells and skeletal homeostasis.

P308-Mo

Long Term Maintenance of Living Human Cancellous Bone Explants ex Vivo

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Introduction: The Zetos culture system1 has potential to keep cancellous bone tissue viable ex vivo through the use of unique chambers and a loading device that allows the tissue to remain in its natural 3D milieu. The system can use human tissue, which overcomes many limitations associated with animal tissue.
Materials and methods: Human femoral heads were excised and cut into 9 mm thick sections. Cores 10 mm in diameter were bored, and then cut parallel to a height of 5 mm. Bone cores were inserted inside loading chambers and placed at 37°C. Explants were circumfused with DME medium supplemented with 10% FCS and loaded daily for 300 cycles at 1 Hz with a jumping waveform, giving each core the equivalent of 4000 microstrain. Static unloaded and fresh bone cores were used as controls. Fluorochrome labelling inserted at day 1 (calcein green) and 11 (alizarin red) provided markers for bone apposition. Both fresh and cultured bone cores from the Zetos chambers were fixed in 70% ethanol, before being dehydrated and embedded in Technovit 9100 New resin[2] for histological and immunohistochemical evaluation. ^{3}H-glycine was placed in the media for 24 h at days 0, 7, and 14 to detect newly synthesised proteins.
Results: Histological sections showed well-preserved tissue with little necrosis. Osteoblasts had produced osteoid seams and osteoclasts were observed in Howship's lacunae. Noncollagenous proteins such as osteopontin, osteonectin and osteocalcein, as well as procollagen Type I were localised through immunohistochemical labelling of sections. Active areas of bone apposition were observed in loaded explants with much less observed in static controls. ^{3}H-glycine was detected in protein through SDS-page and Western blot analysis as well as autoradiography of embedded sections, demonstrating cell viability after 15 days postexplantation.
Conclusion: This system has potential for studying bone remodelling in normal and osteoporotic human bone, bone bio-mechanics, and the effects of drugs, hormones or growth factors on cancellous bone, making it an essential laboratory aid in the future.

References: [1]Jones D.B. et al., (2003). Eur Cell Mater **5**: 48–60. [2]Yang R. et al., (2003) Eur Cell Mater **6**: 57–71.
Acknowledgments: Funded by 3R Foundation grant #78/01. Thanks to Dr Thomas Perren for supplying human tissue Ethic Commission Graubunden (18/02) approval.

P309-Tu

The Effects of Green Tea Catechin on the Expression of Osteoprotegerin (OPG) and Macrophage-Colony Stimulating Factor (M-CSF) in a Cultured Bone Marrow Mesenchymal Stem Cell Line

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Previous studies verified the beneficial effects of catechins in decreasing serum lipid, reducing blood pressure, modulating immune responses and antitumorigenic and antibacterial effects. However, the effects of green tea catechins on OPG/RANKL/RANK system in pluripotent stem cell have rarely been investigated. Osteoprotegerin (OPG) and macrophage-colony stimulating factor (M-CSF), mainly produced by the cells of the osteoblastic lineage, play a key role in regulating osteoclastogenesis via the OPG/RANKL/RANK system. In our study, we found that there was an inhibitory effect of EGCG on M-CSF mRNA expression and a stimulatory effect on OPG mRNA expression. The increase secretion of OPG is further proved by ELISA. These results suggest that osteoclastogenesis may be decreased through the mechanism of diminished M-CSF and intensified OPG by EGCG. Our result from this study is original for the finding of a novel affiliation between EGCG and OPG/RANKL/RANK in a bone marrow mesenchymal stem cell line. Elucidations on the detail mechanisms of EGCG in OPG/RANKL/RANK system require further investigations.

P310-Su

Morphological Changes in Actin Cytoskeleton Induced by Tensile Stress in Mouse Cranial Suture

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Mechanical tensile stress stimulates differentiation of osteoblasts and bone formation in the mouse cranial suture (Ikegame et al., 2001). In order to clarify the role of the cytoskeleton in this mechanism, the morphological changes in actin cytoskeleton in such stressed suture cells were investigated. Tensile stress was applied to the cranial suture of neonatal mouse calvaria in culture. After 3 and 6 h, the

specimens were fixed. They were stained with Alexa phalloidin and observed with confocal laser scanning microscope. As a control, the unstressed, uncultured calvariae were fixed and processed in the same way. The osteoblasts located near the parietal bone edge of control specimens were oval-shaped, in which actin was mainly located throughout their cell-cortices, under the cell membrane. In these controls, fibroblastic cells located near the center of the suture had less actin at the cell-cortex, and were accompanied by a random network of actin fibers. In the fibrous periosteum layer, fibroblastic cells had a great number of actin fibers which roughly ran in the direction crossing the suture. The sutures of the 3-h stressed specimens were expanded in width, with all the cells elongated in the direction of the tension. The actin fibers in the fibroblastic cells of the center of the suture were aligned in the direction of the tensile stress. However, the arrangement of actin fibers in the periosteum layer did not change remarkably. In the sutures stressed for 6 h, most of the cells were further elongated and the actin fibers became thicker. Preosteoblastic cells were increased in number in the area between the osteoblasts and the fibroblastic cells of the center of the suture. The cells had both thick actin layer in the cell-cortices and actin fibers which aligned in the direction of the tension. These results show that tensile stress changes the arrangement of actin fibers principally in the fibroblastic cells in the center of the cranial suture and some of those cells seems to be differentiated into preosteoblasts.

P311-Mo

Exposure of MC3T3-E1 Osteoblast-Like Cells to Arachidonic Acid and Docosahexaenoic Acid Modulates Prostaglandin Synthesis and Secretion of Osteoprotegerin

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Polyunsaturated fatty acids (PUFAs) increase bone formation in animal studies and an anti-resorptive effect on bone has been observed in elderly women after supplementation with PUFAs for 3 years. The cellular mechanism of PUFA action may be due to stimulation or suppression of local bone regulating factors such as prostaglandins (PGs), or to regulation of proteins expressed by the osteoblast such as osteoprotegerin (OPG).

In this study, non-transformed MC3T3-E1 cells derived from murine calvaria that exhibit the phenotype characteristic of osteoblasts in vivo were used. In order to measure PGE2 synthesis, cells were cultured for 24 h. Cells were subsequently exposed to vehicle (0.1% ethanol) (control), and arachidonic acid (AA) or docosahexaenoic acid (DHA) at 20 μg/mL for 4 h and the culture media collected. For measurement of OPG, cells were precultured for 24 h, exposed to vehicle (0.1% ethanol), PGE2 ($10-8$ M), AA or DHA at 2.5 to 20 μg/mL for 24 h and the conditioned media harvested. In some cases, 1 μM indomethacin, a cyclooxygenase blocker, was added to growth media 45 min prior to addition of test substances. Three separate experiments were conducted ($n = 4$).

Exposure to AA increased PGE2 production 18 fold ($P < 0.05$) over that of the control, while DHA had no effect. AA suppressed OPG secretion in a dose-dependent manner, and this effect was abolished by addition of indomethacin. PGE2 reduced OPG secretion by 40% ($P < 0.05$), as compared to control. DHA at higher concentrations also reduced OPG secretion by 20%, but concomitant exposure to indomethacin had a less prominent effect.

MC3T3-E1 cells produced significant amounts PGE2 in response to exogenous AA suggesting significant cyclooxygenase activity. The significant PGE2 synthesis after exposure to AA may also be due to autoamplification by PGE2 via the EP1 receptor, which is attributed to calcium mobilisation. AA decreased OPG secretion possibly via PGE2 formation, as PGE2 alone also significantly reduced OPG secretion. Abolishment of the effect of AA by indomethacin confirmed this observation. The slight reduction of OPG by DHA could be due to endogenous PGE2 production, as DHA itself is not a substrate for PGE2 synthesis. This work needs to be expanded to include measurements of RANKL secretion, as the ratio between OPG and RANKL is important for regulation of the bone microenvironment.

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P312-Tu

Glutamate Signaling Determines the Proliferation and Differentiation Status of Mesenchymal Stem Cells

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Skeletal development, repair and remodelling are dependent on the proliferation and differentiation capacity of mesenchymal stem cells (MSCs), which reside in bone marrow and give rise to different mesenchymal lineages. The regulatory inputs that control MSC fate are unclear. We determined the role of glutamate, an excitatory neurotransmitter, in these processes by expression/function screening of glutamate receptors in human primary MSCs. Using degenerate RT-PCR and fluorescent immunocytochemistry we identified all major subtypes of ionotropic (AMPA, kainate (KA) and NMDA) and group I metabotropic receptors. By confocal imaging of Fluo-3 loaded-MSCs, we demonstrated that AMPA receptor activation induced a rapid increase in intracellular calcium that was sensitive to the AMPA/KA receptor antagonist CFM2, EDTA and mimicked by the calcium ionophore ionomycin. Glutamate and its co-agonist glycine induced an initial rapid rise followed by a slower, more sustained increase in intracellular free calcium consistent with secondary release

from intracellular stores. In MTT and CFU-f assays, AMPA, NMDA and glutamate/glycine significantly increased viable MSC numbers. In contrast, blocking AMPA/KA and NMDA receptors using the specific antagonists CFM2 and MK801, respectively, impaired MSC proliferation and significantly reduced colony formation. MSC apoptosis/necrosis, as determined by Anexin V/propidium iodide flow cytometry and caspase assays, were unaffected by exposure to the glutamate receptor antagonists. We demonstrated by fluorimetric analysis that MSCs spontaneously released glutamate, at a level dependent on their differentiated status, providing evidence for autocrine glutamatergic regulation of MSC activity. In multipotency assays, the effects of glutamate receptor antagonists were determined on the differentiation capacity of MSCs. Following osteogenic induction, CFM2 and MK801 caused a dose-dependent inhibition of alkaline phosphatase activity and prevented the formation of von Kossa-positive mineralised nodules. In contrast, differentiation of oil-red O-positive adipocytes was impaired by MK801 and enhanced by exposure of cells to CFM2. CFM2 also completely inhibited the formation of Alcian blue-positive cells in micromass assays of chrondrogenesis. These data demonstrate that the relative contribution of AMPA/KA and NMDA glutamatergic signaling helps determine the proliferative activity and multilineage differentiation potential of MSCs in vitro.

P313-Su

The Effect of Statins on the Process of Cervical Intervertebral Fusion with Allogenous Cortical Bone Transplantation

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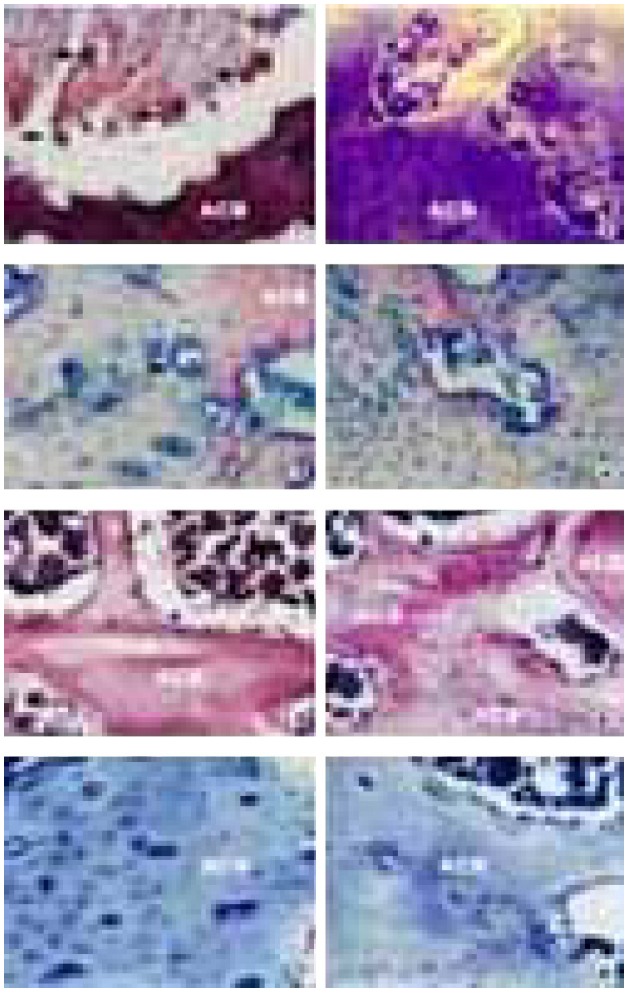
Aim: To investigate the effect of statins on the process of anterior cervical discectomy and fusion (ACDF) with allogenous cortical bone transplantation.
Methods: After evaluating the clinical efficacy of allogenous cortical bone as an alternative for autograft in ACDF procedure, we observed the effect of statins on the proliferation, differentiation, mineralization and the expression of BMP-2 and VEGF in osteoblasts. Further, we detect the influence of statins on the mice calvaria bone through subcutaneous injection over the skull. Finally, animal model of ACDF with allograft bone transplantation was established to study the effect of statins on the fusion process by radiographic and histopathological examination.
Results: Allogenous cortical bone will not attenuate the curative effect of ACDF and provide satisfied cervical lordosis and fusion rate. Statins can increase proliferation, mineralization, and expression level of BMP-2 and VEGF in osteoblasts. Topical administration of statins over the skull will thicken the calvaria bone, as well as promoting angiogenesis within it. Oral statins could suppress the immune rejection to the implant, stimulate bony fusion between the transplant and trabeculae, and enhance intraosseous angiogenesis and osteogenesis in the core region of the graft.
Conclusions: Allogenous cortical bone showed to be an efficacious substitute for autograft bone, and statins demonstrated the potency to promote graft integration with vertebral body.
Keywords: Statins, Allogenous Cortical Bone, transplantation, Bone Morphogenetic Protein, Vascular Endothelial Growth Factor.



P314-Mo

Combination of Bone Morphogenetic Protein-2 and Troglitazone Induces Differentiation of Multipotent TBR31-2 Cells into Adipocytes

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TBR31-2 cells belong to one of the stromal cell lines established from the marrow of transgenic mice harboring the temperature-sensitive SV 40 T-antigen gene. These cells show the characteristics of undifferentiated cells and possess the capacity to differentiate toward adipocytic and osteoblastic cells during long-term culture, or short-term culture under the effect of a differentiation inducer. In the present study, we investigated the effect of bone morphogenetic protein-2 (BMP-2) or troglitazone, which is an inducer of osteoblasts or adipocytes, on the differentiation of TBR31-2 cells.

Differentiation toward osteogenic cells was observed when TBR31-2 cells were cultured for 6 days at 37°C with alpha-MEM supplemented with 10% FBS, 0.2 mM ascorbic acid, 5 mM beta-glycerophosphate, and 400 ng/ml BMP-2. One of the osteogenic differentiation markers, alkaline phosphatase (ALP) activity, increased during 6 days of culture at 37°C in a time-dependent manner.

Troglitazone is one of the thiazolidinediones that activate the PPAR gamma. After the TBR31-2 cells had reached confluency, they were cultured at 37°C with alpha-MEM supplemented with 10% FBS and 10 microM troglitazone for 6 days. Oil droplet accumulation and ALP activity were examined using Oil red O and ALP staining. By the addition of troglitazone, oil-accumulated cells were observed, and the increase of ALP activity was completely suppressed.

RT-PCR analysis was performed to determine the expression of the specific gene for the osteogenic cell differentiation marker, such as Cbfa1, ALP, and type I collagen, and for the adipocyte differentiation marker, such as PPAR gamma, adipsin and lipoprotein lipase. Confluent cultures of TBR31-2 cells were kept at 37°C in differentiation medium with 400 ng/ml BMP-2 or 10 μM troglitazone or both for 3 and 6 days. In the presence of BMP-2, an increase of mRNA expression in the osteogenic cell differentiation markers and a decrease of mRNA expression in the adipocyte differentiation markers were observed. On the other hand, troglitazone treatment produced the opposite tendency. Interestingly, in the presence of BMP-2 with troglitazone, mRNA expression in the adipocyte differentiation markers was more increased than that in the presence of only troglitazone.

Further signal transduction studies are needed to reveal the regulatory mechanisms of mesenchymal differentiation.

P315-Tu

The Effect of Oxidative Stress on the Proliferation and Differentiation of Human Bone Marrow Stromal Cell-Derived Osteoblasts

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Osteoporosis is associated with many etiological causes such as nutrition, cytokines, hormones, and aging. Recently, reactive oxygen species (ROS) are considered to be responsible for the aging process and osteoporosis.

The objectives of our study were to assess the effects of oxidative stress on the proliferation, differentiation and apoptosis of human bone marrow stromal cell (BMSC)-derived osteoblasts in an ex vivo culture and to explore pathways by which osteoblast cell apoptosis was induced.

The bone marrow was obtained from healthy donors. Mononuclear cells including BMSCs were isolated and cultured to osteoblastic lineage. Different doses of hydrogen peroxide (H_2O_2) were added to the culture media. Cellular oxidative stress was measured fluorometrically by monitoring the oxidation of intracellular dichloroscein (DCFH) using a cytofluor reader. In response to H_2O_2, DCF fluorescence increased significantly in BMSCs over the course of 2 h. At the 15th day of the primary culture, H_2O_2 diminished the mean size of CFU-Fs dose dependently. The size and number of ALP(+) CFU-Fs were also decreased by H_2O_2 treatment. With MTT assay, when compared to the control group, H_2O_2 significantly decreased the total number of cells of each culture well. Besides, H_2O_2 significantly diminished expression of osteocalcin mRNA in the cultures of human BMSC-derived osteoblasts. Antioxidant, N-acetylcystein (NAC) blocked the diminution of cell viability and the inhibition of osteocalcin mRNA expression by H_2O_2. Addition of H_2O_2 resulted in the increase of caspase-9 and caspase-3 activity but not caspase-8, and release of cytochrome *c* to the cytosol. These data suggest that, in primary human BMSCs, oxidative stress inhibits proliferation of stromal cells and inhibits the differentiation to osteoblastic lineage. In addition, oxidative stress induces apoptosis of human BMSC-derived osteoblasts and this may be mediated by mitochondrial pathway of apoptotic signal.

P316-Su

Human Fetal Bone Cells Associated with Bioresorbable PLA Composite Scaffolds for Tissue Engineering

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For clinical bone transplantations, tissue engineering techniques based on the delivery of cells to the defect using scaffold materials are investigated. Part of the project of "Lausanne Center for Bone Tissue Engineering" consists in the evaluation of a possible use of fetal bone cells for tissue repair. The aim was to characterize these cells for their potentially inductible osteoblast phenotype and to compare them with adult osteoblasts and mesenchymal stem cells. The possibility of associating fetal bone cells with biodegradable PLA scaffolds for tissue repairing was then tested in vitro. Human fetal bone cells obtained after voluntary interruption of pregnancy were used (Ethical Protocol 51/01). To differentiate them into osteoblasts, cells have been treated with osteogenic factors. Cells were tested for ALP activity and for cbfa-1, ALP, osteocalcin and a1 chain of collagen I gene expression during a time course experiment. Mineralization was measured every week by von Kossa staining. Fetal bone cells were also seeded on PLA foams and treated with osteogenic factors during 4 weeks. The distribution of cells, extracellular matrix, eventual modifications of the scaffold structure were observed by scanning electron microscopy. Doubling time of fetal bone cells was comparable to mesenchymal stem cells, but significantly shorter than adult osteoblasts. ALP enzymatic activity induction was higher for fetal bone cells than adult bone cells, but lower than mesenchymal stem cells. ALP mRNA was highly upregulated for the treated fetal cells. Collagen I a1 and Osteocalcin gene expressions were also increased. Extracellular matrix analysis showed that fetal bone cells without treatment were able to mineralize, but the osteogenic factors strongly increased this phenomenon. Mesenchymal stem cells and adult osteoblasts did mineralize later. In the presence of fetal cells, the porous structure of PLA foams was colonized and covered by extracellular matrix. Crystalline deposits were visible. In this study, we show that the PLA foams obtained by supercritical CO2 foaming are biocompatible with fetal bone cells. We are further investigating the influence of the ceramic contents of the PLA foams on the cell's behavior. This study was supported by grants from the Swiss National Science Foundation (PNR 46 No 404640–101114/1 and FNRS No 2100–066872.04.01), the Fondation Lémanique pour la Recherche sur le Tissu Osseux, and by the Lausanne Center for Bone Tissue Engineering.

P317-Mo

Study of Osteoblast Function Through Cellular Cultures in Primary Osteoporotic Men

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Primary osteoporosis in men is a not well-characterised condition. Clinical and histomorphometric studies reveal that bone formation is probably reduced due to a decreased osteoblast (OB) activity. To assess the OB function, we have carried out an in vitro study. In which, we compare the proliferation and activity of OB of primary osteoporotic men and "healthy" age-matched controls, in primary cultures.
Human bone cells were obtained from 1 to 2 mm explants of trabecular bone, (iliac crest bone biopsies samples). Cells were grown in Dulbecco's modified Eagle medium and 10% Fetal Calf Serum. OB were characterized by alkaline phosphatases activity and osteocalcin synthesis. OB proliferation was measured through cellular proliferation curve. Function was measured through assessment (real time PCR) of gene expression of specific OB markers [type I collagen (COL1A1) and osteocalcin], with/out addition of vitamin D. Statistical analysis was performed using the SPSS 11.5. This included Kolmogorov–Smirnoff test and parametric and non-parametric test. Area Under Curve (AUC) for cellular proliferation was figured out.
Thirty samples were obtained from 14 osteoporotic men and 16 from age-matched healthy men. The two groups were similar in age (Age: 55.75 ± 17.9 years in N1 versus 56.54 ± 12 years in OP1) and in body mass index (BMI: 27.6 ± 3.6 kg/m^2 in N1 versus 26.4 ± 2.8 kg/m^2 in OP1). The OB proliferation of osteoporotic group, measured as AUC, was 34.5% smaller than the control group one (633.619 versus 967.851 cells, $P < 0.05$). No significative differences were found between both groups with regard to: COL1A1 gene expression, with/out addition of vitamin D and osteocalcin gene expression without vitamin D. When vitamin D was added, both groups increased osteocalcin gene expression (2.542% in N1 group and 2.435% in OP1 group, $P > 0.05$ compared with basal levels), but not COL1A1 gene expression (95.8% in N1 group and 110% in OP1 group, P = n.s.). Osteocalcin gene expression was greater in control group than in the osteoporotic group, when both cultures were added with Vitamin D (OP1: 1.58 ± 1.3 vs. N1: 2.76 ± 1.77; $P = 0.037$). In conclusion, in this study, we found that: OB proliferation was decreased in primary osteoporotic men in comparison with OB proliferation of age-matched healthy men. OB function is disturbed in primary osteoporotic men as was shown through the gene expression of Osteocalcin. This difference was only revealed when vitamin D was added.

P318-Tu

Characterization of Bone Cells Cultured from the Human Maxillary Alveolar Ridge

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Introduction: In a number of in vitro studies, bone cells from different origins have been characterized. As a potential source of cells for tissue engineering, it is essential

to define the biology of these different cell populations. In this study, we characterized bone cell cultures (n = 10) derived from the human maxillary alveolar ridge, which could be a potential cell-source for tissue engineering of the severely resorbed maxilla.

Methods: From 10 individuals an osseous core from the maxilla was obtained. Ten explant cultures were cultured until 70–80% confluence. During culture, the morphology of the cells was studied with light microscopy (LM).

Confluent explant cultures were analysed by flow cytometry with respect to size, granularity and surface marker expression. Fluorochrom-conjugated (FITC or PE) monoclonal antibodies (CD13, CD31, CD44, CD90 or CD73) were employed and gating values were used in the size/granularity analysis. Early passage cells (E1P1) were cultured in either standard medium (SCM) or osteoinductive medium (OIM), containing ascorbic acid, dexamethasone and b-glycerophosphate. After 21 days, cells were analysed for alkaline phosphatase (ALP) expression (histochemical) and calcium deposits (Von Kossa assay).

Results: LM demonstrated that cells had a polygonal morphology containing heterogenous granular cytoplasm and a central nucleus with 2–3 nucleoli. Size/granularity analysis demonstrated that no significant difference in these two parameters existed between different explant cultures. Immunophenotypically, these cells were found to be positive for CD13, CD44, CD90 and CD73 and negative for CD31. Cells cultured in SCM for 21 days showed moderate ALP staining and many calcium deposits. Culturing cells in OIM for 21 days significantly increased both ALP staining and the number of calcium deposits.

Discussion: In this study, we have characterized the primary biological characteristics of bone cells derived from 10 maxilla core biopsies. To our knowledge, it is the first time that surface marker expression has been evaluated on bone cells originating from this site. The explant culture resulted in a homogenous cell culture with no significant morphological differences between individuals. Cells were positive for markers characteristic for immature mesenchymal stem cells and had osteogenic differentiation capability. This study indicates that cells derived from maxilla biopsies could be a potential cell source for tissue engineering.

P319-Su

Osteoblast-Like Cell Response to Calcium Phosphate Coating Chemistry and Morphology

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The possibility of creating a micro-environment to promote adhesion, proliferation, differentiation and mineralisation of bone cells in vitro, by successfully combining the chemistry and topography of a micro-fabricated substrate has key clinical significance. Using techniques such as sputter deposition in conjunction with a photolithographic procedure for substrate conditioning, it may be possible to create a successful combination of chemistry and topography that promotes bone growth.

Thin film calcium phosphate (CaP) coatings were deposited on etched n-type silicon surfaces by rf magnetron sputtering. FTIR and XPS spectroscopy and X-ray diffraction were used to investigate CaP coating chemistry. SEM was used to illustrate coating topography, profilometry and atomic force microscopy was used to determine surface roughness and contact angle to examine surface energy.

SaOs-2, MG-63 and hFOB osteoblast-like cells were cultured on silicon (Si), etched silicon (Si Et), as deposited CaP on etched silicon (Si Et AD) and annealed CaP on etched silicon (Si Et AN). Cell characterisation was performed prior to analysis by RT-PCR and immunocytochemistry. Assays including cell adhesion and proliferation (MTT), total cell protein content, osteoblast differentiation (Western blotting and alkaline phosphatase (ALP) assay), gene expression (RT-PCR) and SEM of fixed cultures to determine cell morphology were performed over a 10-day period.

Protein synthesis, ALP activity and cell proliferation were at a maximum at 5 and 10 days on the Si Et AN surface. Western blotting for ALP, collagen type-I and BMP-4 indicated significantly greater differentiation levels for SaOs-2 cells cultured on Si Et AN. Gene expression for ALP, BMP-2, osteocalcin and osteonectin was also upregulated on the annealed CaP etched surface (Si Et AN).

Cell biology analysis suggests that a semi-crystalline CaP coating on a surface with well-defined morphology is capable of supporting osteoblast-like cell attachment, proliferation and differentiation. Optimising the combination of CaP coating chemistry and topography may have clinical significance for future tissue engineering applications.

P320-Mo

Vitamin K2 Inhibited IL-1beta-increased Proliferation of Human Osteoblasts

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Although proinflammatory cytokine interleukin (IL)-1beta is known to stimulate cell proliferation in osteoblastic cells and induce osteoclast formation, we found that some types of osteoblast responded to IL-1beta and others did not. In contrast, vitamin K2 (VK2), which is used as osteoporosis therapeuticums in Japan, inhibits osteoclast formation and 1,25-dihydroxyvitamin D3 (1,25(OH)2D3)-increased cell proliferation in osteoblasts. In addition, menaquinone-4 (MK-4), a vitamin K2 with four isoprene units, enhances the accumulation of gamma-carboxyglutamic acid-containing osteocalcin and 1,25(OH)2D3-increased mineralization by human osteoblasts. The present study was conducted to compare the effect of IL-1beta on cellular proliferation in

human osteoblastic cells (SaM-1) and osteosarcoma-derived cells (SaOS-2, HOS, and MG-63), and to prove the effect of VK2 on IL-1beta-stimulated proliferation in SaM-1 cells. Although IL-1beta significantly stimulated proliferation in SaM-1 and MG-63 cells in a dose dependent, the response was not observed in SaOS-2 and HOS cells. Both SaM-1 and MG-63 cell showed constitutive expression of IL-1 receptor (IL-1R)I mRNA but not IL-1RII and IL-1R antagonist (IL-1RA) mRNA, as determined RT-PCR analysis. The constitutive expressions of these mRNAs were extremely low in SaOS-2 and HOS cells. These data suggest that IL-1beta-stimulated proliferation occurs through IL-1RI in SaM-1 and MG-63 cells. Furthermore, IL-1beta-stimulated proliferation was inhibited by MEK inhibitor PD98059 but not by p38 MAPK inhibitor SB203580 and COX-2 inhibitor NS-398 in SaM-1 cells. These data suggest that IL-1beta-increased the proliferation system involved in MEK but not that in the prostaglandin (PG)E2 synthesis system. Furthermore VK2 inhibited IL-1beta-increased proliferation of SaM-1 cells. In addition, IL-1beta-decreased expression of osteocalcin was elevated by VK2. These findings indicate that the decrease of osteocalcin leads to cellular proliferation and the increase of osteocalcin leads to differentiation, suggesting that osteocalcin plays an important role as a key molecule in cellular proliferation of human osteoblast.

P321-Tu

c-SRC Inhibits ER Alpha Gene Transcription in Differentiating Human Osteoblasts-Like Cells

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c-Src activity is well known to inhibit full differentiation of osteoblasts. Estrogen-dependent signals in these cells, mediated by the Estrogen Receptor alpha (hER alpha), are pivotal for balanced bone tissue turnover, and we investigated whether c-Src is involved in ER alpha gene transcriptional regulation in human osteoblast-like cells. The hER alpha gene is driven by multiple promoters, of which the distal E/F promoter appear specifically active in osteoblasts, albeit at relatively low levels. We stably transfected human osteosarcoma-derived Saos-2 cells with a luciferase reporter gene downstream of the F promoter (Saos F-Luc). Treatment of these cells with PP1, a specific inhibitor of c-Src enzyme activity, resulted in a remarkable (~three-fold) increase of reporter activity. Consistently, a time-dependent promoter induction was also obtained by prolonged over-confluence, a condition whereby cultured osteoblasts show diminished c-Src activity and a more differentiated phenotype. Over-confluence correlated with marked PKC alpha de-activation, and PKC down-regulation, obtained by long-term treatments with PMA (Phorbol 12-Myristate 13-Acetate), also resulted in reporter stimulation (~two-fold) in proliferating, but not in over-confluent, cells. In PP1-treated cells, promoter activity could not be further increased by PMA. The F promoter contains a putative PMA-responsive AP-1 site, but PP1-treated cells with high reporter activity showed inhibited AP-1 activation. Moreover, marked AP-1 inhibition by the generic kinase inhibitor DMAP (4-DiMethylAminoPyridine) resulted in a dose-dependent promoter stimulation, which matched a dose-dependent decrease of Src-activating phosphorylation at its Y416 amino acid residue. Importantly, in proliferating mouse primary osteoblasts ER alpha protein and mRNA were increased after PP1 treatment. In over-confluent counterparts, the full-length receptor levels were unchanged, but a putative isoform of lower (~46 kDa) molecular weight appeared. These results point to a strong c-Src/PKC-dependent pathway modulating ER alpha transcription in osteoblasts, perhaps affecting the balance of alternative receptor isoforms during differentiation.

P322-Su

Purinergic Receptor Expression Changes with Osteoblast Differentiation and Maturation

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Accumulating evidence suggests that extracellular nucleotides, signalling through P2 receptors, may play an important role in bone biology, modulating both osteoblast and osteoclast function. In vitro, ATP and ADP stimulate osteoclastic resorption, whilst ATP and UTP act on osteoblasts to inhibit bone formation. Primary osteoblast cultures were derived from neonatal rat calvariae by trypsin/collagenase digestion. Osteoblasts cultured for up to 10 days were loaded with the intracellular Ca^{2+}-sensing fluorophore, Fluo-4, and a fluorescence imaging plate reader (FLIPR) used to measure the responses of these cells to a wide range of nucleotide agonists. Peak responses were typically observed with 10–20 μM ATP or UTP, which accords with concentrations of these agents we have reported to inhibit bone nodule formation. In this culture system, osteoblast number doubled between days 4 and 10 but the peak intracellular Ca^{2+} response (to 20 μM ATP or UTP) increased 5-fold. These results suggest that osteoblast responsiveness to ATP increases as cell differentiation proceeds. Previous studies, including work from our group has provided evidence for the expression of $P2X_2$, $P2X_5$, $P2Y_1$ and $P2Y_2$ receptors on rat osteoblasts, and $P2X_7$, $P2Y_4$ and $P2Y_6$ receptors on human osteoblasts. Using RT-PCR we detected the presence of mRNAs for $P2X_2$, $P2X_5$, $P2X_7$, $P2Y_1$, $P2Y_2$, $P2Y_4$ and $P2Y_6$ receptors in primary rat osteoblasts and

found the expression varied considerably with time in culture- and thus, state of cell differentiation. For example, expression of $P2Y_2$ receptor mRNA, thought to be involved in mediating the inhibitory effects of ATP and UTP on bone formation, increased very strongly between days 4 and 15; in contrast expression of $P2X_5$ (associated with proliferation) decreased to undetectable over the same period. Immunostaining for P2Y receptors revealed similar changes over time. Thus, mature osteoblasts preferentially express a receptor that functions as an "off switch" for bone formation. These findings shed further light on potential role of extracellular nucleotides in the regulation of bone formation in health and disease.

P323-Mo
Expression and Functional Analysis of Gamma-Secretase Components in Osteoblasts
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The gamma-secretase complex belongs to a family of proteases that cleave their substrates within the hydrophobic transmembrane domain. The enzyme is composed of four protein subunits, with presenilin (PS) at its active site, and targets substrate proteins such as cadherins, amyloid precursor protein (APP) and Notch. PS can also affect β-catenin turnover and modulate Wnt-dependent transcriptional activation. Mutations in PS are the primary cause of familial Alzheimer's disease and PS1 knockout mice suffer huge hemorrhages in the central nervous system as well as severe skeletal abnormalities with marked underossification. Considering the fundamental involvement of gamma-secretase in several relevant signalling networks, we determined the expression, activity and function of the gamma-secretase complex in osteoblastic cells. Using RT-PCR, we identified expression of the gamma-secretase components, APH-1α, APH-1 β, nicastrin, PEN-2, PS1 and PS2 in different osteoblastic cell lines (MG63, SaOS-2, TE85) and primary human osteoblasts. By immunocolocalisation and confocal microscopy, the gamma-secretase complex appeared to form discrete intracellular distribution patterns in osteoblastic cells. Using a specific fluorimetric assay, gamma-secretase activity was confirmed in human and rat primary osteoblasts at levels equivalent to or greater than rat brain controls. Furthermore, gamma-secretase activity increased during osteogenic differentiation of primary rat osteoprogenitor cells and human mesenchymal stem cells (MSCs), which appeared to reflect a temporal change in expression of gamma-secretase component proteins. Specific gamma-secretase inhibitors, which targeted both Notch and APP cleavage, applied to MSCs during osteogenic differentiation, caused a significant, dose-dependent inhibition of alkaline phosphatase activity and reduced formation of von Kossa-positive mineralised nodules, without affecting cell viability. Using RT-PCR, we confirmed that osteoblasts expressed APP and deposits of Congo red-positive amyloid were detected in the osteoid seams of bones taken from 12 week old rats. These data indicate that cells of the osteoblast lineage express active gamma-secretase, at levels similar to or greater than those in neuronal tissues. Gamma-secretase may impact on diverse cellular events and regulate osteogenesis through its effects on Notch, Wnt, APP and related target proteins.

P324-Tu
Divergent Roles for Canonical and Non-Canonical Wnt Signalling in Human Mesenchymal Stem Cells
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We and others have recently identified a role for Wnt signalling in mesenchymal stem cells (MSCs) and post-natal bone biology, which supports a growing appreciation of the involvement of developmentally important signalling pathways in stem cell activity and lineage specification.
Wnts bind to cell surface frizzled receptors and their effects are transduced through complex canonical and non-canonical intracellular pathways. Canonical signalling induces activation of dishevelled (Dvl), which stabilises β-catenin, causing its nuclear translocation and transactivation of TCF-dependent genes. Non-canonical signalling branches at the Dvl level, ultimately resulting in the activation of jun N-terminal kinase. We determined the effects of canonical (Wnt3a) and non-canonical (Wnt5a) activation on proliferation and differentiation of human MSCs.
Exposure of Wnt3a to MSCs induced β-catenin stabilisation and nuclear translocation. This resulted in a 20-fold increase in transcriptional activity of a TCF-responsive luciferase reporter in transfected MSCs, compared to untreated controls, which was reproduced to a similar level following β-catenin overexpression. In MSCs induced to undergo osteogenic differentiation, Wnt3a caused a significant, dose-dependent inhibition of alkaline phosphatase (ALP) activity and dramatically reduced the formation of alizarin red-positive calcified deposits. Dickkopf1 (Dkk1), an inhibitor of canonical signalling, caused a significant increase in ALP activity. Wnt3a also prevented the accumulation of oil red O-stained lipid in MSCs exposed to adipogenic conditions. In contrast, Wnt5a induced up to 14-fold increase in ALP activity and stimulated mineralised nodule formation in osteogenic MSCs and increased number and size of lipid-filled vacuoles in adipogenic MSCs compared to controls. Both Wnt5a and Dkk1 inhibited MSC proliferation, whereas Wnt3a caused a dose-dependent increase in MSC numbers.
We showed by RT-PCR that MSCs expressed several co-regulators of Wnt signalling that act on Dvl, including Par1Aalpha, Par1Balpha, Vangl1 (but not Vangl2), Diversin and Dapper1, which influence the relative contribution of canonical and non-canonical pathways. Our data demon-

strate that different Wnt mechanisms operate in MSCs with opposing effects on proliferation and differentiation indicating that a complex repertoire of extracellular ligands and intracellular components is likely to contribute to Wnt-dependent effects in skeletal tissues.

P325-Su

Osteoblastic Interaction on Calcium Phosphate Surface Topography at the Sub-Micron Level

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A commonly reported problem in bone defect filling is the formation of a necrotic core within the implant. There are countless factors, the majority of which have yet to be identified, which effect the migration, differentiation and expression of a cell when in contact with a surface. However, surface chemistry and structure, through such phenomena as contact guidance, have shown to control some of these cellular responses. If inner pore structure of a scaffold can be manufactured to provide for controlled submicron to nanometer scale topography, it may be possible to provide for specific cell stimulatory conditions in situ and to enhance the prospects for vascularisation by providing cell guidance, thus, reducing necrosis.
Hydroxyapatite (HA) substrates were thermally consolidated at 800°C and 1200°C to induce changes in chemistry and HA particle size. These materials were characterised FTIR and XPS spectroscopy and X-ray diffraction. SEM was used to illustrate coating topography, profilometry and atomic force microscopy was used to determine surface roughness were appropriate.
SaOs-2 and hFOB osteoblast-like cells were cultured on hydroxyapatite substrates abraded and unabraded, thermally annealed at 800°C and 1200°C and also on porous HA scaffolds thermally annealed at 800°C and 1200°C. Assays were performed to evaluate cell adhesion and proliferation (MTT), whole cell protein content and differentiation (Western blotting and alkaline phosphatase activity).
Increased cell viability and proliferation was observed on substrates thermally annealed at the higher temperature of 1200°C compared to the 800°C substrates. Similarly, protein synthesis, i.e., total cell protein and matrix produced protein was significantly increased on the substrates annealed at the higher temperature. ALP activity was also significantly up-regulated on the surfaces at the higher annealed temperature. Images obtained from the crystal violet binding assay for the samples of interest illustrate cells aligning to the cracks and defects caused by the abrasion process.
The work presented here forms the basis of investigation into how inner pore geometry and morphology of HA scaffolds affects osteoblast expression. If inner pore structure of a scaffold can be manufactured to provide for controlled topography, it may be possible to provide for specific cell stimulatory conditions in situ and to enhance the prospects for vascularisation by providing cell guidance on an appropriate scale.

P326-Mo

Interactions Between Transmembrane Matrix Metalloproteinase-1 and Alkaline Phosphatase in Mature Osteoblasts Determine the Formation of Nodules and Deposition of Mineral

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ROB, rat tibia derived pre-osteoblast during differentiation in vitro, express in a developmentally regulated fashion the early activated protein markers of osteogenesis Matrixmetalloproteinase-1 (MMP-14) and Alkaline Phosphatase (AP).
We here report that the temporal overlapping of the expression of these enzymes is accompanied by a requirement for their contemporary functional activity.
Alteration of either enzyme function (obtained by methods which do not affect the synthesis of the protein, such as with specific inhibitors of the enzymatic activity) results in the failure of function of the other enzyme. In both cases of lack of function, the consequence is the inhibition of the formation of nodules and of deposition of Ca in the ECM, i.e., lack of mineralization, confirming that both Ap and MMP-14 function are prerequisites for osteogenic progression and suggesting that the two enzymes have a complementary functional role.
Nonetheless, when studying situations where the expression and function of AP or MMP-14 are not downmodulated, obtained in clones constitutive for the expression of AP or in normal preosteoblasts treated with an activating antibody for MMP-14, also in these cases, we observe inhibition of nodule formation and mineralization. These data suggest that the physiological downmodulation of the enzymes, after their maximal expression in the second phase of osteogenesis, is also a requirement for the proper prosecution of osteogenesis.
It is possible that the effect of cross-inhibition and cross-maintenance of function observed for AP and MMP-14, both located on the cell membrane, is caused the request for their physical interaction for function Other possibilities are also considered.

P327-Tu

Evaluation of Osteogenic Potential of Cultured Human Oral Periosteum-Derived Cells

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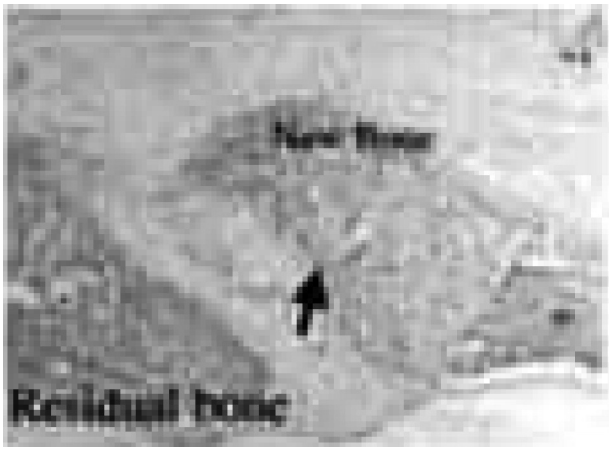
Aim: Periosteum plays an important role in bone development and fracture healing and the osteogenic potential of transplanted periosteal cells has been reported. Clinical application of cultured periosteal cells for the repair of bone and joint defects is considered possible; however, little is known about the osteogenic potential and osteogenic mechanism of cultured oral human periosteal cells in vivo. In the present study, we examined radiographically and histologically the osteogenic potential of cultured human oral periosteum-derived cells that were grafted into rat calvarial critical-sized defects.

Materials and methods: Primary human periosteal cells were isolated from intraoral mandibular periosteum (5 × 7 mm) of 25 Japanese patients undergoing routine oral maxillofacial surgery. Periosteal cells were cultured for 14 days. After confluence, periosteal cells/collagen complex were prepared with osteogenic medium containing with 10−8 M dexamethasone, 10 mmol glycerol phosphate, 0.3 mmol ascorbic acid and 10% fetal bovine serum. Gingival fibroblasts taken from oral gingiva were used as the negative control. Cell/collagen complex was grafted into the critical-sized calvarial defect (7 mm in diameter) of Sprague–Dawley (SD) rats (7-week-old males). To avoid immunorejection, the immunosuppressant FK506 (1.0 mg/kg/day; Tacrolimus, Fujisawa Co Ltd., Osaka, Japan) was intramuscularly administered daily. Specimens were extirpated at 14, 21 or 45 days post-grafting and examined.

Results: At 14 days post-grafting, new bone formation was detected at the center of the defect. By 21 days, new bone developed. Radiographic findings showed the development of calcification in the defect. By 45 days, increased amounts of new bone were present in the defect, but had not fused to residual bone. Radiographically, calcification had developed. In controls, no new bone was observed in the defect (Total Bone/Total Volume of defect = 57.1 ± 7.5%).

Conclusion: Our preliminary study suggests that while cultured human periosteal cells show osteogenic potential in vivo, the amount of new bone produced is currently not sufficient for the repair of bone defects.



P328-Su

Critical Regulation of BMP-Induced Osteoblastic Differentiation by Delta1/Jagged1-Activated Notch1 Signaling

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Functional involvement of the Notch pathway in osteoblastic differentiation has been previously investigated using the truncated intracellular domain, which mimics Notch signaling by interacting with the DNA binding protein CBF-1. However, it is unclear whether Notch ligands Delta1 and Jagged1 also induce an identical cellular response in osteoblastic differentiation. We have shown that both Delta1 and Jagged1 were expressed concomitantly with Notch1 in maturating osteoblastic cells during bone regeneration and that overexpressed and immobilized recombinant Delta1 and Jagged1 alone did not alter the differentiated state of MC3T3-E1 and C2C12 cells but augmented bone morphogenetic protein-2 (BMP2)-induced alkaline phosphatase (ALP) activity and the expression of several differentiation markers, except for osteocalcin, and ultimately enhanced calcified nodule and in vivo ectopic bone formation of MC3T3-E1. In addition, both ligands transmitted signal through the CBF-1-dependent pathway and stimulated the expression of HES-1, a direct target of Notch pathway. To study the requistric role of Notch signaling in BMP2-induced differentiation, Notch signaling was inhibited by the dominant negative extracellular domain of Notch1, specific inhibitor, or siRNA. These treatments decreased ALP activity as well as the expression of other differentiation markers and inhibited the promoter activity of Id-1, a target gene of the BMP pathway. These results indicate the functional redundancy between Delta1 and Jagged1 in osteoblastic differentiation, whereby Delta1/Jagged1-activated Notch1 enhances BMP2-induced differentiation through the identical signaling pathway. These data also suggest that functional Notch signaling is essential not only for BMP2-induced osteoblast differentiation but also for BMP signaling itself. However, contradictory results have been reported from a retrovirus or an ordinary stable transfection study in which NICD introduction impairs osteoblastic differentiation of stromal cell ST-2, MC3T3-E1, and Kussa cells. This suggests that the effect of Notch activation may vary depending on the period of Notch activation; transient and short-term activation of the Notch pathway may enhance osteoblastic differentiation as shown in our study, whereas long-term continuous activation may lead to the inhibition of osteoblastic commitment.

P329-Mo

Fibroblast Growth Factor 8 is a Novel, Potent Regulator of Mesenchymal Stem Cell Differentiation into Mature Osteoblasts

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Fibroblast growth factor 8 (FGF-8) is widely expressed in developing skeleton and thus may regulate cartilage and/or bone formation. Other FGFs, such as FGF-2 and FGF-18 have been shown to regulate both osteoblast and osteoclast differentiation and function. In this study, we have induced osteoblast formation from NMRI mouse bone marrow cells. FGF-2 and bone morphogenetic protein 4 were used as controls. FGFs were either present in the primary culture (days 0–7) or in the beginning of the secondary culture (days 7–10). Osteoblast formation was induced by culturing marrow cells in alpha-MEM supplemented with 15% FCS, 10 mM sodium-β-glyserophosphate, 0.05 mM ascorbic acid-2-phosphate and 10 nM dexametasone (Dex). Dex was present only in the primary culture, since it was found to inhibit the bone formation capacity of osteoblasts formed in the culture. At the end of the culture, bone formation was assessed by calcium determination and by von Kossa staining. Both FGF-8 and FGF-2 at 25 ng/ml present in the primary culture stimulated bone formation for approximately 15- and 10-fold, respectively, compared to the control. Interestingly, if either one of FGFs was present all the time in the culture, it significantly inhibited bone formation activity. In the proliferation assay, FGF-8 was found to stimulate the proliferation of mouse mesenchymal cells and MG-63 osteosarcoma cells. These results show that FGF-8 has mitogenic effect on mesenchymal stem cells (MSCs) and it increases their osteogeniety in the early phase of MSC differentiation into mature osteoblasts. Importantly, it appears that FGF-8 is even more potent than FGF-2 in enhancing the osteogenic potential of MSCs.

P330-Tu

Optimization of Mesenchymal Stem Cell Culture Conditions for 3-D Culture

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Our goal is to develop a mesenchymal stem cell (MSC)-containing carrier, which can be used to treat bone defects. However, we still lack the knowledge of optimal conditions for the growth of MSCs and their induction into functional osteoblasts. For in vitro cultures, either human or mouse bone marrow-derived MSCs were first cultured in a-MEM supplemented with 15% FCS for 1–2 weeks to establish highly-proliferative, multipotent, non-differentiated stem cells for further cultures. After trypsinization and cell counting, mouse cells were applied into type I collagen sponges (1 cm × 1 cm × 1 cm, Spongostan, Johnson and Johnson, UK) at different cell densities and cultured for 3 weeks in a-MEM supplemented with 15% FCS, 10 mM sodium-β-glyserophosphate and 0.05 mM ascorbic acid-2-phosphate. For human bone marrow-derived MSCs, the culture period was 4 weeks instead of 3 weeks. Dexamethasone (10 nM) was present in the secondary culture only at days 1–7 to induce optimal stem cell proliferation and differentiation into osteoblasts. At the end of the culture, sponges were fixed with 3% paraformaldehyde in PBS and stained either for alkaline phosphatase (ALP) or with von Kossa staining. In both of these culture systems, MSCs were effectively differentiated into ALP-positive cells, which had high bone formation capacity. HEPES (20 mM) was found to inhibit bone-formation capacity of osteoblasts suggesting that pH plays important role during bone formation. pH values in the cultures at the time of bone formation with and without HEPES were 7.59 and 8.06 on an average, respectively. Fibroblast growth factor (FGF)-2 was also tested in the mouse system in order to increase the proliferation and recruitment of MSCs either in the primary culture or in the beginning of the secondary culture. FGF-2 stimulated significantly the proliferation and osteogenic capacity of MSCs. If FGF-2 was present all the time in the culture, the overall effect was inhibitory in bone formation. Isolated human MSCs were multipotent, since in addition to osteoblasts, they also had the capacity to differentiate into chondrocytes and adipocytes as analyzed by Alcian blue and oil red O staining, respectively. To summarize, MSCs can proliferate and differentiate into osteoblasts with high bone formation activity in 3-D scaffold, such as type I collagen sponge and this carrier can be used as a reference material for developing new 3-D carriers for MSCs.

P331-Su

Regulation of Osteoblast Attachment and Morphology on Calcium Phosphate Coatings by Surface Chemistry and Pre-Adsorption of Fibronectin

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Joint replacement surgery has been a major advance in the treatment of arthritis, and more than 100,000 hip, knee, shoulder and elbow joint replacement operations are carried out in the U.K. each year. However, revision surgery is forming an increasing proportion of the workload of modern orthopaedic surgery with a 10% failure rate occurring in a significant proportion of primary TJR cases. Thus, the long-term effectiveness of TJR prostheses is a major issue in determining overall cost and benefit. The development of a tissue-engineered implant surface with superior long-term

performance has the potential to eliminate the need for revision operations. The nature of the bone-implant interface is highly dependent on the characteristics of the implant surface. Thus, control of surface chemistry and topography is pertinent in directing the cell response and securing optimum bone formation at the interface.

Thin film calcium phosphate (Ca-P) coatings were deposited onto glass Petri dishes by rf magnetron sputtering. Chemical analysis of coatings was performed using FTIR, XPS, and XRD. SEM was used to illustrate coating topography and atomic force microscopy was used to determine surface roughness. Prior to cell culture coating, surfaces were conditioned with pre-adsorbed fibronectin, a cell adhesion protein. hFOB osteoblast cells were cultured on conditioned and non-conditioned Ca-P coating surfaces. A fluorescence-based assay was used to examine cell attachment and morphology was illustrated using fluorescent microscopy. Western blotting was used to monitor fibronectin adsorption to the calcium phosphate surfaces.

Surfaces conditioned with fibronectin showed increased cell attachment. Fluorescent microscopy illustrated rounded cell morphology on non-conditioned surfaces. Differences in cell response to the various coatings were also observed. Furthermore, Western blotting revealed changes in fibronectin adsorption to the coating surfaces.

Results suggest that the pre-adsorption of fibronectin on Ca-P coating surfaces can enhance osteoblast adhesion. The observed differences in fibronectin adsorption can indicate that surface chemistry may be responsible for enhanced osteoblast adhesion. Optimising the surface chemistry combined with apposite fibronectin adsorption may have the potential to secure a stronger and continuous bone-implant interface as well as significance for future tissue engineering applications.

P332-Mo

Role of Endothelin-1 and VEGF in a Co-Culture Model of Microvascular Endothelial Cells and Fetal Rat Calvarial Cells

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Endothelin-1 and vascular endothelial growth factor (VEGF) are important factors expressed by vascular endothelial and osteoblast progenitor cells and play a role in inter-cellular signalling, angiogenesis and bone microcirculation. We have shown that exogenous endothelin-1 stimulates proliferation and differentiation of fetal rat calvarial (FRC) cells in vitro and leads to downregulation of VEGF expression in FRC cells. We therefore asked if there is any correlation between the expression of endothelin-1, its receptors (ETR-A and B) and VEGF in a co-culture model of rat microvascular endothelial (RMVEC) cells and FRC cells.

RMVEC and FRC cells were isolated and co-cultured in six well plates with fitted inserts. FRC cells were treated with or without dexamethasone 10 mM prior to starting the 14-day co-culture period. Co-cultured FRC cells revealed a significantly higher proliferation rate at day 4 and day 7 and a lower Alkaline Phosphatase enzyme activity at day 14 compared to control FRC cells, as well as a qualitative difference in matrix deposition. Both RMVEC and FRC cells expressed mRNA for endothelin-1, VEGF isoforms and their respective receptors during the entire experimental time course as determined by RT-PCR. There was a strong correlation between expression of endothelin-1 and its receptor ETR-A in all groups of both FRC and RMVEC cells. However, no direct correlation between the expression of endothelin-1 and VEGF isoforms in either RMVEC, FRC or in the corresponding co-culture groups could be determined by statistical analysis using GraphPad Prism.

We conclude that in a co-culture system of RMVEC and FRC cells, the presence of vascular endothelial cells promoted osteoblastic cell proliferation (preferentially over differentiation); that ET-1 expression was correlated to expression of its receptor, but that there was no obligatory inter-regulation between endothelin-1 and VEGF expression. As such, ET-1 and VEGF are influenced by other factors that are objectives of future investigations.

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P333-Tu

Ectopic Overexpression of Adipogenic Transcription Factors Induces Transdifferentiation of MC3T3-E1 Osteoblasts

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Osteoblasts and adipocytes originate from a common mesenchymal progenitor cells. We have investigated whether mouse osteoblastic MC3T3-E1 cells can be induced to transdifferentiate into mature adipocytes by the ectopic expression of adipogenic transcription factors, PPARγ, C/EBP-α, or both. Retrovirus-mediated overexpression of PPARγ alone or both PPARγ and C/EBP-α resulted in reduced alkaline phosphatase activity and osteoblast-specific gene expression. Moreover, foci of adipocytes were identified in conditions favoring osteoblast maturation. Upon treatment with insulin, dexamethasone and IBMX, cells overexpressing PPARγ alone or both PPARγ and C/EBP-α showed marked transdifferentiation to mature adipocytes expressing molecular markers of adipocytes. Cells expressing both PPARγ and C/EBP-α showed more robust phenotype of adipocytes than the cells expressing PPARγ alone. Overexpression of C/EBP-α alone did not result in adipogenesis. These results suggest that PPARγ is a key molecular switch for the transdifferentiation to adipocytes, whereas C/EBP-α may differentiate MC3T3-E1 cells into osteoblasts and adipocytes.

P334-Su

Osteoblastic Cell Differentiation and p38 Map Kinase

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The complex nature of osteoblastic cell differentiation and its regulation by interacting signaling pathways is still not completely understood. The mitogen-activated protein kinase p38 (p38MAPK), which has been documented as an important regulator for cellular responses to environmental stimuli, has been shown to be essential for the differentiation of several cell types, but its role in osteoblastic cell differentiation has not been completely delineated. The goal of this study was to elucidate the role of p38 MAPK in the differentiation process of osteoblasts using the murine cell line, MC3T3-E1 cells line, as well as primary human osteoblastic cells. The human cells were obtained from explants of mandibular bone fragments that would have been otherwise discarded during normal oral procedures. MC3T3-E1 and human osteoblastic cell cultures were each cultured, after confluency, with osteogenic alpha MEM medium, which contains three differentiation and calcification-inducing substances; beta glycerol phosphate (1.8 g/L) dexamethasone (10 nM) and ascorbic acid (2 mg/L). In some experiments, the activity of p38 MAPK was inhibited by treatment with the specific inhibitor, SB203580. Alkaline phosphatase activity, measured biochemically, and osteocalcin expression, detected with RT-PCR analyses, were used as indicators of osteoblastic cell differentiation. Expression of protein kinase C (PKC) isoforms, studied with Western blots, was also evaluated in controls and cells treated with SB203580, in order to delineate a possible relationship between specific isoforms and p38 MAPK activity in differentiating cells. The results of these studies indicated that p38 MAPK, analyzed with Western blotting, was activated throughout the differentiation of the MC3T3-E1 cells as well as the human osteoblastic cells. Continuous treatment with SB203580 reduced alkaline phosphatase activity and osteocalcin expression. Inhibition of p38 MAPK with the drug significantly increased the expression of PKC alpha and PKC eta with slight decreases in PKC delta. All the studies were repeated with at least four different cultures and analyzed for significant differences employing ANOVA. These studies suggest that increases in p38 MAPK occur as osteoblastic cells differentiate and that PKC isoforms may be selectively regulated by the kinase.

P335-Mo

c-ABL Augments BMP2 Induced Id1 Expression and Participates in Senescence of Osteoblasts

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Non-receptor tyrosine kinase c-Abl is activated by growth factors and genotoxic stress. Mice lacking c-Abl exhibit a variety of complex phenotypes including aging associated osteoporosis. Here, we report that c-Abl$^{-/-}$ osteoblasts showed accelerated senescence, manifested by reduced proliferation capacity, increased expression of senescence associated β-galactosidase (SA-β-Gal), and elevated levels of p16INKa, a known mediator of senescence. Up-regulation of p16 correlated with reduced expression of Id1, a negative regulator of p16 transcription. c-Abl positively regulates Id1 expression as c-Abl deficiency or inhibition by Sti571 reduced Id1 levels, while c-Abl overexpression upregulated Id expression at both the protein and the mRNA levels. These findings were further supported with increased endogenous Id1 levels observed when using the BCR-ABL-expressing cell line K562 in comparison to HL60, a myeloblastic cell line. In addition, we have found that c-Abl was required for maximal Id1 induction under BMP2 stimulation in osteoblasts. Reconstitution of c-Abl in c-Abl deficient cells rescued BMP2 induced activation of smads 1/5/8 and Id1 expression. These results suggest that c-Abl is a positive regulator of the BMP pathway. The premature senescence phenotype of c-abl−/− osteoblasts is p53 dependent, as p53−/− c-abl−/− osteoblasts, like p53−/− osteoblasts, underwent indefinite proliferation even though they expressed elevated level of p16. Although c-abl−/− osteoblasts were hypersensitive to oxidative stress, which is known to induce senescence, we found that the oxidative stress may not be linked to the premature senescence of c-abl−/− osteoblasts as oxidative stress did not up-regulate p16 expression and that oxidative stress did not involve p53 in c-abl−/− osteoblasts. We conclude that c-Abl regulates osteoblast senescence and oxidative stress response via different mechanisms. Abl$^{-/-}$ mice present another model that correlates premature cell senescence with aging phenotypes.

P336-Tu

Histone Deacetylases are Involved in the Transcriptional Regulation of Siblings During Osteoblast Differentiation

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Bone sialoprotein (BSP), osteopontin (OPN), dentin matrix protein 1 (DMP1), dentin sialophosphoprotein (DSPP) and matrix extracellular phosphoglycoprotein (MEPE) are all members of a unique family named SIBLINGs (Small Integrin-Binding Ligand, N-linked Glycoprotein). These secreted phosphoglycoproteins are components of the mineralized extracellular matrices of bone and teeth. Histone deacetylases (HDACs) represent a large family of

enzymes identified as key regulators of nucleosomal histone acetylation, a major event that controls eukaryotic gene transcription. Recent studies have shown that HDACs are involved in the regulation of osteoblast-mediated mineralization and bone formation. In this study, we have investigated the potential role of HDACs in the regulation of SIBLINGs expression during osteoblast differentiation. We used Saos-2 human osteosarcoma cell line which is a useful model of osteoblastic differentiation since the expression of bone matrix proteins is up-regulated in these cells as they become confluent. Expression of HDACs 1 to 8 was suppressed through the transfection of specific siRNAs into Saos-2 cells. Using quantitative real time RT-PCR, we observed that expression of BSP was up-regulated by the inhibition of HDAC3 synthesis and down-regulated by the inhibition of HDAC2. OPN was also upregulated by the inhibition of HDAC3 synthesis but was upregulated in the presence of HDAC6 siRNAs. DSPP expression was upregulated when HDAC6 expression was inhibited and strongly downregulated by the inhibition of HDAC1 synthesis. DMP1 expression was not modulated by the inhibition of anyone of the HDACs analyzed to date. In accordance with the upregulation of BSP and OPN expression observed in Saos-2 cells in which HDAC3 expression was abolished, we observed a dramatic decrease of HDAC3 mRNA expression in differentiated Saos-2 cells when compared to non-differentiated ones. Together, these results indicate that specific HDACs are involved in the regulation of osteoblastic cell's differentiation process by either inducing or repressing SIBLINGs expression.

P337-Su

Indirect Action of VEGF on Osteoblast Behaviour: Prostaglandin-Mediated Regulation of Endothelial Cell: Osteoblast Cross-Talk

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Vascular endothelial growth factor (VEGF) is implicated in the coupling of osteogenesis to endothelial cell (EC) behaviour. Although central to bone pathobiology, the mechanisms coupling osteoblast (OB) to EC behaviour remain to be elucidated. We have previously found: (i) that OB produce more VEGF than EC, and unlike EC increase their VEGF secretion in response to prostanoids, like PGE2, (ii) that EC exhibit greater intracellular signalling responses to VEGF than OB, and (iii) that both OBS and EC produce PGE2 in response to VEGF treatment. We hypothesise that EC facilitate osteogenesis in response to OB-derived VEGF and that this cross-talk is regulated by PGs. We have examined whether coupling requires soluble mediators or direct cell contact. To distinguish between these, we used: (i) a non-contact system in which human umbilical vein EC (HUVEC) and human OB (HOBS) are separated by a 0.4-μM filter, or (ii) a direct-contact co-culture system. Non-contact co-culture enhanced proliferation of both cell types. Long-term co-culture also promoted increased HOB alkaline phosphatase (ALP). Treatment of HUVEC, but not HOB, with VEGF165 in non-contact co-culture conditions promoted further increases in HOB differentiation. Direct contact in co-culture also enhanced ALP of HOB. In this instance exogenous VEGF165 failed to promote this influence. Thus, VEGF exerts an indirect EC-mediated effect on OB behaviour, unless direct cell contact between OB and EC is possible. To determine the autocoids contributing to this crosstalk, we used a pharmacological approach. This showed in direct contact co-culture: (i) that L-NIO, reduced but did not abolish HUVEC-related increases in HOB ALP, (ii) that a VEGF-receptor blocker had no effect, but (iii) that NS398, significantly enhanced those increases in HOB AP activity induced by HUVEC and that this enhancement was reversed by exogenous PGH2 (COX-2 product). Inhibiting COX-2 in co-culture suggests that PGs are vital negative regulators of OB:EC cross-talk when these cells are in close proximity. Disruption in this controlled crosstalk may underpin bone pathologies.

P338-Mo

The Influence of Implants Surface Properties on Cellular Adhesion, Proliferation and Synthetic Activity

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Introduction: The cellular response by which cells react to the presence of some commonly used and newly developed commercial implant materials was studied under in vitro conditions. The influence of some physicochemical implant parameters on response of cells growing in the implant immediate vicinity, namely the influence of tested materials on their adhesion, proliferation and synthetic activity were compared. The effect of surface characteristics of studied materials on the blood clot formation and the presence of some expressed cell markers were evaluated too.
Methods: Embryonal human lung fibroblast LEP Sevapharma, Prague, Czech republic) and osteoblasts NHOst (Cambrex, Walkersville, USA) were used for cultivation experiments under the standard conditions (1). We monitored a direct influence of the tested materials on the cells viability using the MTT test according to the Laughton (1984). The presence of some inflammatory mediators we evaluated in the cultivation medium using Elisa methods. The freshly drawn blood was time dependently fixed on the surface of tested materials and evaluated microscopically. The surface-free energy (SFE) of the tested materials were estimated by a static contact angle (CA) measurement in the three different solvents using the drop sessile method. The

advancing and receeding contact angles were estimated by the dynamic Wilhemy plate method in water and in cultivation medium.

Results: According to the SFE and CA measurements on selected implant surfaces there are two main groups with extremes in cellular adhesion. In the first groups (zirconium ceramic, and titanium polished surfaces), the increased polar part of SFE, the highest cell density, the lowest inflammatory cytokines production, but no fibres in the clotting blood were found. On the contrary, the second group of materials with very low polar part of SFE (polyethylen, carbon composite) performed distinctly higher expression of inflammatory mediators, the low cell proliferation, but a quicker formation of the fibres in blood coagulum.

References:

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P339-Tu

Improved Bone Cellular Activity Through the use of Calcium Phosphate Coated Polymeric Scaffolds

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Biodegradable polymers, in general, lack cell-recognition signals, which lead to poor cell adherence. Hydrophobic surface sites lead to an inhomogeneous distribution of adherent cells. These features tend to cause cells to react biologically in a different way to these polymers than to extracellular matrix tissue. This study focuses on optimisation of the polymeric scaffold for bone cellular activity using a calcium phosphate coating. Coated scaffolds were produced by an alternate soak method. Bone marrow cells were harvested from trabecular bone marrow samples of haematologically normal patients. After the fourth passage, cells were trypsinised and seeded on top of each of the scaffolds. The scaffolds were then placed into the incubator (37°C, 5% CO_2) to allow the cells to adhere and culture medium was added after 4 h. 1 week after the start of culturing, the complete medium was replaced by osteogenic medium (-α-minimal essential medium with 10% foetal calf serum, 50 units/25 μg/ml penicillin/streptomycin, 10^{-8} M dexamethasone, 100 μM ascorbic acid and 10 mM β-glycerophosphate). Osteogenic medium was changed every 2 days. The scaffolds were analysed for cellular activity using alamarBlue™ and alkaline phosphatase assays using a microplate spectrofluorometer. Scanning electron microscopy was also used to visualise cell attachment on the material surface. Material analyses were carried out using wavelength dispersive X-ray analysis and X-ray diffraction analysis. In addition, to study biocompatibility, scaffolds were implanted into subcutaneous sacs in BALB/c mice. The mice were killed and biopsies analysed 2 and 4 weeks postoperatively. The results from alamarBlue™ and ALP assays showed better cell viability in the coated scaffolds. SEM results also showed that more cells were attached to the coated scaffolds, forming multi-layers, as compared to the uncoated scaffolds. WDX analysis showed that the calcium phosphate coating layer had a Ca/P ratio of 1.68, which is similar to that of hydroxyapatite. This was further confirmed by the XRD results. Biocompatibility studies showed no sign of adverse tissue inflammatory response when the scaffolds (coated and uncoated) were implanted in vivo. Hence, current results indicate that the calcium phosphate coated polymeric scaffolds show improved cell attachment, proliferation and differentiation properties, which would be potentially useful in the field of bone tissue engineering.

P340-Su

Rapid Activation of CREB and Elk-1 and Actine Reorganization in Female Osteoblasts in Response to a Low Dose of Daidzein

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The isoflavone daidzein acts on transcription via the intracellular estrogen receptors (ER), mainly ERbeta, in various cell types, including osteoblasts, the cells responsible for bone formation. The few published data on non-classical effects of this isoflavone concern its use at high (1–10 μM) concentrations. We have identified the multi-step processes involved in the rapid actions of low (1 pM–1 nM) doses of daidzein in female osteoblasts. The first membrane protein activated was the pertussis toxin (PTX)-sensitive Gbeta 1 sub-unit coupled to PLC-beta2, which triggered a rapid (5 s) mobilization of calcium from the endoplasmic reticulum. Daidzein activated ERK1/2 within 15 s, but had no effect on p38 MAPK or JNK/SAPK. Several inhibitors (PD98059 (MEK1/2), Gö6976 and chelerythrine (protein kinase C, PKC), wortmannin and LY294002 (phosphatidylinositol 3-kinase, PI3K), PP1 (Src) and PTX) all blunted the Dz-stimulated phosphorylation of ERK1/2. Incubating cells with daidzein (100 pM) rapidly increased (20 s) the phosphorylation of the two transcription factors Elk-1 and CREB, and this was inhibited by PTX and PD98059. Lastly, daidzein induced rapid (1 min) changes in the actin cytoskeleton via PI3K/Src/MEK1/2 activation. The pure estrogen antagonist, ICI 182,780, did not alter the responses to daidzein. Thus, daidzein rapidly triggers the activation of transcription factors involved in cellular proliferation and differentiation. Moreover, re-arrangement of the actin skeleton by modifying cell–cell substratum may control many cell functions such as motility and division, and the disassembly of stress fibers and focal adhesions may stimulate cell motility. The cascade

of rapid effects of daidzein may involve the classical ERs located at the plasma membrane or an uncharacterized form of ERs that is insensitive to nuclear antagonists.

P341-Mo

Exposure of Mouse Pre-Osteoblast Cells to Pulsed Electro-Magnetic Fields Rapidly Activates the mTOR Signaling Pathway

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The mouse pre-osteoblast MC3T3-E1 cell line was used under serum-free culture conditions to investigate the effect of pulsed electromagnetic fields (PEMF) on autocrine growth factor production and to identify signal transduction pathways that are activated soon after PEMF exposure. PEMF exposure increased the amount of TGF-beta in the conditioned medium after 1 day of exposure, but had no effect thereafter, and PEMF exposure also had no effect on the amount of prostaglandin E2 in the conditioned medium. To identify early cellular responses to PEMF exposure, we assayed a set of signal transduction pathway components for activation after 1 h of PEMF exposure. Further analysis revealed that the mTOR-signaling pathway is activated after 10 min of PEMF treatment, as evidenced by increased phosphorylation of mTOR, p70 S6 kinase, and the ribosomal protein S6. Inhibition of PI3-kinase with the chemical inhibitor LY294002 blocked this PEMF-dependent activation.

P342-Tu

Gene Array Analysis of Adipogenic Transdifferentiation of Committed Osteoblasts

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In human bone marrow, the age-related expansion of adipose tissue at the expense of osteogenic differentiation may partly account for diseases such as osteoporosis and osteonecrosis. The molecular basis for this adipogenic degeneration process is largely unknown. We recently established a cell culture system of human bone marrow-derived mesenchymal stem cells, in which the cells can be reprogrammed (trans-differentiated) during their differentiation from osteoblasts into adipocytes. RT-PCR analysis of the cells revealed that after adipogenic transdifferentiation of committed osteoblasts (cells differentiated in osteogenic medium for 2 weeks), only adipogenic markers, but no osteogenic markers, were expressed. To elucidate the underlying molecular pathways of this reprogramming process at the level of global gene expression patterns, we performed an Affymetrix gene array analysis by comparing transdifferentiated adipocytes with committed osteoblasts. To aim for the identification of potential control factors initiating the transdifferentiation process, RNA was isolated 3 and 24 h after the initiation of transdifferentiation (i.e., addition of adipogenic medium to committed osteoblasts) and compared with RNA from committed osteoblasts. The gene array analysis identified 201 regulated genes (at least 2-fold change at one or both time points examined). Regulated gene products mainly consisted of transcription factors and signaling molecules. 18 of 22 selected genes (based on at least 4-fold regulation and functional potential; e.g. core promoter element binding protein, cysteine rich angiogenic inducer 61, kruppel like factor 4, and fatty acid binding protein 4) proved to be regulated by semi-quantitative RT-PCR. The majority of these gene products was also regulated in independent experiments. Our findings demonstrate reproducible molecular changes associated with the trans-differentiation of mesenchymal stem cell-derived osteoblasts into adipocytes. Further functional investigation of selected gene products could reveal novel signaling processes initiating transdifferentiation or even acting as "molecular switches". Thus, novel targets could be detected for therapeutic interventions in order to prevent adipogenic degeneration and to stimulate osteogenic differentiation.

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P343-Su

Increased Bone Formation Associated to Decreased Adipogenesis Participates to Mechanical Loading-Induced Bone Adaptation

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Osteoblasts and adipocytes develop from common bone marrow mesenchymal precursors. Aging and unloading inhibit bone formation and increase adipogenesis. It is not known whether mechanical loading has the ability to reverse such situation. Pluripotent C3H10T1/2 cells and bovine primary marrow stromal cells, cultured on a permissive osteoblastic and adipocytic medium were expended 72 h on type I collagen-coated-silicone membranes then daily submitted to cyclic strain (10 min, 0.5%, 1 Hz; FLEXCELL FX-3000 unit). In primary cells, stretch-induced cell growth increase the first 3 days. After 14 days of stretching, protein evaluation showed that alkaline phosphatase (ALP) activity and osteocalcin (OC) increased by 147% and 76%, respectively. After 3 days of stretching, Runx2 increased by 67%

then reached NS level. At 14 days, alkaline phosphatase-positive osteoblasts were more numerous in stretched cells (S) (+35%) than in non-stretched cells (NS), peroxisome proliferator-activated receptor (PPAR)g2 decreased by 80%, GAPDH by 50% and red oil positive adipocytes were less numerous (−65%) in S than in NS. In the C3H10T1/2 cell line, growth in S was not affected after 3 and 7 days, and slightly decreased thereafter. At 7 and 14 days, osteoblastic markers represented by ALP, Runx2 and OC protein levels increased in S compared to NS, whereas PPARg2 nuclear activity was decreased. Gene expressions of runx2, msx2, osterix and OC increased in S compared to NS while expression of adipogenic markers such as ADD1/SREBP1, PPARg2, aP2 and adipsine decreased at different time points of the experiment. As a consequence, osteoblasts were more numerous in S than in NS (+18%), whereas the contrary was seen for adipocytes (−46%) at 14 days. To test whether PPARg2 is at crossroad, we added an agonist (rosiglitazone, BRL49653, 1 μM) or an antagonist (GW9662, 1 μM) every other day during medium changes. Rosiglitazone, while increasing adipogenesis, reduced osteoblastogenesis. Mechanical regimen partially inhibited rosiglitazone-induced adipogenic stimulation without any major effects on osteoblastic repression. GW9662 had the reverse effects than rosiglitazone. In this case, mechanical regimen partially restored osteoblastogenesis (runx2 and msx2 expression) and further inhibited PPARg2 expression. Our mechanical regimen is able to enhanced osteoblastogenesis at the expanse of adipogenesis and this reciprocal fate is largely dependent on PPARg2 expression and activity.

P344-Mo

Comparative Characterisation of Hair Follicle Dermal Stem Cells and Bone Marrow Mesenchymal Stem Cells

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Mesenchymal stem cells (MSCs) give rise to various lineages, including osteoblasts, adipocytes and chondrocytes. These cells form a rare population in the bone marrow, but have also been reported at other tissue sites. In the present study, we demonstrated the simple isolation and expansion of dermal stem cells from rat whisker hair follicles and compared their characteristics with MSCs derived from the rat bone marrow. Both cell types were adherent and morphologically similar to interfollicular dermal fibroblasts. A single hair follicle generated approximately 1×10^4 adherent cells within 5 days of isolation and both follicle and bone marrow-derived cells proliferated rapidly with population doubling times of 27 and 30 h, respectively. Both cell types initiated fibroblastic colony-forming units and differentiated into various mesenchymal lineages in multipotency assays. Following osteogenic induction, hair follicle cells and bone marrow MSCs expressed osteonectin, osteopontin, osteocalcin and twist mRNA and formed von Kossa-positive mineralised nodules. Adipogenic conditions induced lipoprotein lipase and PPAR-gamma expression and the accumulation of lipid-filled vesicles was detected by oil red O staining. Chondrogenic differentiation appeared more efficient in bone marrow MSCs than in hair follicle-derived cells following Alcian blue staining and analysis of aggrecan expression in chondrogenic pellets. In contrast, we identified more efficient myogenic differentiation in hair follicle dermal cells compared to bone marrow MSCs through the formation of myo-cellular alignments and expression of β-myosin heavy chain and myocyte enhancement factor 2D. Control dermal fibroblasts failed to differentiate. The question as to whether dermal stem cells resided in a specialised compartment in the hair follicle was examined by culturing intact hair follicles under osteogenic conditions and identifying early mineralization events by von Kossa staining. Mineralization initiation sites were dispersed throughout the outer dermal layer and in the dermal papilla of the follicle. No mineralization of epidermal tissue was observed, nor in hair follicles kept in control medium. These results demonstrate the presence of MSCs in the hair follicle with similar characteristics to bone marrow derived MSCs. The hair follicle may therefore represent an important and accessible source of MSCs for research and therapeutic application in musculoskeletal disorders.

P345-Tu

Evaluation of Different Methodologies to Generate Mesenchymal Stem Cells (MSC) from Human Bone Marrow

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Mesenchymal stem cells (MSC) are pluripotent and thus represent an invaluable tool both for the study of cell differentiation and for therapeutic applications. These cells can be found on different tissues, and have been isolated from bone marrow, blood from the umbilical cord and peripheral blood. They can differentiate to osteoblasts, adipocytes and chondrocytes, among other cell types. Yet, the differentiating potency and cell division capacity of these cells diminish with the number of divisions that they have undergone in vitro. Therefore, they may have a limited therapeutic value. The isolation, tissue culture and generation of large number of such cells and the maintenance of their pluripotency is therefore a challenge nowadays.

We have compared two methodologies for the isolation and tissue culture of MSC from bone marrow. The first approach is used for the isolation of mononuclear cells from bone marrow by means of a Ficoll gradient. Such cells were grown on plastic flasks. After several media replacements, the MSC colonies were observed. In the second method used [Kotobuki et al. 2004], the bone marrow was centrifuged to discard the plasma. The pellet including both mononuclear cells and erythrocytes was then grown on plastic flasks. The cells not attached were removed after several media replacements, and eventually the MSC colonies were observed.

The procedure by Kotobuki et al., generated 10 times more MSC cells from the same amount of bone marrow, after 12 days of tissue culture than the mononuclear cell isolation alternative by the Ficoll gradient protocol. To further optimize the Kotobuki methodology, the tissue culture media (containing cells obtained from bone marrow) was supplemented with the fibroblast growth factor (bFGF) at concentrations ranging from 1 to 3 ng/ml. After 9 days of tissue culture (when the colonies were confluent), the cultures with bFGF showed three times more cells than the controls. No dose-response effect was observed between the bFGF concentration and the number of cells. Our results show that the procedure by Kotobuki et al. is a simple, yet efficient approach to generate a large number of MSC cells from bone marrow. Furthermore, the addition of bFGF allows the production of a large number of pluripotent MSC in a short period of time. MSC can be then used for research or therapeutic applications.

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P346-Su

Raloxifen and Tamoxifen Act as Anti-Estrogen on Osteoblastic Differentiation but Estrogen-Like on Adipocytic Differentiation of Bone Marrow Stromal Cells

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Osteoblasts and adipocytes originate from the common precursor bone marrow stromal cells (BMSC). We previously reported that 17beta-estradiol (E2) promotes early osteoblastic differentiation whereas inhibits adipocytic differentiation of these cells. We suggested that this preferential differentiation of BMSC in osteoblastic lineage contributes to protective effect on bone by E2. Because selective estrogen response modulators (SERMs) such as raloxifen (RAL) and tamoxifen (TAM) also protect bone, they are considered to act estrogen-like on bone in vivo. However, their effects on bone mineral density (BMD) are weaker than that of E2. Since SERMs effects on BMSC differentiation have not been studied in vitro, we examined them using a mouse BMSC line, ST-2, that were stably transfected with an expression vector of human estrogen receptor (ER) alpha, ST2ERalpha, or of ERbeta, ST2ERbeta. Osteoblastic differentiation was assessed by measuring alkaline phosphatase (ALP) activity in response to bone morphogenetic protein-2 (BMP-2), whereas adipocytic differentiation was evaluated by oil red-O staining in response to troglitazone. As reported, 1 nM E2 enhanced BMP-2 stimulation of ALP activity, whereas both RAL and TAM dose-dependently decreased BMP-2 induced ALP activity. Furthermore, RAL or TAM at 100 nM completely abolished E2 (1 nM) enhancement of ALP activity. In contrast, both RAL and TAM, like E2, dose-dependently decreased the number of oil red-O-positive adipocytes, which were reversed with ICI182780. There were no differences in the effects of SERMs between ST2ERalpha and ST2ERbeta. These results indicate that RAL and TAM act as anti-estrogen on osteoblastic differentiation whereas act like estrogen on adipocytic differentiation. These distinctive effects of SERMs on BMSC differentiation may in part account for their apparently weaker effects on BMD compared to E2.

P347-Mo

Minipig Bone Cells for Bone Tissue-Engineering Purposes

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Jawbone augmentation is usually performed using autogenous bone. Autogenous bone contains cells with an osteogenic potential and serves as a scaffold with osteoconductive properties. However, donor site morbidity may be an additional outcome of the harvesting. Bone substitutes are cell-free and mainly osteoconductive. Bone tissue engineering, cultivating cells from a minor autogenous bone sample in combination with a matrix material, could be the solution to the morbidity problems.

The purpose of the study was to establish a method to cultivate minipig bone cells in combination with a bovine bone substitute.

In general anaesthesia, trabecular bone chips were harvested from the sternal bone in 8 minipigs. Cell cultures were established under conventional conditions. Suspension with cells was transferred to particles of a bovine bone substitute (Bio Oss) after the second passage and cultured for further 4 weeks. Particle surface and presence of cells were evaluated by scanning electron microscopy.

Results 1: Almost no cells could be seen on scanning electron microscopy images. A single swollen and dirty

osteoblast could occasionally be noted. The surface of the bone substitute looked dirty too.
The study was repeated with the following changes: instead of trypsinating the cells after the second passage, a cell scraper was used to bring the cells into suspension and, the bone substitute particles were rinsed extensively in medium before the suspension was transferred.
Results 2: Scanning electron microscopy after 4 weeks of cultivation showed multiple cell layers on the particles. Transmission electron microscopy after 4 weeks revealed accumulation of mature collagen fibrils in the intracellular and extracellular spaces and showed multi layered rough endoplasmic reticulum as well as mitochondria-rich cells surrounded by dense extracellular matrix. Biochemical analysis revealed formation of cross-links of mature collagen. Thus, it is possible to obtain mature osteoblasts from minipig bone marrow. Further, it is shown that it is possible to get minipig osteoblasts to adhere to a bovine bone substitute and produce collagen fibres that are deposit onto the bone substitute. However, the success of the procedure is highly dependent on the method used, and it seems important to avoid trypsin and to ensure that the surface of the matrix material is as clean as possible before seeding the bone cells.

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Differential Changes of Function and Gene Expression of Bone Marrow Stromal Cells After Extracorporeal Shock Wave Application (SWA)
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Despite the successful use of extracorporeal shock waves in the treatment of delayed fracture unions and nonunions, the biological mechanism of its osteogenic effect is still unclear. Knowledge about the direct effects of SWA on single cell types is limited. Thus, there is considerable debate as to its appropriate usage and efficacy. Our objective was to examine the dose-dependent alteration growth and gene expression of BMSC after in-vitro application of ESWT.
Materials and methods: Cultures of BMSC were isolated from seven patients undergoing reconstructive surgery of bone nonunions. BMSC were treated with 500 SW-impulses of two energy doses 0.06 and 0.5 mJ/mm^2 and cultured for a further period of 24 and 96 h. Cell proliferation was measured in order to detect SWA-associated changes of cell growth using the MTT-Assay. Osteoblastic differentiation of BMSC was measured using a quantitative alkaline phosphatase (AP) assay. Microarray analysis was performed to detect global gene expression changes by SWA (Affymetrix Inc). Significant results of differential expression were confirmed by semiquantitative PCR.
Results: BMSC showed a dose-dependent increase of proliferation rates by 168.7% (0.06 mJ/mm^2, $P = 0.002$)-181.6% (0.5 mJ/mm^2, $P = 0.001$). AP activity was increased by 24,3% (0.06 mJ/mm^2, $P < 0.015$) and 34% (0.06 mJ/mm^2, $P < 0.001$). Expression of fourteen different genes was found to be most significantly influenced by ESWT (peptidases, transcription factors, transporters, at least 2-fold expression, $P > 0.001$). Particularly, two novel markers, which has not been previously described in bone metabolism, were highly activated after SWA: (1) Asporin: a member of the SLRPs (small leucin rich protein), which may play a role in cellular growth control, as described by ability of different growth factors and cytokines to modulate its expression. (2) Calmegin, a calcium binding chaperon with a 54% homology to calnexin; its expression has been exclusively described in germ cells.
Discussion: Our results indicate that SWA does directly induce the proliferation, osteoblastic differentiation, and gene expression of BMSC. We could provide additional information about SWA-associated changes in gene expression of specific factors, which could explain the underlying osteoinductive effects of shock wave treatment. Further studies will help to understand the function of these factors, and also to find the appropriate energy dose and treatment regimens needed for the clinical practice.

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(−)-Epigallocatechin-3-Gallate (EGCG) Enhances Osteogenic Differentiation in a Murine Bone Marrow Mesenchymal Stem Cell Line
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Green tea was reported to possess antioxidant, antitumorigenic, antibacterial activity and regulate endocrine system. Previous epidemiological studies found that the bone mineral density (BMD) of post-menopausal women with a habit of tea drinking was higher than that of the women without habitual tea consumption. However, the effects of green tea catechins on osteogenic function have rarely been investigated. In this study, we tested (−)-epigallocatechin-3-gallate (EGCG), one of the green tea catechins, 1 μmol/L and 10 μmol/L, on the mRNA expressions of relevant osteogenic markers, protein secretion, alkaline phosphatase (ALP) activity, mineralization, incorporation of [H3]-thymidine and TUNEL stain. In a murine bone marrow mesenchymal stem cell line, D1, core binding factors a1 (Cbfa1/Runx2), osterix, osteocalcin (OC), ALP exhibit increases in their respective mRNA expression upon 48 h of EGCG treatment. Increased OC secretion was detected by ELISA. ALP activities were also significantly augmented upon an EGCG treatment of 4 to 14 days. Furthermore, mineralizations assayed by Alizarin Red S and von Kossa

stain were enhanced after EGCG treatment for 2 to 4 weeks in D1 cell cultures. However, there was no obvious cytotoxic effect of EGCG by flow cytometry. On the contrary, incorporation of [H3]-thymidine showed that there were mild inhibitory effects in cell proliferations by EGCG. The results demonstrated that EGCG increases the expressions of osteogenic genes and OC secretion, elevates ALP activity and eventually stimulates mineralization without obvious toxicity. This finding suggests that EGCG induces mesenchymal stem cells toward osteoblastic differentiation without increasing proliferation may be one of the mechanisms that allow tea drinkers to possess higher BMD.

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Osteogenically Induced Bone Marrow Stem Cells Heal Mouse Craniofacial Bone Wounds

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To study cell differentiation of bone marrow stem cells (BMSCs) into osteoblastic cells and their roles in bone tissue repairing and regeneration, we used a mouse calvarial wound model. We also tested the feasibility of monitoring transplanted cell migration and behavior in wound sites. We collected BMSCs from femurs and tibia of transgenic mice in which a luciferase reporter gene was linked to a 9.0-kb murine bone sialoprotein (BSP) promoter. The cells were subsequently expanded ex vivo in an osteoinducing medium. The BMSCs were then seeded into type I collagen spongy or silk matrix scaffolds and transplanted into calvarial wounds (4 mm in diameter) or mandibular defects (2 × 2 mm) of 8-week-old nude mice. Control groups included matrix only or empty defects left untreated. The wound healing process was followed by radiography and an IVIS imaging system. The animals were sacrificed 5 and 8 weeks after surgery, respectively, and the harvested bone tissues were subjected to immunohistochemical analysis. Each group contained five (5) mice. X-ray films showed radiopaque image in the defect area in the animals in the cell transplantation group. There was bone formation in the matrix only group but the formed bone was less radiographically intense. The bone wounds were still widely open which was seen in the untreated group. Fluorescence was detected when the tissue specimens were examined in a Xenogen imager indicating an expression of the luciferase in the transplanted BMSCs. This also suggested the osteogenic differentiation of BMSCs into bone forming cells. Consistent with these findings, immunohistochemical study using antibodies against luciferase demonstrated a strong staining in the cells incorporated in the silk and collagen matrix scaffolds. We thus concluded that the BMSCs are capable in healing craniofacial bone defects. Transplanted BMSCs from BSP and luciferase transgenic mice can be identified and traced in a bone tissue-engineering model. Type I collagen and silk scaffolds are excellent matrix materials for accommodating BMSCs.

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Osteoprogenitor Regulation by Oxygen Tension, Hepatocyte Growth Factor and 1,25-Dihydroxyvitamin D

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Hepatocyte growth factor (HGF) and 1,25-dihydroxyvitamin D (1,25OHD) act cooperatively to regulate cell proliferation. While 1,25OHD usually slows growth, HGF stimulates proliferation. Recently, we isolated a unique subpopulation of cells from human bone marrow capable of differentiating in vitro into functionally mature cells derived from all three germ layers. We named these cells marrow-isolated adult multilineage inducible (MIAMI) cells. Here, we report the role that oxygen tension plays in regulating osteoprogenitor differentiation in concert with the effects of HGF and 1,25OHD.

Although ambient air contains 21% oxygen, the physiologic oxygen level in the body is much lower-from 1% in cartilage and bone marrow to 10–12% in arteries, lungs and liver. Cells are routinely isolated from bone marrow and cultured in incubators in 21% oxygen. Since the oxygen level in the bone marrow is much lower (1–7%), we report here on studies of MIAMI cells under more physiologic conditions. The number of cells obtained with 3% oxygen was consistently higher (3- to 6-fold) independent of age or gender (3–59 years old, male and female) after 7–15 days. Alkaline phosphatase (AP) activity was significantly lower in cells exposed to 3% oxygen compared to cells grown in air after 7–20 days. Biomineralization was not seen in long-term cultures with 3% oxygen even using osteogenic conditions.

HGF treatment of MIAMI cells in air (21% oxygen) for 7–10 days significantly increased cell proliferation, with no effect on AP activity. 1,25OHD treatment under similar conditions significantly inhibited proliferation. Although AP activity was stimulated 7-fold by 1,25OHD, no mineralization was seen. HGF together with 1,25OHD increased both cell proliferation and AP activity, and mineralized nodules were seen after 18 days. MIAMI cells grown under low oxygen (3%) showed increased expression of embryonic stem cells markers (OCT-4 and REX-1), even under conditions that promote osteoblastic differentiation.

Real Time quantitative RT-PCR showed that osteocalcin expression (a marker of more mature, differentiated osteogenic cells) is significantly reduced in MIAMI cells grown under 3% oxygen even in the presence of 1,25OHD or HGF/1,25OHD combined (which in air induces the cells to differentiate).

Our data are consistent with in vivo physiological conditions where MIAMI cells in the marrow miroenvironment are at low oxygen, while osteoblasts, closer to blood vessels are thus exposed to increased oxygen.

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The Level of Adipogenesis Occurring During Osteoblast Differentiation of Human Bone Marrow Cells can be Controlled by Cultivation Protocols

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During implantation, a complex interaction between different human bone marrow derived cells (HBMC) and the surface of the bone implant takes place. HBMC-derived mesenchymal stem cells (MSCs) are capable of self-renewal and have the potential to differentiate into cells like, chondrocytes, adipocytes (AC), fibroblasts and osteoblasts (OB). The differentiation into OB is crucial for implant osteointegration. Because of that HBMCs are commonly used for studying cell–material interactions. Also, the existence of such multipotent cells has opened the field of tissue engineering of autologous bone or to support bone regeneration in case of bone repair procedures. For this, adherent HBMCs are isolated, expanded in culture and subsequently brought to differentiation by the addition of specific agents. Generally, first-passage preparations HBMCs from donors vary between individuals in terms of content of MSCs and the ability to differentiate into mature OB upon stimulation. Usually, osteogenic medium is characterized by the presence of fetal bovine serum, beta-glycerol-phosphate, ascorbic acid-2-phosphate, 1,25 $(OH)_2$ vitamin D_3 and dexamethasone. In the presence of these supplements, the cells acquire an OB morphology with up-regulation of alkaline phosphatase (ALP) activity and deposition of a Ca-rich mineralised extracellular matrix. The occurrence of ACs may, however, negatively affect such differentiation-so it is important to know which factors are crucial for the induction of AC and OB.

In the present study, the fate of HBMCs under varied OB differentiation conditions with a focus on the occurrence of AC- and OB-like cells was evaluated. In first experiments, it was observed that the amount of AC (Oil Red O-positive) and OB (bone-specific ALP-positive)-like cells are dependent on the cell density as well as the time point of addition of OB differentiation stimuli. When after an expansion period all agents are simultaneously added to HBMCs, AC were found to appear 7 days thereafter. After 14 days – a time point at which mineralization can be expected to be seen via staining of Ca deposition with Alizarin Red S – the cultures were often completely overgrown by AC. Adipogenesis is much delayed if not inhibited completely when beta-glycerol-phosphate is omitted from the osteogenic medium for the initial 14 days of the differentiation period and only added thereafter to serve as phosphate source.

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Serum Levels of Cathepsin K Decrease with Age Both in Women and in Men

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Backgound: Cathepsin K is a cysteine protease which plays an important role in degradation of the organic matrix of bone. Since bone resorption increases with age, the aim of this investigation was to evaluate potential age- and gender-related changes of serum levels of cathepsin K.
Methods: 25 young premenopausal healthy females, 24 young healthy males, 26 elderly females (age: 85 ± 1 years), and 25 elderly males (age: 83 ± 1 years) took part in this study. Serum levels of cathepsin K were measured by a recently developed enzyme immunoassay.
Results: Elderly women and men had significantly lower cathepsin K values than their young counterparts (women: 3.7 ± 0.4 pg/ml vs. 10.8 ± 2.3 pg/ml, $P < 0.001$; men: 5.1 ± 0.8 pg/ml vs. 12.3 ± 2.7 pg/ml, $P < 0.01$). In women as well as in men, a negative correlation existed between serum levels of cathepsin K and age. In men, there was a statistically significant negative correlation between the serum levels of cathepsin K on one side and those of osteoprotegerin and the bone resorption marker CTx on the other side. No association between the serum levels of cathepsin K and bone-specific alkaline phosphatase, osteocalcin, or 25 hydroxy-vitamin D levels could be detected.
Conclusion: Despite the age-related increase of bone resorption, this study shows lower cathepsin K values in elderly women and men than in young subjects. It is tempting to speculate that another enzyme could compensate for the decline of cathepsin K during old age.
Keywords: bone metabolism, cathepsin K, age, gender.

P354-Tu

Involvement of the Urokinase Receptor in Bone Homeostasis

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The plasminogen activator system (PAS) is an intricate system of serine proteases, protease inhibitors, and protease receptors. In particular, urokinase receptor (uPAR) is actively involved in the regulation of important cell functions like adhesion and migration and it has been

shown to interact with integrins. PAS is also implicated in the pathogenesis of a remarkable array of important human degenerative diseases, tumor dissemination, vessel wall diseases, and rheumatoid arthritis. The skeleton is particularly rich in extracellular matrix, and remodeling is central to skeletal physiology throughout life. Furthermore, it has been previously shown that the major players of bone remodeling, osteoblasts (Obs) and osteoclasts (Ocs) produce urokinase (uPA) and express its receptor (uPAR).

The purpose of this study was to investigate the role of uPAR in bone remodeling. We analyzed the bone phenotype in uPAR-null female mice. Morphological analysis of the skeleton showed a significant decrease in tibia length in mutant mice as compared to Wt. Bone mass analysis of uPAR Ko by peripheral quantitative computed tomography (pQCT), revealed an increase in bone mass with respect to wild type ($P < 0.02$). The histomorphometric analysis of the tibia in uPAR Ko showed a reduction in the bone volume compared to wild type (more bone density but less bone volume). Moreover, mechanical tests showed a reduction in the capability to sustain a given load in uPAR Ko tibias as compared to tibias from wild type female animals ($P < 0.02$). Moreover, ovariectomy induced a higher rate of bone loss in Ko mice (35%) compared to wild type animals (15%).

To explore the cellular basis of the bone defect in uPAR Ko mice, we cultured osteoblasts in vitro. The results showed a proliferative advantage and a higher susceptibility to matrix mineralization in uPAR Ko as compared to Wt cells. The production of proteins involved in the mineralization process, like alkaline phosphatase (ALP), was increased only during the first weeks of osteoblasts maturation. After 4 weeks, ALP was similar in both genotypes. On the contrary the number of Osteoclasts formed in vitro using uPAR Ko monocytes was decreased compared to wild-type. Together, our preliminary data indicate that uPAR may play an important role in bone physiology and in estrogen-dependent bone loss.

P355-Su

Serum Cathepsin K: A Novel Bone Resorption Marker in the Evaluation of Postmenopausal Women Treated with Alendronate

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Cathepsin k is a member of the cysteine protease family that plays a critical role in osteoclast function and the degradation of protein components of the bone matrix. This protease cleaves both helicoidal and telopeptide regions of collagen type I, the major type of collagen in bone. Postmenopausal osteoporosis is characterized by an increase of bone resorption, resulting in a high turnover state that may be identified by measurements of biochemical markers. Alendronate therapy induces sharply reductions in bone remodelling but there is no data on serum Cathepsin K changes after this treatment.

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 months of treatment with alendronate and a control group of healthy premenopausal women.

Patients and methods: We selected 30 patients (64 ± 7 years) with densitometric criteria of osteoporosis (T-score <2.5 SD) that started alendronate treatment (70 mg/week). Serum samples were obtained at baseline and after 3 months for measurements of biochemical markers of bone remodelling. Serum cathepsin K levels were measured by ELISA (Biomedica Medizinprodukte GhbH and Co KG Wien, Austria). The reference range was 0–300 pmol/l. The detection limit was 1.1 pmol/l, and the intra and inter-assay coefficients of variation (CV) were 4% and 6%, respectively. The control group consisted of 12 nonselected healthy premenopausal women (26 ± 3 years).

Results: We found increased levels of cathepsin K compared with the premenopausal control group ($P < 0.01$). After 3 months of alendronate treatment, cathepsin K decreased significantly ($P < 0.001$). Baseline and 3 months levels were significantly correlated (r: 0.69; $P < 0.0001$). We not found correlation between cathepsin levels, age or years since menopause. Baseline concentrations of cathepsin K and tartrate-resistant acid phosphatase (TRAC) were significantly correlated (r: 0.35; $P < 0.045$).

Conclusions: Our data suggest that serum cathepsin K measurements seem to be a valuable parameter in the evaluation of women with osteoporosis treated with antiresorptives.

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Expression of RAB Genes in Bone Resorbing Human Osteoclasts

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Rab proteins constitute the largest subfamily of small GTPases, since human genome contains at least 60 different RAB genes. Individual Rab proteins are assigned to distinct intracellular compartments where they regulate transport between organelles by cycling between GTP-bound (active) and GDP-bound (inactive) state. This cycling offers temporal and spatial regulation to membrane transport. Bone-resorbing osteoclasts are multinucleated highly polarized cells that have four functionally distinct membrane domains: sealing zone (SZ), ruffled border (RB), functional secretory domain (FSD) and basolateral membrane. During resorption, bone degradation products are endocytosed at RB area and transcytosed to FSD to be released into extracellular fluid. SZ is an organelle free area through which osteoclasts are attached tightly to bone surface. Maintaining the polarity and

orchestrating the endocytotic and exocytotic vesicle movements is essential for osteoclast function. We have shown earlier that Rab7 is an important regulator of RB formation. In order to find out what Rab-GTPases are responsible for the other specific trafficking events, we have started to catalogue changes in the expression of various RAB genes during osteoclast differentiation and function. We studied mRNA levels of different RABs with gene-specific primer pairs as well as using oligoarrays. In resorbing human osteoclasts, we found 28 out of 43 tested RAB genes to be expressed whereas 15 were absent. The expression levels of some these RAB genes were further quantified during the human osteoclast differentiation from peripheral blood CD 14-positive monocytic cells. Rab13 and Rab32 expression levels were found to be highly induced in mature osteoclasts, expression increasing 26- and 2.5-fold, respectively. Strong upregulation of these two Rab proteins in osteoclasts during their differentiation and activation suggests a functional role for them in the resorption process. Preliminary immunolocalization of Rab13 suggests that it is possibly involved in the regulation of tight attachment of the cell to the bone surface via SZ area. More studies at protein level are needed to verify the role of the identified Rab proteins in osteoclast function.

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Transmigration, A New Property of Mature Osteoclasts, is Dependent on c-Src, MMPs and is Regulated by Risedronate

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Osteolytic metastases predominate in breast cancer and during their establishment, histologic analysis and scanning electron microscopy indicate that bone destruction is mediated by an excessive number of osteoclasts (OC) rather than by tumour cells. Moreover, mature OC are found in close contact to bone but whether they form on the bone surface or in its microenvironment is still a matter of discussion. It is assumed that multinucleated OC are found adherent onto bone matrix but in many instances they can be found in the bone microenvironment. OC actin organisation is characterised by the formation of unique actin structures namely podosomes when adherent onto plastic or glass substrates, whereas they form a sealing zone when adherent on apatite mineralized substrates. Podosomes which are characterised by a core of polymerized actin surrounded by proteins such as vinculin have been also found in malignant cells bringing a lot of interest in their putative role in tissue invasion. In this context, our working hypothesis was that OC might possess invasive properties. By confocal microscopy analysis and using Z cut sections extracted from stacks of confocal images, we report that multinucleated mature OC (from murine spleen cells or from a macrophage RAW-GFP cell line constitutively expressing GFP) can transmigrate through multilayers of various cell types including pre-osteoblast, endothelial or adipocyte cell lines. This transmigration is a very efficient process taking place in few hours and is restricted to mature osteoclasts. Moreover, our analysis has shown that this transmigration is more efficient than metastatic cells such as MDA. Interestingly, we did not see any podosome structures upon cell–cell contact (mature OC-Osteoblast) suggesting that they are not involved in that process. On the other side, we found that family members of c-Src and metalloprotease (MMPs) are involved in mature OC transmigration as treatment by PP2 and GM6001 respectively reduced that process in vitro. Whether newly formed OC use this property to migrate from the micro-environment towards the bone surface remains to be further investigated. In addition, we show that an amino-biphosphonate (risedronate) can also block in a dose-dependent manner that transmigration process suggesting that action of BPs on OC could also help to prevent the migration of OC in the vicinity of the tumour decreasing, therefore, bone destruction in bone metastasis.

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Constitutive Expression of *Sparus aurata* Osteocalcin in a Chondrocytic-Like (VSA13) and Osteoblastic-Like Cell Line (VSA16): Effect on Mineralization Rate and Levels of Calcification-Related Gene Expression

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Two cell lines, VSa13 and VSa16, derived from vertebra of the gilthead sea bream, *Sparus aurata*, and capable of mineralizing their extracellular matrix, were recently developed in our laboratory (Pombinho et. al., 2004. Cell Tissue Res 315:393). VSa13 cells were found to be similar to chondrocytes and to express matrix Gla protein (MGP) under normal growth conditions, whereas VSa16 cells were found to be similar to osteoblasts and to express osteocalcin/bone Gla protein (BGP) under mineralizing conditions.

We have developed four clones using each cell line for stable transfection experiments in which we have either over/force-expressed BGP (VSa16/VSa13 cell line, respectively) generating clones which constitutively express BGP under the cytomegalovirus immediate-early promoter (CMV). In this work, we describe for each clone, and in relation with the mineralization status, the effect observed in (i) mineralization rate, (ii) gene expression of Sparus aurata calcification-related genes. Our data indicate that genes expressed by each of the two stably transfected cell lines differ in response to mineralization status and levels of BGP expression. Ongoing experiments aim at understanding the regulatory pathways involved in BGP-induced variation in gene expression in VSa13 and VSa16 cell lines.

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Gene Expression of Monocytes after Exposure to Metal Ions

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Total hip joint replacement is a widely used and highly beneficial orthopaedic procedure. Despite its wide use, loosening of the implants is a frequent complication. Different processes, such as the corrosion of metal ions, may affect the longevity of the implant. In the present study, we investigated the changes in the gene expression and the release of cytokines by monocytes treated with metal salts.
Low-density arrays (LDA) provide a method allowing for the investigation of up to 384 genes simultaneously by real-time PCR. We used LDA to investigate changes in gene expression of human peripheral blood monocytes treated with metal salts [$TiCl_3$; $CoSO_4$; $NiCl_2$] at a concentration of 0.1 mM for 18, 40, and 64 h at 37°C. The LDA was composed of 96 genes, including endogenous control genes, cytokines, transcription factors, and enzymes, which may be involved in osteoclastogenesis. The strongest effect on transcription levels was observed after treatment of the monocytes with Co^{2+} and Ni^{2+}, while the reaction of the cells on Ti^{3+} was weaker. The transcriptional regulation of the majority of genes affected by the exposure of monocytes to Co^{2+} and Ni^{2+} was similar. Some genes critically involved in the processes of inflammation and bone resorption, however, were found to be differentially regulated by these bivalent cations. CSF-1 was exclusively upregulated by Co^{2+}, while the upregulation of PTGS2 and VEGF was stronger in cells exposed to Ni^{2+}. The expression of transcripts encoding the osteoclastogenic cytokines IL-1, IL-6, and IL-8 was increased by treatment of the monocytes with either $Co^{2+}$and Ni^{2+}. In contrast, the levels of mRNA encoding TNF-α were unchanged in most samples. Furthermore, the release of TNF-α, IL-1-α, IL-1β, IL-6, and IL-8 in the supernatants of monocyte cultures was determined by ELISA. The levels of TNF-α, IL-1, IL-6, and IL-8 were increased in the cell supernatants after treatment with $Co^{2+}$and Ni^{2+}, while Ti^{3+} had virtually no effect on the cytokine release.
The data demonstrate that exposure of monocytes to Co^{2+} or Ni^{2+} induces a profound change in gene expression, leading to the release of factors that may eventually contribute to the haematopoietic microenvironment regulating the recruitment and activation of osteoclasts. This suggests that the reaction of monocytes to metal ions may contribute to the focal resorption of periimplant bone.

P360-Tu

RANKL Acts Upstream of Caspases to Prevent Bisphosphonate-Induced Osteoclast Apoptosis in Vitro

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Bisphosphonates can cause osteoclast apoptosis, an effect that contributes to their ability to inhibit bone resorption. Alendronate (ALN) causes osteoclast apoptosis by inhibiting FPP synthase and causing the loss of prenylated small GTPases, whereas clodronate (CLO) causes apoptosis due to the intracellular accumulation of the cytotoxic metabolite $AppCCl_2p$. Since RANKL promotes osteoclast survival in vitro, we examined whether RANKL could also suppress apoptosis of osteoclasts induced by bisphosphonates, and whether this affected the ability of bisphosphonates to inhibit bone resorption in vitro.
Osteoclasts were isolated from the long bones of 2–3 day old rabbits and seeded onto ivory discs, or into multiwell plates before purification using pronase/EDTA. These cultures were then treated for 48 h with 100 μM ALN or CLO in the absence or presence of 50 or 100 ng/ml RANKL. RANKL alone did not significantly stimulate osteoclast resorption (quantified by reflective light microscopy) or alter the number of adherent or apoptotic osteoclasts. It also had no effect on the ability of ALN to inhibit protein prenylation (determined by the accumulation of unprenylated RAP1A). The number of adherent osteoclasts in culture dishes was reduced to ~52% and ~57% of control cultures after treatment with 100 μM ALN or CLO, respectively. This was increased to ~77% and ~85% of control cultures in the presence of RANKL ($P < 0.01$). In addition, ~17% of adherent osteoclasts were apoptotic after bisphosphonate treatment, which was reduced to 9% in the presence of RANKL ($P < 0.001$). RANKL also caused a reduction in cells with active caspase-9, from 30% to 18% after ALN treatment, determined using the Apofluor green single-cell caspase assay. However, it was not associated with a change in the level of caspase inhibitory proteins cIAP1, cIAP2 and XIAP, determined by Western blotting.
These observations demonstrate that RANKL suppresses bisphosphonate-induced osteoclast apoptosis by mechanisms that prevent activation of the caspase cascade but that occur independently of changes in IAP proteins. These observations may be of relevance when considering the effectiveness of bisphosphonates in the treatment of inflammatory diseases such as rheumatoid arthritis that involve increased levels of RANKL.

P361-Su

Tumor Necrosis Factor-Alpha and Vitamin D Together Inhibit the Development of Osteoclasts in Vitro

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TNFalpha is a major mediator of inflammatory processes and a potent stimulator of bone resorption. The cytokine was suggested to be involved in bone loss associated with rheumatoid and degenerative diseases of the skeleton. Within the present study, the effects of TNFalpha on the development of osteoclasts in vitro in combination with

osteoclastogenic agents such as $1,25(OH)_2D_3$, PTH, and PGE_2 was investigated. For this purpose, cocultures of primary murine calvaria osteoblasts (ob) from *wt* and p55 TNFalpha receptor-deficient mice, and *wt* bone marrow cells (BMC) were grown in the presence of $1,25(OH)_2D_3$ (10^{-8} M), PTH (10^{-8} M), PGE_2 (10^{-7} M) and Dex (10^{-8} M). Conditioned media (CM) from *wt* and $p55^{-/-}$ ob were prepared by treating the cells with the factors and collecting the culture supernatants after 72 h. In cocultures of *wt* ob and BMC (+$1,25(OH)_2D_3$), TNFalpha inhibited the development of osteoclasts at concentrations of 0.1 ng/ml and 1.0 ng/ml. In the absence of $1,25(OH)_2D_3$, TNFalpha induced low levels of osteoclast development at 5 ng/ml, but failed to do so efficiently at 10 and 20 ng/ml, due to cytotoxic effects on ob. In cocultures of $p55^{-/-}$ ob and *wt* BMC, TNFalpha induced the development of osteoclasts both in the presence and absence of $1,25(OH)_2D_3$ at concentrations of 1 ng/ml up to 20 ng/ml. Furthermore, the inhibition of osteoclastogenesis was mediated through CM from *wt* ob treated with $1,25(OH)_2D_3$ and TNFalpha (1 ng/ml), while CM from *wt* ob treated with TNFalpha in the absence of $1,25(OH)_2D_3$ or from $p55^{-/-}$ ob (± $1,25(OH)_2D_3$) failed to block this process. Cultures with inactivating antibodies against TNFalpha demonstrated that the observed effect of the CM was independent of the cytokine, while the effect was blocked when the antibodies were added together with TNFalpha to the cocultures and to the ob during the conditioning phase. CM from ob treated simultaneously with Dex, $1,25(OH)_2D_3$ and TNFalpha, did not inhibit the development of osteoclasts. The data demonstrate that TNFalpha, through binding to the TNFp55R, in the presence of $1,25(OH)_2D_3$, induces ob to release an inhibitor of osteoclastogenesis, while stimulating the same process simultaneously through a direct action on osteoclast lineage cells in the bone marrow. PTH and PGE_2 could not substitute for $1,25(OH)_2D_3$, while Dex, probably through its action on IkappaB, counteracts the TNFalpha effect. TNFalpha therefore exerts dual effects on osteoclastogenesis and may therefore not be considered solely as a stimulator of bone resorption.

P362-Mo

Rheumatoid and Crystal Arthritic Synovial Fibroblasts Induce Osteoclast Formation

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Receptor Activator of Nuclear Kβ Ligand (RANKL) is expressed by synovial fibroblasts in pathological joint conditions such as rheumatoid arthritis (RA) where there is formation of marginal erosions. In this study, we have determined whether synovial fibroblasts in other forms of arthritis, i.e., pyrophosphate arthropathy (PPA) and osteoarthritis (OA) also express RANKL and examine the mechanism whereby these cells support osteoclast formation. Immunohistochemistry of the synovium from OA, RA and PPA joints showed RANKL and OPG expression. Isolated synovial fibroblasts cultured from RA, OA, and PPA joints expressing the fibroblast markers vimentin and proline hydroxylase were analysed by Western blotting and showed expression of RANKL and osteoprotegerin (OPG) a decoy receptor for RANKL, but not tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) which binds OPG. Increased RANKL expression was detected in fibroblasts isolated from RA and PPA whereas RANKL was only weakly expressed in OA cases. Conditioned medium of cultured OA, RA and PPA synovial fibroblasts induced osteoclast formation from normal human peripheral blood monocytes (PBMC) in the presence of M-CSF, and OPG. Osteoclast differentiation was determined by the expression of specific osteoclast markers TRAP, VNR, F-actin ring formation and evidence of lacunar resorption. As synovial fibroblasts do not express TRAIL, and OPG does not inhibit osteoclast formation, our studies indicate that synovial fibroblasts in RA/Crystal arthritis may induce osteoclast formation through a RANKL independent signalling pathway.

P363-Tu

The Chemokine CCL-9 is Highly Expressed by Differentiating Osteoclasts in Vivo and Promotes Osteoclastogenesis in Vitro

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In the *toothless* (*tl*) rat mutation, a frameshift mutation in the *Csf-1* gene prevents the differentiation of osteoclasts, resulting in severe osteoclast deficiency and osteopetrosis. Undifferentiated mononuclear precursor cells remain capable of becoming mature osteoclasts, as evidenced by their rapid differentiation into active osteoclasts when supplied with exogenous CSF-1. Thus, the *tl* rat provides a powerful system to identify changes in gene expression during osteoclast formation in vivo. We exploited this system to perform microarray analyses of long bone RNA in *tl* rats following CSF-1 treatments. Three-week-old rats were injected daily and long bones were collected at 2, 4, and 6 days, a time that covers a rapid and robust increase in osteoclast number and activity. One gene, the chemokine CCL-9 (also called MIP-γ), was strongly and rapidly up-regulated, especially during the first 2 days when osteoclast differentiation is in its early stages. The peak of CCL-9 mRNA expression is earlier than other osteoclast-specific markers, such as tartrate-resistant acid phosphatase, suggesting an important role for CCL-9 in early events, for example, chemotaxis and fusion of precursor

cells. In situ hybridization and immunohistochemistry were used to establish that CCL-9 is highly expressed by osteoclasts in vivo. Despite low CCL-9 mRNA levels in tl rats prior to injections, immunohistochemistry revealed significant CCL-9 protein levels in a small population of cells within the bone. Consistent with earlier reports of CCL-9 expression and a role for it in mouse osteoclast differentiation in cell cultures, we could inhibit the differentiation of primary rat osteoclasts from bone marrow precursors with anti-CCL-9 antibodies. Together, these studies demonstrate that CCL-9 is strongly expressed in vivo at very early stages of osteoclastogenesis in the rat, and that it plays an important role in steps required for fusion of mononuclear precursors.
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P364-Su
Characterization of the Grey-Lethal Protein and its Role in Osteoclast Maturation and Activation
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Bone homeostasis relies on a tightly regulated equilibrium of bone formation by the osteoblast, a mesenchymal derived cell and bone matrix resorption by the osteoclast, a multi-nucleated cell of hematopoietic origin. Malignant autosomal recessive osteopetrosis is caused by lack of bone resorption and has been associated with a defect in osteoclast. We are studying the grey-lethal (gl) mouse mutant, the most relevant animal model of malignant recessive osteopetrosis. We have recently identified the gl gene that encodes a novel 38-kDa protein. The relevance and importance of the gl gene in human has been demonstrated in osteopetrotic patients carrying mutation in the human Gl gene. The mutation affecting the gl gene is a deletion in the promotor region that abolish both mRNA and protein expression. The osteoclasts from the gl/gl mouse are unable to resorb bone due to a defect in the activation of the cell, reflecting the essential role of the gl gene in this pathology. Cellular analysis of osteoclasts from the gl/gl mice shows a defect in cytoskeletal rearrangement and ruffled border formation preventing resorption of the bone matrix. Therefore, the Gl protein is essential for proper maturation and activation of osteoclasts. The Gl protein (338 aa) has a potential signal peptide and a putative transmembrane domain. This protein structure suggests that Gl is part of the osteoclast vesicular compartment and participates in protein trafficking in association with cytoskeletal rearrangement and exocytosis. To characterize and define the function of the Gl protein in the osteoclast, we aim to define its localisation in the osteoclast and establish its specific interactions with partner proteins. We demonstrated that the Gl protein was highly glycosylated and is localized within the osteoclast cytosolic membrane compartment. In addition, several potential Gl partners were isolated in a yeast two-hybrid screen and we are in the process to confirm these interactions in mammalian cells. In conclusion, these complementary approaches will help define the role of the novel Gl protein in osteoclast maturation and activation in association with specific interacting partners.

P365-Mo
Abstract Withdrawn

P366-Tu
Effect of PPAR Agonists, on Human Osteoclast Formation and Activity in Vitro
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Fibrates and statins are used to treat hyperlipidemia. Epidemiology studies suggest they may also have effects on bone. Statins can inhibit osteoclastogenesis and osteoclast function through inhibition of protein prenylation. Fibrates act through 'lipid-sensing' peroxisome proliferator-activated receptors (PPARs) with three possible isoforms: PPAR-alpha, -beta and -gamma, each with specific functions. We have investigated the expression of PPAR receptors and the effects of PPAR-specific and non-specific agonists, fibrates, on osteoclastogenesis and osteoclast function. Human peripheral blood mononuclear cells (PBMCs) were stimulated with human recombinant RANKL and M-CSF to generate osteoclasts. RNA was extracted at days 0, 7, 14 and 21 and RT-PCR for all three PPAR receptor isoforms demonstrated their expression thought the culture period. In parallel cultures, PPAR agonists (1×10^{-9} M to 10^{-5} M) were added from the beginning of the culture till day 14, when mature osteoclasts were formed and the number of osteoclasts assessed by counting TRAP-positive cells with 3 or more nuclei. PBMCs were also grown on dentine wafers without the addition of any compounds until day 14. Once mature osteoclasts were formed, the PPAR agonists (1×10^{-9} M to 10^{-5} M) were added for 7 days and the extent of resorption was measured. Activation of all PPAR isoforms with specific agonists (ciglitizone, L165041 and GW9578) resulted in significant dose-dependent inhibition of osteoclastogenesis ($P < 0.05$). Dose-dependent inhibition of osteoclast resorption was observed with, ciglitizone, a PPAR-gamma-specific agonist ($P < 0.05$). Whereas L165041, a PPAR-beta-specific agonist, resulted in significant dose-dependent stimulation of osteoclast resorption ($P < 0.05$). GW9578, a PPAR-alpha-specific agonist, suppressed osteoclast resorption when 1×10^{-7} M was

added to the culture. Higher and lower concentrations of GW9578 gave a dose-dependent loss of suppression of osteoclast resorption. In contrast to specific PPAR agonists, bezafibrate and fenofibrate, which non-specifically activate all PPAR isoforms, had no significant effect on osteoclastogenesis and resorption activity. While pure PPAR agonists have specific effects on osteoclast generation and activity, the current available fibrates have mixed effects possibly due to stimulation of more than one PPAR isoform.

P367-Su

Apoptotic Osteocytes Support Osteoclastogenesis

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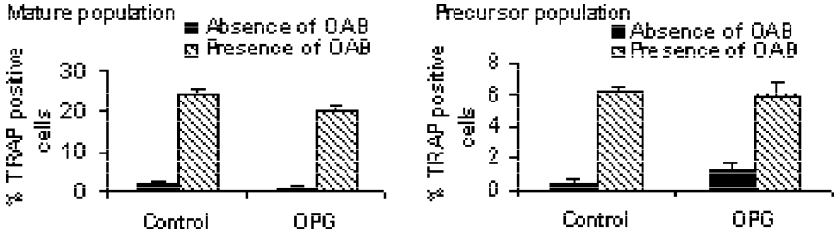
While it has been demonstrated that osteoclastic resorption occurs at sites of high osteocyte death to date no evidence supporting a causal link between the apoptotic process and osteoclastic activity has been shown. Here, we have challenged mature murine bone marrow osteoclasts and their precursor cells with MLO-Y4 osteocyte apoptotic bodies (OAB) for 72 h in vitro. In the absence of exogenous RANKL and MCSF, the percentage of TRAP-positive multinuclear cells derived from the mature osteoclast population was increased from 2 ± 0.9% in control to 5 ± 0.6% in OAB treated cultures while in the precursor population treatment with OAB increased TRAP-positive multinuclear cells to 5.1 ± 0.9% vs. 0.3 ± 0.3% in control. Osteoclastic activity was determined by the number of resorption pits and resorption area on dentine slices. Treatment with OAB resulted in a 300% and 200% increase in pit number in the mature osteoclast and precursor cell population respectively. The total resorption area was increased in the mature population on OAB treatment from 339 ± 52 μm^2 in control to 2076 ± 10 μm^2 and in the precursor cell cultures from 257 ± 47 μm^2 in control to 1664 ± 76 μm^2. Treatment with OPG did not abrogate the effects of OAB on osteoclastogenesis suggesting a RANKL independent effect (Fig. 1). Experiments involving the use of a range of neutralizing antibodies pointed to the involvement of specific cytokine delivery or production in this response. Apoptotic bodies derived from osteoblasts (both primary and cell line) did not support osteoclast differentiation pointing to the osteoclastogenic factors being specifically related to the apoptotic osteocyte. These in vitro data point to the potential for OAB to profoundly alter the bone environment in terms of resorptive capacity. The future identification of the pro-resorptive factors generated by dying osteocytes may contribute to the development of intervention strategies related to the control of deregulated bone targeting by osteocytes.



P368-Mo

Identification of the Genes Differentially Expressed in Human Osteoclasts, Dendritic Cells, Endothelial Cells and Macrophages from a Common Precursor by Microarray Analysis

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$CD14^+$ human peripheral blood mononuclear cells are cable of differentiating into several distinct cell lines. We have established an assay in which human $CD14^+$ human peripheral blood mononuclear cells (PBMC) are differentiated on plastic wells into mature osteoclasts, endothelial cells, macrophages and dendritic cells. The aim of the study was to characterize genes that are differentially expressed between these cell lines at the end of the culture period (day 9). In this study, we used a 60-mer in situ synthesized Human 1A Oligo Microarray (V2) from Agilent Technologies. The data obtained was analyzed by using Silicon Genetic's program GeneSpring 7.0. We were able to identify numerous genes differentiating studied cell lines, giving us the possibility to present "cell line gene sets" for differentiating osteoclasts, dendritic cells, endothelial cells and macrophages at the end of the culture period. At the same time, we were able to identify genes differentially expressed within mature individual cell lines, as native human $CD14^+$ PBMC were used as a common control sample in each array. Our data revealed 31 genes differentiating the cell lines in our assay. We were also able to identify the gene expressions for the known cell-specific markers and to compare these in between the cell lines. qPCR from selected genes was used to verify the microarray data and several 2-D gel protein studies were ran to further exploit the cell lines. Gene expressions for the proteins found were also separately analyzed from the array data. The data described here gives us an unique possibility to study the molecular features of each cell line and leads us towards better

understanding of molecular factors behind PBMC differentiation. Keywords: PBMC, DNA microarray, osteoclast, dendritic cell, macrophage, endothelial cell.

P369-Tu

Physiological Role of Melatonin in the Scale of Teleost

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The teleost scale is a calcified tissue that contains osteoclasts and osteoblasts similar to those found in mammalian bone. We recently developed a new in vitro assay system using fish scales. This system can detect the activities of both scale osteoclasts and osteoblasts at the same time with tartrate-resistant acid phosphatase and alkaline phosphatase as makers. Using this system, we, for the first time, demonstrated that melatonin has a suppressive function in both osteoclasts and osteoblasts in vertebrates. On the other hand, it has been reported that melatonin is produced in a number of extrapineal sites, where it could act as an intracellular mediator or paracrine signal in addition to having endocrine effects. As melatonin controls bone metabolism in the scale, we investigated the presence of melatonin and synthesizing enzymes in goldfish scales. Melatonin was identified in the scales of male and female goldfish using reversed-phase high-performance liquid chromatography coupled with fluorometric detection and a radioimmunoassay. In addition, mRNA expression of arylalkylamine N-acetyltransferase, which is the rate-limiting enzyme in the synthesis of melatonin, was found in the scales of goldfish. Therefore, we examined the seasonal change in the melatonin content in the scales of female goldfish. Scales in the dorsal, lateral, or ventral region were collected, and melatonin was measured by reversed-phase high-performance liquid chromatography. The melatonin levels in female scales exhibited significant annual variations in the dorsal ($P < 0.05$) and ventral ($P < 0.05$) regions, with higher levels in April (reproductive season). In female teleosts, estrogen enhances the synthesis of vitellogenin, which is a major component of egg protein and a calcium-binding protein. At the same time, estrogen promotes calcium resorption from the scales by activating osteoclasts. Therefore, melatonin synthesized in the scale functions to suppress osteoclastic activity for the protection of the scale from the excess degradation during vitellogenesis.

P370-Su

Sirnas Designed by Sirna_Profile to Silence Human Tartrate Resistant Acid Phosphatase

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RNA interference (RNAi) has been accepted as a highly specific and efficient gene silencing method in mammalian cells. Role of short interfering RNA (siRNA) design has been elevated in the whole RNAi methodology.

We have developed a new interactive siRNA_profile program based on thermodynamics and asymmetric properties of functional siRNAs. Our siRNA_profile program has multiple options for users to control the siRNA sequence search based on their own needs or to use the suggested parameters to find optimal antisense siRNA sequences activating RNAi pathway. Selection of the functional siRNA sequences from target mRNA is based on the differences of functional and non-functional siRNA sequences. Functional siRNAs has shown low average internal stability at the 5′ terminus and high average internal stability at the 3′ terminus, which suggested the essential role for the helicase activity choosing the strand for RNA induced silencing complex (RISC). In the energy profile, the stability for a nucleotide position is taken as the sum of stability of the pentamer toward the 3′ terminus and is calculated by using the nearest neighbor method. To prove the accuracy of our program, we used published silencing efficiencies of 180 siRNAs targeting firefly luciferase and human cyclophilin B (Reynolds, A. et al. 2004). The results show that siRNA_profile selects 7 siRNAs out of the 180 that have average target gene expression 29.8% of the control, minimum and maximum values of 1% and 125%, respectively, and median is 18%. Average target gene expression with these 7 selected siRNAs was lower than 11% (minimum 2%, maximum 30%, median 7%).

Serum TRACP has been a useful biochemical resorption marker, however TRACP has unknown biological functions in osteoclasts. Here, we have used the siRNA_profile program to design three siRNAs to silence TRACP and the aim was to study the knock-down effects in vitro human osteoclast culture. CDS of human TRACP sequence (NM_001611) was used for TRACP siRNA design. Parameters of siRNA_profile for TRACP siRNA search were chosen based on the thermodynamical recommendations. Silencing effect of TRACP siRNAs was followed in cellular phosphatase activity by using fluorescent TRACP substrate.

P371-Mo

Cytosolic Entry of Bisphosphonates into Macrophages and Osteoclasts Requires Fluid-phase Endocytosis and Endosomal Acidification

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It is now apparent that nitrogen-containing bisphosphonates (N-BPs) inhibit bone resorption by inhibiting the intracellular enzyme FPP synthase and thus preventing protein prenylation. However, the route by which N-BPs are

internalised into osteoclasts and other cells remains unclear. To address this, we synthesised a fluorescently-labelled analogue of alendronate (AF-ALN). Using confocal microscopy, we found that AF-ALN was internalised into endocytic vesicles by rabbit osteoclasts and J774 cells in vitro, and colocalised with TAMRA-dextran (a marker of fluid-phase endocytosis) but not with wheatgerm agglutinin-633 (WGA, a marker of adsorptive endocytosis) or transferrin-633 (a marker of receptor-mediated endocytosis). Furthermore, when J774 cells were treated with these agents for 1 h at 4°C then washed and incubated for 3 h at 37°C, neither AF-ALN nor dextran were internalised, whereas WGA and transferrin bound to the cell surface and were then internalised at 37°C. These observations demonstrate that initial uptake of AF-ALN occurs by fluid-phase endocytosis and does not involve binding to the cell-surface. Consistent with this, 500 μM dansylcadaverine, an inhibitor of endocytosis, reduced the uptake of AF-ALN to 51% of AF-ALN alone (quantified by flow cytometry) and prevented the inhibition of Rap1A prenylation by ALN.

Endocytosis of BPs appears to be enhanced by Ca^{2+}, since EGTA or other BPs reduced the internalisation of AF-ALN and prevented ALN-induced accumulation of unprenylated Rap1A, an effect that was reversed by addition of Ca^{2+} ions. By contrast, the monophosphonate analogue of ALN (which does not chelate Ca^{2+}) had no effect on AF-ALN uptake and did not affect inhibition of Rap1A prenylation by ALN.

To investigate the mechanism by which BPs exit endocytic vesicles and enter the cytosol, we examined the effects of 20 μM monensin (MON) and 50 nM bafilomycin A (BAF), inhibitors of endosomal acidification. Neither MON nor BAF affected vesicular uptake of AF-ALN or uptake of [^{3}H]zoledronic acid (ZOL), but completely prevented the inhibitory effect of ALN or ZOL on Rap1A prenylation in macrophages and osteoclasts.

Taken together, these results demonstrate that BPs are internalised initially by fluid-phase endocytosis in a calcium-dependent manner. Vesicular acidification is then essential for the BPs to enter the cytosol, presumably by causing protonation of the negatively-charged phosphonate groups to allow movement across the vesicular membrane.

P372-Tu

Initiation of Bone Resorption Regulated by Mechanically Damaged Osteocytes

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Bone is continuously subjected to repetitive loading, which leads to microdamages even if the strain level is within physiological range. The damaged site must be sensed and promptly removed by remodeling process; otherwise, the accumulation of microdamages can lead to clinical bone fractures. Osteocytes have been considered to provide a cellular basis for mechanosensing and bone remodeling. They are embedded deep inside mineralized bone matrix and are connected to each other via gap junctions. This structure is ideal to detect changes in local mechanical environment. However, very little is known about their role in the initiation of remodeling process, i.e., osteoclastic resorption of the damaged bone area. The aim of this study was, therefore, to demonstrate that the damaged osteocytes could induce the initiation of bone resorption. MLO-Y4 osteocyte-like cells were incubated in collagen gel for 3–5 days so that they could form three-dimensional cellular networks. In order to apply mechanical damages to the osteocytes, we established a loading apparatus in which the gel-embedded cells were subjected to cyclic stretching in wide strain range. The intermittent stretching of 10000 micro-strain with frequency of 2 Hz was applied to the gel-embedded MLO-Y4 cells for 24 h. Viability assay indicated that the stretching of 10000 micro-strain mechanically damaged the cells and significantly increased the number of dead cells in the gel. After the loading regime, supernatants of culture media were collected and mixed with fresh medium at the concentration of 20%. Bone marrow cells collected from mouse long bones were incubated with the conditioned media, and then TRAP (tartrate-resistant acid phosphatase) activity was quantified after a week of culture. As a result, the conditioned medium obtained from damaged osteocyte culture significantly increased TRAP positivity in bone marrow cells. It would demonstrate that the mechanically damaged osteocytes have a potential to promote osteoclastic cell formation, and further suggests that the local death of osteocytes provides an important mechanism to target remodeling to microfractures.

P373-Su

Risedronate and other Nitrogen-containing Bisphosphonates are Efficiently Internalised from Mineralised Surfaces by Osteoclasts, but not other Cell Types, in Vitro and in Vivo

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Bisphosphonates (BPs) target to bone due to their high affinity for calcium ions. During osteoclastic resorption, BPs are released from the acidified bone surface and internalised by osteoclasts, where they act by inhibiting the prenylation of small GTPases essential for osteoclast function. However, it remains unclear whether osteoclasts are the only cells in the bone microenvironment that can internalise BPs from the bone surface. We have begun to investigate this question using a novel, fluorescently-labelled alendronate analogue

(FL-ALN) and by examining changes in the prenylation of the small GTPase Rap1A following treatment of cells with risedronate (RIS). Bone marrow cells isolated from rabbits were cultured for 24 h on dentine slices pre-coated with FL-ALN or RIS. Confocal microscopic analysis showed that FL-ALN was avidly internalised by resorbing osteoclasts into intracellular vesicles throughout the cell. This pattern of uptake also occurred with FL-ALN in the absence of dentine. Accordingly, unprenylated Rap1A accumulated to the same extent in osteoclasts cultured on RIS-coated dentine or with RIS in solution. By contrast, J774 macrophages avidly internalised FL-ALN and RIS from solution, but took up little from the surface of dentine, due to their inability to resorb dentine. Calvarial osteoblasts and MCF-7 tumour cells internalised even less FL-ALN and RIS, both from solution and from the surface of dentine, in accordance with their reduced endocytic activity compared to macrophages. To examine uptake of BPs by bone cells in vivo, osteoclasts were isolated from rabbit bone marrow by immunomagnetic bead separation using an anti-vitronectin receptor (VNR) antibody. Prenylation of Rap1A was inhibited in the osteoclast fraction from rabbits that had been injected subcutaneously with 1 mg/kg risedronate, alendronate, ibandronate, pamidronate or zoledronic acid, but there was negligible effect on Rap1A prenylation in the general population of non-osteoclast, VNR-negative bone marrow cells. 0.3 mg/kg cerivastatin (which lacks affinity for bone) inhibited protein prenylation in both fractions. These data demonstrate that osteoclasts internalise large amounts of BP due to their ability to release the BP from the bone surface during resorption and high endocytic activity. In comparison, non-resorbing cell types internalise only small amounts of BP (dependent on the endocytic capacity of the cell) that constitutively detaches from the bone surface.

P374-Mo

Continuous Suppression of Tartrate-resistant Acid Phosphatase Isoform 5b (Reflecting Osteoclast Activity) in Patients with Multiple Myeloma Receiving Bisphosphonate Treatment

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Introduction: Osteoclast activity plays a major role in the pathophysiology of osteolysis in multiple myeloma. Serum activity of tartrate-resistant acid phosphatase 5b (TRACP-5b) reflects osteoclast-induced bone resorption and correlates positively to the extent of bone disease before starting bisphosphonate (BIS) therapy. Monitoring TRACP-5b activity may allow to study the efficacy of BIS treatment in patients (pts) with multiple myeloma.
Methods: Serum TRACP-5b activity was measured by ELISA (BoneTRAP-Assay, medac Wedel, Germany) in 29 pts with multiple myeloma who received monthly BIS therapy (zoledronate or ibandronate) since at least 4 months (20 male, median age 63 years, range 47–77, 1 pt had Durie and Salmon stage I A, 2 pts II A, 22 pts III A, 4 pts III B). Osteolytic lesions were pre-existent in 25 pts. Additional cytotoxic chemotherapy was given to 10 pts during the study period. TRACP-5b activity and other serum markers were monitored bimonthly in each pt over 8 months.
Results: At study entry, TRACP-5b activity was within in the normal range in 25/29 BIS-pretreated pts. A trend towards higher values was seen in pts with shorter duration of BIS pretreatment. During observation time, a significant decrease of TRACP-5b activity was seen in the study population ($P = 0.004$) and all achieved normal values. Only 2 of 8 pts with proven progression of osteolytic lesions showed an increase of TRACP-5b activity (2.2 and 2.3 fold), but within the normal range. TRACP-5b activity did not predict disease progression during BIS treatment. Among a variety of other serum markers including β-crosslaps (C-terminal crosslinking telopeptide of type-I-collagen), β-2MG, and paraprotein levels, β-2MG most significantly correlated with disease progression ($P = 0.004$). Within-subject variability was remarkably low for TRACP-5b activity compared to serum β-crosslaps.
Conclusions: 1, BIS reliably suppress TRACP-5b activity in pts with multiple myeloma. 2, A maximum effect may only be achieved after several months of treatment. 3, Progression of osteolysis during BIS treatment was independent of TRACP-5b activity in most patients indicating the clinical relevance of additional pathways of bone resorption. 4, Given the efficacious suppression of osteoclast activity, the current practice of continuous monthly BIS-treatment of myeloma pts for several years needs reevaluation. TRAP-5b activity can be used to monitor pts in whom prolongation of the intervals of BIS therapy is studied.

P375-Tu

Osteoclasts from Gunmetal Mice are Defective in Rab Prenylation but not in Bone Resorption

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Gunmetal (gm/gm) mice have an autosomal recessive mutation in the gene encoding Rab geranylgeranyl transferase (Rab GGTase), which causes a four-fold reduction in the level of this enzyme. Consequently, the geranylgeranylation of a subset of Rab proteins is reduced in some cell types in these mice, particularly melanocytes, megakaryocytes and platelets. As a result, gm/gm mice suffer from prolonged bleeding, thrombocytopenia and reduced platelet granule contents. We recently found that a weak anti-resorptive phosphonocarboxylate analogue of risedronate,

NE10790, specifically inhibits Rab GGTase. This compound prevents prenylation of Rab proteins in osteoclasts in vitro at concentrations that inhibit bone resorption, demonstrating that reduced Rab GGTase activity disrupts the bone-resorbing capacity of osteoclasts. We therefore investigated whether osteoclasts from gm/gm mice have a defect in the prenylation of Rab proteins and whether this is associated with abnormal osteoclast function or a skeletal phenotype. Bone marrow cells were isolated from gm/gm and heterozygous (+/gm) mice (which have no defect in Rab prenylation) and stimulated to form osteoclasts in vitro using RANKL and M-CSF. As expected, Rab GGTase activity in gm/gm osteoclasts was markedly reduced compared to +/gm osteoclasts. The unprenylated form of numerous Rab proteins accumulated in the gm/gm, but not the +/gm osteoclasts, and the gm/gm osteoclasts were much more sensitive to further inhibition of Rab prenylation with NE10790. However, there was little difference in either the formation or resorptive activity of gm/gm and +/gm osteoclasts, indicating that gm/gm osteoclasts can function normally. Accordingly, pQCT analysis demonstrated that tibiae from gm/gm mice showed no signs of osteopetrosis compared to +/gm mice. Indeed, cortical bone content was reduced by 11% in the gm/gm mice, due to an increased endosteal circumference (and therefore decreased cortical thickness). By contrast, there was little difference in trabecular bone parameters between gm/gm and +/gm mice. These results show that osteoclasts from gm/gm mice retain sufficient Rab GGTase activity to maintain osteoclast function, therefore suggesting that more complete inhibition of Rab prenylation is required to disrupt osteoclast activity. The subtle bone phenotype of gm/gm mice may be caused by functional defects in other cells in the bone microenvironment such as megakaryocytes.

P376-Su

Estrogen and Testosterone Use Different Cellular Pathways to Inhibit Osteoclastogenesis and Bone Resorption

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Estrogen (E2) deficiency is associated with the development of both postmenopausal and senile form of osteoporosis in elderly women. Testosterone (Te) deficiency can also cause osteoporosis in men. Osteoporosis is associated with increased bone resorption caused by enhanced osteoclast formation, increased osteoclast activity and reduced osteoclast apoptosis. However, the mechanisms by which E2 or Te act on bone are not fully understood and one of the central questions is whether these hormones act directly on osteoclast precursors or whether their action is mediated through osteoblasts. In this study, we have cultured human peripheral blood CD14+ osteoclast progenitors in the presence of RANKL, M-CSF, TNF-α and dexamethasone. To study the possible osteoblast mediated effects, osteoclast progenitors were co-cultured with human MG-63 or SaOS-2 osteoblast cells in the presence of parathyroid hormone. These cultures were treated with different concentrations of E2 or Te for 7 days. Osteoclasts were recognized by tartrate-resistant acid phosphatase (TRACP) histochemical staining and by measuring TRACP 5b from the culture media. The resorption activity of osteoclasts was determined by measuring carboxyl-terminal telopeptide of type I collagen from the culture media. E2 did not have any significant effect on osteoclast formation in CD14+monocyte culture, while it slightly inhibited bone resorption. Te inhibited both osteoclast formation and bone resorption in a dose-dependent manner. In the co-cultures, where osteoblasts were present, E2 inhibited osteoclast formation in a dose-dependent manner. At the same time, E2 treatment in osteoblast-containing cultures stimulated significantly the formation of osteoprotegerin (OPG) measured from the culture medium as compared to untreated cultures. The effects of E2 and Te on osteoclast formation and bone resorption were completely antagonized by an E2 receptor (ER) antagonist, ICI 182780, and an androgen receptor (AR) antagonist, flutamide, suggesting ER and AR-mediated mechanisms in these cultures. In conclusion, our results suggest that Te acts directly on human osteoclast progenitors and mature osteoclasts by inhibiting both osteoclastogenesis and bone resorption, while the effect of E2 on osteoclast progenitors and osteoclasts is mostly mediated by osteoblasts.

P377-Mo

Zoledronic Acid Treatment Enhances Hard Callus Formation Without Delaying Endochondral Repair in a Rat Fracture Model

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Previously, osteoclasts have been considered key mediators of endochondral ossification. However, recent studies have indicated that endochondral ossification can proceed normally at the growth plate in the absence of osteoclasts. Accordingly, we speculated that soft callus removal during fracture repair (i.e., endochondral ossification) would not be delayed by the potent osteoclast inhibitor zoledronic acid (ZA). We also sought to compare fracture callus remodelling using different, clinically relevant dosing regimes.

Bolus or weekly ZA dosing commenced 1 week post-surgery in a closed rat femoral fracture model. Treatment groups included: saline, 0.1 mg/kg ZA as a bolus dose or divided into 5 weekly doses. Harvests were at 2, 4, 6 and 12 weeks post-fracture.

QCT at 6 weeks revealed significant increases in both ZA groups over saline in both callus BMC and volume ($P < 0.01$). Between 4 and 6 weeks, callus volume decreased by 8% with bolus dosing, indicating remodelling was occurring. In contrast, weekly dosing delayed remodelling, with callus volume increasing by 24%. By 12 weeks post-fracture, callus volume in both groups had decreased by 23–26%, suggestive of remodelling after cessation of ZA dosing at 6 weeks. However, the weekly group still maintained a larger callus volume (67% over saline) compared to the bolus group (30% over saline). Thus, weekly dosing had an adverse effect on remodelling compared to bolus.

Mechanical testing at 6 weeks of both ZA groups showed significantly increased fracture loads of 39–43% over saline. There was no difference in fracture load between the ZA groups, indicating the delay in remodelling seen with weekly dosing did not negatively affect callus mechanical properties. Crucially, histomorphometry revealed no significant difference in the percent of avascular cartilaginous (soft) callus between all treatment groups. All groups showed complete ossification (hard callus) by 6 weeks. Thus, the process of soft callus removal proceeded normally regardless of treatment.

In conclusion, ZA did not delay fracture callus endochondral ossification, indicating that osteoclast function is not essential to soft callus removal. Single dose bolus treatment provided a better outcome over weekly dosing, allowing for hard callus remodelling while still increasing callus BMC, volume and strength. This study highlights the safety of ZA treatment during fracture repair: as it provides a larger, stronger callus without delaying endochondral repair.

P378-Tu

Characterization of DRAK1 in Osteoclasts by Fluorescence Microscopy

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Osteoclasts are known to die by apoptosis at the end of the bone resorption process and control of apoptosis may be a key step in the regulation of bone resorption. It has been suggested that the drugs such as bisphosphonates and estrogens, act as inducers of osteoclast apoptosis. Kojima et al. (J. Biol. Chem. 276, 19238(2001)) has previously reported that a novel serine/threonine kinase, rabbit death-associated protein (DAP) kinase-related apoptosis-inducing protein kinase 1 (rDRAK1) is involved in osteoclast apoptosis. rDRAK1 has a high homology with human DRAK1 (hDRAK1), which belongs to the DAP kinase subfamily of serine/threonine kinases. In this work, we have studied expressions of DRAK1 by Western blot and confocal microscopy to characterize the presence and localization of antibodies generated against different rabbit and human DRAK1 sequences. We used resorbing and non-resorbing rabbit osteoclasts cultured on bovine bone, ivory and plastic cover slips. To overexpress DRAK1, we used adeno-associated viruses (aav) carrying rDRAK1 and hDRAK1 cDNAs. Apoptosis was studied by DAPI staining and by detection of DNA fragmentation by TUNEL. Cells were also stained with fluorescent phalloidin to characterize actin rings. Western blots of rabbit bone marrow samples using antibodies n-19 and c-20 against n-terminal and c-terminal amino acid sequence of hDRAK1, respectively, and antibody CRQ against c-terminal amino acid sequence of rDRAK1, showed a protein band with molecular weight around 55 kD. Overexpression of rDRAK1 and hDRAK1 by adenoassosiated viruses also showed protein bands with similar molecular weight. Fluorescence and confocal microscopic studies showed that actin organization affected to rDRAK localization. CRQ was localized in nuclei of osteoclasts growing on plastic cover slips and on bovine bone. When the cells cultured on plastic cover slips had podosomal structures or when the cells cultured on bovine bone slices had actin ring, CRQ staining seemed to disappear from nuclei. Antibodies against hDRAK1 had different localization and apoptosis affected to this localization. These results suggested the DRAK1 not only plays an important role in osteoclast apoptosis but is strongly associated with the life cycle of osteoclast on the bone from the early stage of its differentiation. Study of their dynamic property is under progress for understanding the life cycle and apoptosis of osteoclast. The details will be reported.

P379-Su

Independent Pathways in the Modulation of Osteoclastic Resorption by Intermediates of the Mevalonate Biosynthetic Pathway: The Role of the Retinoic Acid Receptor

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Suppression of the mevalonate biosynthetic pathway by statins or nitrogen-containing bisphosphonates (NBPS) inhibits osteoclastic bone resorption. Both statins and NBPS, which suppress the enzymes HMGCoA reductase and farnesyl pyrophosphate synthase, respectively, inhibit the formation of the mevalonate pathway intermediate geranylgeranyl pyrophosphate (GGPP). GGPP is utilized for the prenylation of GTP binding proteins, which is essential for cytoskeletal integrity- and intracellular signalling of osteoclasts. Previously, in fetal bone explants, we have shown that GGOH (the alcohol form of GGOH) stimulates bone resorption, but the mechanism is not known. Previous studies indicate that all-trans GGOH can be metabolised into all-trans geranylgeranoic acid (GGA) and that this metabolite stimulates retinoic acid receptor (RAR) expression and pRARCAT activity. Therefore, we

hypothesized that GGOH stimulates bone resorption via GGA that subsequently activates RAR. We found that GGOH, GGPP and GGA all stimulated osteoclastic bone resorption in bone explants in vitro. This action, as well as the stimulating effect of retinoic acid (RA) on bone resorption, could be blocked by co-treatment with the RAR antagonist AGN-193109 (obtained from Allergan Inc). In addition, PCR analysis showed that RA, GGOH and GGA all significantly increased RARb mRNA expression whereas in control explants no expression was detected.

We further examined the reversibility of NBP (ibandronate) inhibited bone resorption by GGOH, GGPP and GGA. Whereas, as expected, GGOH and GGPP rescued osteoclasts and reversed the antiresorptive effect of ibandronate, GGA had no effect similar to RA. This suggests that GGA is not involved in protein prenylation.

Thus, intermediates of the mevalonate pathway such as GGPP and GGOH can modulate bone resorption not only by the prenylation of proteins, but also by another mechanism involving RAR.

P380-Mo

Modifications to the Phosphonate Groups of Bisphosphonates Affects their Potency and Target Enzyme Specificity

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Nitrogen-containing BPs inhibit bone resorption by inhibiting farnesyl diphosphate (FPP) synthase, thereby preventing the synthesis of isoprenoid lipids required for prenylation of small GTPases in osteoclasts. However, more recently, we showed that a weak anti-resorptive phosphonocarboxylate analogue of risedronate, NE10790, inhibits Rab geranylgeranyl transferase (Rab GGTase), thereby selectively preventing prenylation of Rab proteins in cells in vitro. We have now examined the effects of other modifications to the phosphonate groups of BPs, i.e., removal of one of the phosphonate groups, and replacement of one of the hydroxyl groups on one or both of the phosphonate moieties with a alkyl group (to produce a phosphonoalkylphosphinate (PAP) or bisphosphinate (BPI), respectively). The monophosphonate analogue of risedronate inhibited prenylation of Rabs in J774 macrophages and osteoclasts at 4 mM but had no effect on the prenylation of Rap1A. This compound therefore inhibits Rab GGTase rather than FPP synthase. This analogue also inhibited bone resorption in vitro, but unlike NE10790 also disrupted actin rings, suggesting that the anti-resorptive effect may not be due solely to inhibition of Rab prenylation. By contrast, the monophosphonate analogues of pamidronate or alendronate did not inhibit the prenylation of either Rab6 or Rap1A. Two BP analogues of risedronate inhibited prenylation of Rap1A and Rab6 with similar potency to risedronate (complete inhibition with 100 μM). The PAP analogue of one of these also inhibited prenylation of Rap1A and Rab6, indicating inhibition of FPP synthase, but was approximately 4-fold less potent. By contrast, the PAP analogue of the other BP was completely inactive. Neither of the BPI analogues of these BPs inhibited protein prenylation. In summary, the ability of bisphosphonates to inhibit FPP synthase does not absolutely require the two phosphonate moieties in the P-C-P structure, but is dramatically reduced by the replacement of a hydroxyl group with an alkyl group on one or both of the phosphonate moieties, and is abolished by the removal of one of the phosphonate groups. Furthermore, alterations to risedronate, including removal of one of the phosphonate groups or replacing a phosphonate with a carboxylate group alters target enzyme specificity, generating compounds that specifically inhibit Rab GGTase.

P381-Tu

Endostatin Inhibits VEGF-A Induced Osteoclastic Bone Resorption in Vitro

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Background: In bone development, modeling and remodeling, angiogenesis and osteoclastic resorption are essential processes and they are closely associated with each other. Signaling between endothelium and bone cells may be crucial for the regulation of bone remodeling.

Materials and methods: To address the potential mediators between bone cells, we examined the effect of vascular endothelial growth factor-A (VEGF-A) and endostatin, which are the most critical regulators of angiogenesis, on osteoclastic survival and bone resorption activity using the rat pit formation assay. Immunostainings of VEGF-A receptor 1, Flt-1, were performed and the localization was visualized with confocal laser microscopy.

Results: The number of tartrate resistant acid phosphatase positive (TRACP) cells did not differ after VEGF-A or endostatin treatments indicating that they have no effect on the survival of osteoclasts. VEGF-A stimulated osteoclastic bone resorption, whereas endostatin had no effect on the basal osteoclastic function. Although endostatin had no effect on the basal resorption, addition of endostatin blocked the stimulatory effect of VEGF-A. Immunostainings were performed to investigate the precise localization of Flt-1 in bone cells, and it was clearly detectable both in osteoclasts and in osteoblasts.

Conclusions: These data indicate a clear stimulatory effect of angiogenic VEGF-A on bone resorption and that antiangiogenic endostatin can suppress this stimulus.

P382-Su

Dissolution of the Inorganic Phase of Bone is Important for Osteoclast Survival and the Normal Coupling of Bone Formation to Bone Resorption

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The coupling process is understood as a bone formation response that is the consequence of bone resorption, with an amount of bone formed that is equal to that resorbed. In some but not all osteopetrotic mutations, the coupling principle has been challenged. Osteopetrotic patients due to defective acidification of the osteoclastic resorption lacunae (ClC-7 or V-ATPase), have increased numbers of non-resorbing osteoclasts. These patients as well as animals treated with ClC-7 inhibitors (J Bone Miner Res. 2004 Jul; 19(7):1144-53) have normal bone formation activity, despite the defective resorption.

We investigated if the osteoclast number was dependent on acidification of the osteoclastic resorption lacunae, dissolution of the inorganic phase of bone and calcium.

Normal bone resorption is followed by apoptosis of the osteoclast. To investigate the role of the inorganic phase of bone with respect to osteoclast function and life-span, we used synthetic inhibitors of the chloride channel ClC-7 (NS5818), the V-ATPase (bafilomycin A1), the calcium channel antagonist Ryanodine in combination with cultures of human osteoclasts on cortical bone slices and cortical bone slices decalcified by EDTA.

Both bafilomycin and NS5818 dose dependently inhibited acidification of the osteoclastic resorption lacunae, investigated by Acridine orange. In alignment, a dose-dependent decrease in resorption (100%) was observed, which inversely correlated to a dose-dependent increase in the survival of multinuclear osteoclasts (300%), evaluated by counting of calcitonin receptor-positive cells. To further investigate the inorganic phase of bone we compared osteoclastic survival on normal bone slices to that of decalcified bone slices. On normal bone slices but not on decalcified bone slices osteoclast survival was increased. To specifically study the role of calcium in osteoclast survival, we used the calcium channel antagonist Ryanodine and calcium. We found that inhibition of calcium channels by Ryanodine dose dependently increased osteoclast survival by 100% at 90 mM, and that calcium alone, at 20 mM, decreased osteoclast survival.

In conclusion, we present data suggesting that inhibition of acidification leads to increased numbers of non-resorbing osteoclasts. We suggest that the dissolution of the inorganic phase of bone is a key player in the control of osteoclast survival, and thereby important for the normal coupling of bone formation to bone resorption.

P383-Mo

The Use of Hydroxyapatite Column Chromatography as a Novel Method to Reveal Differences in Relative Binding Affinities of Bisphosphonates

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Bisphosphonates (BPs) continue to be the most widely used class of antiresorptive agents for the treatment of metabolic bone diseases, especially bone metastases and osteoporosis. There is a growing appreciation that small but significant and potentially important differences exist among the BPs, not only in their relative potencies but also in their duration of action. Our recent studies suggest that unexpected differences in mineral binding affinities contribute both to potency and to reversibility of action.

In order to study the differences in mineral binding that may relate more directly to their retention and diffusion through bone, we have developed a novel method based on FPLC using columns (3 × 25 mm) of hydroxyapatite to which BPs adsorb and can be eluted subsequently by using increasing phosphate buffer gradients (1–1000 mM) at pH 6.8. The individual BPs emerge as discrete peaks detectable by their UV absorbance and their identities confirmed by using mass spectrometry. Under the conditions used the retention time (min; mean ± SEM) for zoledronate was 22.0 ± 0.1, compared with 16.16 ± 1.17 for risedronate, and 7.33 ± 0.08 for NE10790, a risedronate analogue in which one of the phosphonate groups is replaced by a carboxyl group. These elution patterns were consistent and the elution times statistically different ($P < 0.05$). Moreover, the sequence of elution was in the same order as that recently described for kinetic affinity constants (KL) for binding to HAP determined by a crystal growth inhibition assay in which the KL were 3.47, 2.0, 0.03 × 106 L/mol for zoledronate, risedronate, and NE10790 respectively (Ebetino, Nancollas et al: JBMR, 2004, 19:(supp. 1) S157).

These results confirm that differences in hydroxyapatite binding affinities are an important feature of BPs used in clinical practice, and suggest that the side chains may contribute to mineral binding affinities in addition to the P-C-P backbone. These differences may help to explain the

variations in retention and persistence of effects of BPs observed in animal studies as well as in clinical studies.

P384-Tu

Osteoclast Function Relies on Raft-Dependent Vesicle Trafficking Between The Ruffled Border and the Functional Secretory Domain

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We analyzed the role of rafts in vesicular trafficking and maintenance of distinct membrane domains in resorbing rat osteoclasts in vitro by studying the delivery of Influenza Hemagglutinin and recombinant vesicular stomatitis G-proteins. Our results show that there is a continuous and bi-directional membrane flow between the ruffled border and the FSD and that rafts are involved for these targeting processes. Replacing the cytoplasmic tail of the vesicular stomatitis virus G-protein with that of CD4 resulted in partial insolubility in Triton X-100 and retargeting from the peripheral non-bone facing plasma membrane to the FSD. The recombinant G-proteins revealed endocytosis from the FSD and a raft-dependent delivery to the ruffled border, which likely compensates for membrane loss during degraded bone matrix uptake. Furthermore, several vesicle families rise from the ruffled border and most of them contain rafts. Thus, rafts are vital in membrane trafficking to the late endosomal/lysosomal ruffled border and in the post-lysosomal pathways towards the FSD, and removal of rafts results in disturbance of ruffled border formation and maintenance and finally, bone resorption.

P385-Su

Inhibitory Effect of Ribbon Type NFκB Decoy Deoxyoligonucleotides on Osteoclast Induction and Activity

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Purpose: We examined the effect of ribbon (circular) type NFκB decoy deoxyoligonucleotides (ODN) on induction and activity of osteoclasts.
Method: We extracted bone marrow cell from the femur of rats and non-adherent cells were incubated with RANKL (100 ng/ml) and M-CSF (20 ng/ml). First, cells were incubated with FITC labelled ribbon type NFκB decoy ODN without reagent and 24 h later transfer efficiency of decoy was examined by counting the number of the fluorescent cells. To examine the effect of ribbon type NFκB decoy ODN (RDODN) on induction and activity of osteoclasts, cells were incubated with or without decoy and TRAP staining was performed on day 7 and the number of TRAP-positive multinucleated cells was counted. To study the effect of RDODN on the bone resorbing activity of mature osteoclasts, we performed the bone-resorbing assay. Non-adherent bone marrow cell were cultured on Osteologic Bone Cell Culture System. On day 8 cells were incubated with decoy and on day 10 calicified matrix resorption area on each disc was measured using the MacSCOPE image analyser.
Result: About 80% of the cells showed the fluorescence of FITC. The total number of TRAP-positive multinucleated cells were significantly decreased in RDODN treated group compared to no decoy treated group or ribbon type scrambled decoy treated group ($P < 0.01$). The average resorbed area of calicified matrix was significantly decreased in ribbon type NFκB decoy treated group compared to other groups ($P < 0.05$).
Conclusion: These data demonstrated the high transfer efficiency of RDODN to osteoclasts and the inhibitory effect of RDODN on induction and activity of osteoclasts and suggested the therapeutic application of RDODN in joint destruction of arthritis.

P386-Mo

StemPro34 Medium, IL-3, IL-6 and Stem Cell Factor Enhance the Yield of Osteoclast Progenitors from Human Adult Peripheral Blood

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Osteoclast (OC) function, in balance with that of osteoblasts, leads to bone remodelling central to the mechanical properties of the skeleton, the control of mineral homeostasis and the development of bone marrow and nervous system contained in the bone cavities. Much insight into the mechanism of bone reabsorption has been achieved since when OCs were isolated from tissue and investigated ex vivo. However, the OC yield, particularly from human sources, is not yet satisfactory. Impaired OC function causes osteopetrosis, and the harvest of a high number of OC progenitors would help settling the basis for cell therapy of this orphan disease. Therefore, we tested several OC culture conditions and identified a combination of treatments that increases the yield of OCs from human adult peripheral blood mononuclear cells (PBMCs). Healthy donor PBMCs were purified by the Ficoll/Histopaque procedure. Equal numbers of cells were cultured in 96 well multiplates, w/wo bone sections, in standard conditions (DMEM, 10% FBS, 25 ng/ml hrM-CSF, 30 ng/ml hrRANK-L, for 2 weeks for OCgenesis and 3 weeks for bone resorption), or were pre-treated for 1 week with 20 ng/ml hrIL-3, hrIL-6 and 50 ng/ml Stem Cell Factor (SCF), followed by 1 week treatment with

20 ng/ml hrGM-CSF prior to addition of hrM-CSF and hrRANK-L as above. Cultures were also performed in FBS-free Gibco/Invitrogen StemPro34 medium, specifically formulated to support the growth of human haematopoietic cells. Compared to the best results obtained in DMEM, the number of total OC nuclei was 6.8-fold in StemPro34 medium, distributed in more than a doubled number of OCs, with and average of 10.6 ± 1.6 nuclei/cell vs. 3.1+/0.1 nuclei/cell in DMEM. OCgenesis occurred earlier in StemPro34 medium and multinucleated OCs appeared at 4 days from administration of hrM-CSF and hrRANK-L, versus 10 days in DMEM cultures. Accordingly, OCs obtained in StemPro34 medium resorbed 3-fold more bone, evaluated by the pit assay, vs. OCs in DMEM, and pre-treatment with hrIL-3/IL-6/SCF enhanced StemPro34 medium performance increasing by 1.6-fold both OC number and nuclei/cell. In conclusion, StemPro34 medium offers a significant advantage to OC progenitors from adult PBMC fraction, thus suggesting that its use could enhance the benefit for research purposes and open an avenue for the development of cell therapy in osteopetrosis, based on the employment of healthy donor or genetically engineered OC progenitors.

P387-Tu

Mechanically Induced Osteocyte Signals Explain Modeling and Remodeling of Tissue Structure in Cortical and Trabecular Bone

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There is a strong relationship between mechanical forces and bone tissue architecture. This relationship is established by bone-resorbing osteoclasts and bone-forming osteoblasts that are responsible for modeling and remodeling of the tissue. The pathways by which mechanical forces are expressed in these (re)modeling processes are not understood. We developed a conceptual mechano-biological theory to relate mechanical forces to osteoclast and osteoblast activity in bone modeling and remodeling, for cortical as well as trabecular bone. Its basic assumptions are that (a) osteocytes are mechanosensors that send signals, through the canalicular network, to the bone surface. (b) Osteoblast bone formation is initiated where the amount of osteocyte signal is high. (c) Osteoclast resorption is repressed by these signals, whereas (d) lack of signal, due to disuse or microdamage, initiates osteoclast activity. We tested this theory with computer simulations in which we accounted for the positions and activities of osteocytes, osteoclasts and osteoblasts and the assumed relationships between these cells. Thus we were able to show that our theory explains bone modeling in growth to a homeostatic trabecular-like structure with trabeculae that are aligned to the external loading direction. The proposed regulatory mechanism also explains, at a more refined topological scale, how osteoclasts and osteoblasts collaborate in trabecular BMUs (Basic Multicellular Unit) in mature trabecular bone. In that case, osteoclast resorption is directed along the trabecular surface and osteoblasts follow in a coordinated manner creating hemi-osteons of renewed tissue. Simulations of the cortical bone remodeling process show that the regulatory mechanism also explains how osteoclasts and osteoblasts collaborate in cortical BMUs. Osteoclasts in the tip of the cutting cone excavate a tunnel in the main loading direction, and also here they are followed by osteoblasts in a closing cone to gradually fill the tunnel and create a secondary osteon. In conclusion, we can explain modeling and remodeling of trabecular and cortical bone under influence of mechanical forces by assuming that mechanosensitive osteocytes control osteoclast and osteoblast activity at the bone surface. Thus, we can explain how the tissue structure relates to external loading, but also, at a more refined topological scale, how osteoclasts and osteoblasts collaborate in trabecular and cortical BMUs. All with one theory.

P388-Su

DAP12 and the FC-Receptor Gamma-Chain Regulate Osteoclast Differentiation and Function

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Osteoclasts, the only bone-resorbing cells, are central to the pathogenesis of osteoporosis, yet their development and regulation are incompletely understood. Multiple receptors of the immune system use a common signaling paradigm whereby phosphorylated immunoreceptor tyrosine-based activation motifs (ITAMs) within receptor-associated adapter proteins recruit the Syk tyrosine kinase. Here, we demonstrate that a similar mechanism is required for osteoclast development and function. Mice lacking two ITAM-bearing adapters, DAP12 and the Fc receptor γ-chain (FcRγ), are severely osteopetrotic. DAP12$^{-/-}$ FcRγ$^{-/-}$ bone marrow cells fail to differentiate into multinucleated osteoclasts or resorb bone in vitro and show impaired phosphorylation of the Syk tyrosine kinase. Syk$^{-/-}$ progenitors are similarly defective in osteoclast development and bone resorption. Even when osteoclast development is partially restored by high-dose M-CSF treatment, DAP12$^{-/-}$ FcRγ$^{-/-}$ and Syk$^{-/-}$ cells still fail to resorb bone. Intact SH2-domains of Syk, introduced by retroviral transduction, are required for functional reconstitution of Syk$^{-/-}$ osteoclasts, whereas intact ITAM-domains on DAP12 are required for reconstitution of DAP12$^{-/-}$ FcRγ$^{-/-}$ cells. These data indicate that recruitment of Syk to phosphorylated

ITAMs is critical for osteoclastogenesis and osteoclast-mediated bone resorption. Although DAP12 appears to be primarily responsible for osteoclast differentiation in cultures directly stimulated with M-CSF and RANK ligand, DAP12 and FcRγ have overlapping roles in supporting osteoclast development in osteoblast-osteoclast cocultures, which mirrors their overlapping functions in vivo. These results provide new insight into the biology of osteoclasts and suggest novel therapeutic targets in diseases of bone remodeling.

P389-Mo

BSP and RANKL Act Synergistically to Induce Osteoclastogenesis and Bone Resorption

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Bone sialoprotein (BSP) is a member of the SIBLING (small integrin-binding ligand N-linked glycoprotein) family of secreted proteins, mostly produced by mature osteoblasts, osteocytes, platelets and cancer cells. BSP plays a role in mineralization and can act as a nucleator of hydroxyapatite in vitro. BSP can also induce osteoblast and osteoclast adhesion and bone resorption in vitro. BSP contains an RGD-sequence and partly mediates its biological functions through binding to integrin alphavbeta3, although other non-RGD sequences might also be involved in its biological functions. Receptor activated of NFκB ligand (RANKL) is a cytokine that induces osteoclastogenesis and bone resorption by binding to its receptor RANK in the osteoclast. Several diseases characterized by an excessive osteoclastic activity, including osteoporosis, melanoma, and breast or prostate cancers have been associated with the upregulation of BSP as well as of RANKL. The aim of this study was to analyse the possible mechanisms by which BSP and RANKL regulate osteoclastogenesis and bone resorption by using the RAW264.7 monocyte/macrophage cell line. To test the effects of these proteins in osteoclastogenesis we performed Tartrate-resistant acid phosphatase (TRAP) cytochemical staining on fixed cells, and counted the number of TRAP-positive cells with 3 or more nuclei. Our results indicated that human recombinant BSP (20–100 ng/ml) could induce the formation of TRAP-positive multinucleated cells in a dose-dependent fashion. Furthermore, the BSP osteoclastogenic effects were synergistic to those mediated by RANKL at concentrations of BSP ranging 1–100 ng/ml. BSP and RANKL were also synergistic in inducing the formation of resorption pits at concentrations ranging 1–100 ng/ml. BSP and RANKL synergistic effects in bone resorption were partly blocked by PP2 (an inhibitor of Src activation) and by the calcium chelator BAPTA. BSP and RANKL synergistic effects in osteoclastogenesis were partly blocked by BAPTA and the JNK inhibitor SP600125. Taken together our results suggest that BSP and RANKL may act synergistically to induce osteoclastogenesis and bone resorption in pathologies characterized by the overexpression of these proteins.

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P390-Tu

Modulation of MAPK Signalling and Rho GTPase Activation in Osteoclasts by Pasteurella Multocida Toxin (PMT) Results in Differential Effects on Differentiation and Activation of Murine and Human Osteoclasts

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Rho GTPases and MAPK cascades are well-known regulators of the osteoclast cytoskeleton and differentiation, respectively. We have recently shown that the unique bacterial toxin, Pasteurella Multocida toxin (PMT), which activates the heterotrimeric G-protein, Gq, leading to stimulation of Rho and actin rearrangements, and activation of phospholipase C, protein kinase C and the Ras/MAP kinase pathway, inhibits osteoblast differentiation and bone nodule formation via activation of Rho and its effector Rho kinase (ROK). However, PMT also targets osteoclasts, since the main in vivo effect of PMT is the porcine bone resorbing disease, atrophic rhinitis, resulting in pathological bone resorption. Therefore, in this study, we have investigated the effects of PMT and Rho-ROK activation on osteoclast differentiation and activity, as well as on RANKL-stimulated MAPK activation.

In RANKL- and MCSF-based cultures of human PBMCs or murine bone marrow cells, PMT inhibited osteoclast differentiation and resorption in a dose-dependent manner. This correlated with an inhibition of expression of the osteoclast markers, calcitonin receptor and cathepsin K, but there was no effect on RANK or c-fms receptor expression. Biochemical studies demonstrated that PMT markedly induced the levels of active GTP-bound Rho in both mouse and human osteoclast precursors and mature osteoclasts, with no effects on Rac and only a slight increase in active Cdc42. To investigate the mechanisms of PMT action, we analysed the pathways downstream of RANKL signalling and Rho GTPase. PMT treatment of murine osteoclast precursors markedly blocked RANKL-stimulated p-p38, p-JNK and p-ERK levels. Interestingly, however, treatment of human or murine osteoclast precursors with the ROK inhibitor, Y-27632, rescued the inhibition of human osteoclast differentiation, with little effect on murine osteoclasts. In contrast to the inhibitory effects on differentiation, PMT appeared to promote resorption of mature osteoclasts as addition of PMT to mature human osteoclasts increased the proportion of F-actin ring-containing, vitronectin receptor-positive osteoclasts, with a concomitant increase in resorption.

Overall, these data suggest a novel inhibitory role for the Rho-ROK pathway in osteoclast differentiation and we are currently investigating how the divergent effects of PMT on osteoclast differentiation, activation and MAPK stimulation, converge to produce the bone loss observed in vivo.
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P391-Su
What is the Function of Glutamate Transporter Variants in Osteocytes?
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L-glutamate activates glutamate receptors during excitatory neurotransmission and is regulated by excitatory amino acid transporters (EAATs) that remove glutamate from the synapse. Identification of the glutamate transporter, GLAST-1 (EAAT1), as a mechanically-regulated gene in osteocytes, implicated glutamate as an osteogenic signal in bone (1). It is now established that glutamate receptors are functional in both osteoblasts and osteoclasts, and that their activation alters bone cell phenotype.
GLAST-1, a member of the 'high affinity' glutamate transporter family (EAATs 1-5), is expressed in a wide range of tissues and acts as a transporter and an ion channel. We have cloned a novel splice variant of the GLAST gene, called GLAST-1a, in which 46 amino acids are removed. GLAST-1a is expressed in bone, cerebellum and retina, and may orientate in reverse in the plasma membrane (2). Alignment of EAATs 1–5 and analysis of intron/exon structure and splice sites reveal that most retain the potential for an equivalent splicing event in spite of extensive sequence divergence. This evolutionary conservation led us to investigate GLAST-1a function in *Xenopus* oocytes and osteocytes.
Oocytes microinjected with GLAST-1a cRNA take up radiolabelled-glutamate at similar rates to those expressing GLAST-1. Furthermore, whole cell clamping of GLAST-1a expressing oocytes reveals both glutamate transport and glutamate gated ion channel activity. Intriguingly, co-injection of GLAST-1 and GLAST-1a cRNA into the same oocyte altered responses to glutamate. This is supportive of a regulatory role for the splicing event that may explain its evolutionary conservation.
In MLO-Y4 osteocytes, expression and localisation of GLAST variants respond to extracellular glutamate concentrations. GLAST protein distribution is also responsive to mechanical load in osteocytes in vitro. However, these responses to glutamate are not mediated by classical glutamate receptors as osteocytes do not exhibit glutamate-dependent increases in intracellular calcium.
We propose that GLAST-1 and GLAST-1a directly mediate mechanical signalling in osteocytes by acting as ion channels or receptors in their own right. Exploitation of this signalling mechanism may allow us to mimic the osteogenic potential of mechanical loading, thus offering a novel approach to increase bone mass.

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P392-Mo
Forearm Single X-Ray Absorptiometry in the Identification of Postmenopausal Women with Osteoporosis at the Hip and Spine: A Correlation Study
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The International Society for Clinical Densitometry has recently approved the use of the World Health Organization classification of osteoporosis in bone mineral density (BMD) measurements of the 33% radius.
Objective: In this study, we assessed the value of the forearm site in identifying subjects with spinal and femoral osteoporosis, and we defined the 90% sensitivity point for the DTX-100 bone densitometer.
Patients: 264 postmenopausal Bulgarian women (mean age 63.02 ± 9.88 years) participated in this study.
Methods: Forearm BMD was measured on a DTX-100 device (Osteometer Meditech, USA) followed by forearm and axial measurements on a QDR 4500 A densitometer (Hologic Inc., Waltham, MA 02154, USA). Linear BMD correlations among sites were analysed. The forearm site sensitivity and specificity in identifying osteoporotic subjects at axial sites were determined.
Results: BMD correlations of the forearm and the axial sites ranged from 0.35 to 0.78; and were slightly better at younger age. The forearm site sensitivity for detecting axial osteoporosis increased slightly with age, but specificity decreased. The distal site showed 56.2% specificity for spinal osteoporosis at the 90% sensitivity BMD cut-point of 0.415 g/cm^2 and 40.1% for hip osteoporosis at 0.340 g/cm^2 BMD. At the ultradistal site the corresponding values were 58.1% at 0.325 g/cm^2 for spinal osteoporosis and 41.6% at 0.275 g/cm^2 for femoral osteoporosis.
Conclusion: The best performance of forearm densitometry might be between age 50 and 70. Forearm densitometry could be used in large-scale screening programmes to reduce the number of subjects needing axial measurements.

P393-Tu
Prevalence of Low Central Bone Mineral Density in a Bulgarian Female Referral Population: A Pilot Study
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Osteoporosis is one of the most important health problems among elderly women. The prevalence of central osteopo-

rosis in Bulgaria is still unknown. We tried to determine retrospectively the prevalence of osteopenia and osteoporosis at the spine and hip in a female referral population. 2600 consecutive Bulgarian women (age 25–87) referred for bone densitometry screening were included. Information about known risk factors for low bone mass was recorded. Bone mineral density, BMD, was measured by dual-energy X-ray absorptiometry (Hologic QDR 4500 A) at the lumbar spine (in 2547 participants, 1954 of whom postmenopausal) and left hip (in 723 participants, 605 of whom postmenopausal). T-scores were calculated from Hologic provided and own Bulgarian peak BMD data. Peak BMD data were based on 122 healthy premenopausal 25–39-year-old women. Peak BMD in the spine was 0.994 g/cm^2 (SD 0.095 g/cm^2) and thus lower than the manufacturer-provided peak BMD of 1.047 g/cm^2. The peak BMD in the total hip was 0.959 g/cm^2 (SD 0.9 g/cm^2) and thus higher than the manufacturer-provided BMD of 0.942. T-scores differed according to the database used. In the spine the osteoporosis threshold of −2.5 SD was reached in age group 70–74. Left hip T-scores showed a much slower decline with age. In women aged 50 and older, the prevalence of osteoporosis of the spine reached 37.31%, and of the left hip- 16.14%. The prevalence of osteopenia reached 39.74% in the spine and 65.57% in the total hip. This is the first Bulgarian study looking for the prevalence of central osteopenia and osteoporosis in a female referral population. It may become the starting point for future epidemiological work.

P394-Su

Protective Effect of Vitamin D3 on Methylprednisolone Actae Induced Loss of Bone Metabolism Markers and Bone Mineral Density in the Lumbar Spine of Rat

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Introduction: Although some vitamins have been shown to prevent glucocorticoids induce osteoporosis in short time, the magnitude of this effect remains to be clarified. The aim of this prospective study is the evaluation of protective effect of vitamin D3 on methylprednisolone Acetate induced osteoporosis in rats.
Methods and materials: Total duration of the experiment was 4 weeks. Twenty-four male Sprague–Dawly rats (8 week old and 180 g weight) were randomly divided into four groups: Group A (n = 6), was a base line control or normal animals. Group B (n = 6), was treated only normal saline (0.9%), group C (n = 6), was treated methylprednisolone acetate (0.2 mg/kg) subcutaneously for 4 weeks (3 times per a week) and finally group D (n = 6) were administered Methylprednisolone acetate resemble to group C and treated by Vitamin D3 (0.1 μg/kg dissolved in ethanol daily). For evaluation of biochemical agents changes in the serum, such as calcium, osteocalcine and Acid phosphatase were measured before and after treatment. Also, bone mineral density (BMD) of lumber vertebrae was measured by dual energy X-ray absorptiometry (DEXA).
Results: The results showed that, the serum calcium level unaffected ($P > 0.05$) by methylprednisolone acetate in all groups before and after treatment, but, the serum osteocalcine level and bone mineral density of lumbar vertebrae were significantly ($P = 0.05$) decreased in group C compared with groups A and B. In group D serum osteocalcine level increased again significantly ($P < 0.05$) but increasing of bone mineral density and bone mineral content were not significant ($P > 0.05$). Also, the serum acid phosphatase level increasing in group C was treated by vitamin D3 (in Group D), but was not significantly ($P > 0.05$).
Conclusions: The findings of present investigation indicate that by using of vitamin D3 in methylprednisolone acetate induced rats could increase bone formation and decrease bone resorption.
Keywords: Methylprednisolone acetate, Vitamin D3, Osteoporosis, Bone Markers Metabolism, BMD and Rat.

P395-Mo

Dual-Energy X-RAY Absorptiometric In Vivo Bone Densitometry-Boon or Bete Noire?

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Advances in bone fragility research appear hampered, misdirected, and otherwise frustrated by a growing number of perplexing anomalies and unresolved inconsistencies that serve to make equivocal present understanding of the underlying causes, assessments (diagnostics/prognostics), and remedial or preventative treatments of osteoporosis. Among these are: (a) the apparent failure of anabolic parathyroid hormone (PTH) and anti-resorptive therapies, when taken concurrently, to have a combined, synergetic remedial efficacy exceeding that of either therapy administered singly, (b) the inability to establish a biological/biochemical relationship between body fat mass and bone mineral areal density (BMD) or between serum leptin levels and bone mineral areal density, both of which are generally considered to be positively correlated with body fat mass, (c) the seeming lack of meaningful concordance between bone fragility (non-traumatic, osteoporotic fracture propensity) and BMD, and (d) the lack of a coherent connection linking bone mineral density, bone remodelling, and skeletal fragility. Although each of these confounding incongruities appears to pertain to somewhat different aspects of bone fragility and treatment, a single, common factor links these and several other disparate facets of the overall bone fragility/bone density/bone remodelling conundrum: the seemingly unqualified reliance and near-universal dependence upon dual-energy X-ray absorptiometry (DXA) methodology to provide accurate, quantitative in vivo bone mass/bone mineral areal density determinations. It is shown that the

underlying systematics of the sizable DXA in vivo BMD inaccuracies, previously demonstrated to be inherently unavoidable in this methodology, are fully and quantitatively consistent with being the cause of most all of the principal anomalies and ambiguities misleading and/or obstructing progressive development of a coherent understanding of bone fragility. It is also demonstrated that the underpinnings of all these major anomalies and inconsistencies that form the bone fragility/bone density/bone remodelling conundrum are quantitatively consistent with being manifestations of the systematic inaccuracies inherent in DXA in vivo (in situ cadaveric) BMD measurements arising from soft tissue anthropometric and X-ray absorptiometric particulars within the DXA scan region of interest, and are not of bone-biological origin.

P396-Tu

Preventing Fractures: An Integrated Hospital Service

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Given the high risk of subsequent fractures, identifying and treating patients with osteoporosis who have a fracture is an important part of a fracture prevention strategy. In 2003, at our hospital (population served 310,000), we set up an integrated clinical service, The Fracture Liaison Service (FLS). The service included a 'one-stop' clinical/falls assessment and DXA scan of patients with fragility fracture. Experience and data from 12 months are presented.

Methods: All fracture patients 50–75 years identified from ward or fracture clinic had a DXA scan (Hologic, Discovery) and were seen by a specialist nurse on the same day. All data were compiled, and reports generated, using Formis (RIOMED). Referrals to falls or metabolic bone clinics were made if appropriate. Drug treatment (non-calcium/vitamin D) was recommended according to protocol for patients: age < 60 with *T* score < −2.5; age > 60 with *T* score < −1.5. Clinical discretion was applied where other risk factors were considered significant, e.g., previous fractures, steroid use.

Results: 543 patients age 50–75 years were identified (456 [84%] female). 74 patients did not attended. The most frequent fractures were: forearm (168/469 36%), lower leg (21%), hip (18%) and humerus (7%). DXA data were available for 419 fracture patients. BMD criteria for osteoporosis (LSp and/or hip *T* score < −2.5) were met for 60/168 (36%) forearm, 18/98 (18%) lower leg, 12/22 (55%) hip, 13/32 (41%) humerus and 26/99 (26%) spine/'other' fracture patients. Of a subset of patient's age 50–75 years, 112/409(27%) presented with (at least) their 2nd fracture. Treatment was recommended for 272/469 (58%) patients- oral bisphosphonates (+calcium/vitamin D) in virtually all. New major diagnoses were made in 23/408 (6%) patients. Bone-relevant diagnoses were made in 18/23 patients including hyperparathyroidism (8), male hypogonadism (3), osteomalacia, hyperthyroidism, thyroxine abuse and alcoholism.

Conclusions: A Consultant-led, Nurse-run FLS can work efficiently within an acute care hospital. The FLS is an efficient way of targeting treatment for patients at high risk of fracture but the assessment of secondary fracture risk would be enhanced by accurate estimates of absolute fracture risk. By our criteria a majority of patients age 50–75 years with fragility fracture are at sufficiently high fracture risk to recommend bisphosphonates. A small but significant number of new diseases (6% patients) were usefully identified by a FLS.

P397-Su

BMD and Fracture Risk in Women Stopping HRT

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In December 2003, in Britain, The Department of Health issued a statement that it was inappropriate for post-menopausal women to be recommended HRT for osteoporosis alone. Subsequently, as women stopped HRT, there was an increase in referrals to our, and other units, for DXA scans. Resources for scans in Britain are limited and the capacity of our DXA unit is 500 scans/100,000 population/year. We hypothesised that DXA scans may be unnecessary in many women who had been on HRT a number of years as BMD may be predictably high.

Methods: We collected hip and LSp DXA (Hologic Discovery) and basic clinical data (by questionnaire) on women (sequentially) referred, requesting DXA scan "to confirm BMD status in women stopping HRT" (or similar wording for same indication) for 6 months. Clinical data obtained included information on osteoporosis risk factors and total duration of HRT. Following the scan a 10-year fracture risk was estimated for each woman (Kanis et al. Osteoporosis Int 2001;12:989–95).

Results: Data were collected on 36 women ages 48–74 years. All women had been taking HRT for >5 years (5–19 years; mean 9 years) and had either stopped HRT within the previous 6 weeks or were planning to stop. Four women had had previous fractures- only one was a fragility fracture (74-year-old women with a forearm fracture 20 years previously). No one had been on steroids or had a condition associated with osteoporosis. Though the DXA scan referral stated "family history of osteoporosis" for 13 women, no woman had a history of maternal hip fracture <70 years old. Mean BMI was 25 (range 18–35). Mean BMD T scores were: LSp + 0.20 ± 1.5; total hip −0.28 ± 0.99; femoral neck −0.68 ± 1.07. The 10-year risk of (any type of) fracture was <6% in 7 women overall (19%), <11% in 28 women overall (78%), <16% in 31 women (86%) and <21% in all 36 women. Mean age of those with 0–5% risk of any fracture over the next 10 years was 55 years, of a 5–10% risk was 58 years, of a 10–15% risk was 61 years and of a 15–20% risk was 67 years.

Conclusions: The 10-year fracture risk in a large majority (78%) of postmenopausal women age 48–74 years who have no major osteoporosis risk factors but have had HRT

for >5 years is 10% or less. Where there are limited resources, DXA scans are not necessary in the majority of women <60-year-old stopping HRT if the HRT has been taken for 5 years or more.

P398-Mo

Bone Mineral Density Fails to Predict the Presence of Significant Vertebral Deformities in type 2 Diabetic Women

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Background: Type 2 diabetics tend to have higher bone mineral density than non-diabetics; however, non-vertebral fracture risk is increased in diabetic patients. We previously demonstrated that unsuspected significant vertebral deformities are highly prevalent in type 2 diabetic women, yet over 65% of those affected had normal antero-posterior spine and total hip T-scores.
Objectives: To determine the bone mineral density site that best predicts the presence of significant vertebral deformities in type 2 diabetic women at our center.
Methods: We retrospectively identified 116 type 2 diabetic women (median: age 67, BMI 32.27) having multi-site Dual X-ray Absorptiometry scans and a single energy lateral Instant Vertebral Assessment scan performed in 2003. A single team performed, analyzed and interpreted scans. Quantitative morphometry of vertebral height defined a significant deformity as >20% reduction in height. Patients' bone mineral density scans were classified at each anatomic site as normal (T-score > -0.99) or abnormal (T-score < -1.00).
Results: 75 (64.7%) had significant deformities (median: age 68, BMI 32.35); 41 (35.3%) had none (median: age 64, BMI 32.18). See table for relationship of bone mineral density/deformities.
Conclusions: No anatomic site had sufficient sensitivity or specificity to assist in ruling in/out the presence of significant vertebral deformities. Of all anatomic sites, an abnormal bone mineral density scan at the total hip had the greatest positive predictive value for a patient to have a significant vertebral deformity. No anatomic site had adequate negative predictive value, such that a normal bone mineral density scan did not rule out the presence of a significant vertebral deformity. Instant vertebral assessment is vital to rule in/out the presence of vertebral deformities in type 2 diabetic women.

Table
Bone mineral density at anatomic sites

Anatomic site	Sensitivity (%)	Specificity (%)	+pred value of abnormal T	−pred value of normal T
A-P spine	30.1	69.2	65.7	34.2
Lateral spine	80.8	26.5	62.7	47.4
Femoral neck	46.7	63.4	70.0	46.4
Total hip	34.7	85.4	81.3	41.7
Total forearm	54.1	64.1	74.1	42.4

Relationship to significant vertebral deformities.

P399-Tu

PQCT Accuracy and Prediction of Bone Strength at the Distal Radius

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Quantifying the determinants of bone strength is essential to understanding if or how the structure will fail under load. Determining failure requires knowledge of material and structural properties. However, characterizing the relative contributions of structural parameters of bone to overall bone strength has been difficult to date because of limitations in imaging technology. Peripheral quantitative computed tomography (pQCT) can estimate bone strength in the limbs; it is a relatively safe technique to differentiate cortical from trabecular bone and assess bone geometry and density. However, in a compromised osteoporotic skeleton, cortices are thin and low scan resolution can result in an inability to provide an accurate analysis. Therefore, in this 3-part investigation we scanned ten pairs of fresh-frozen radial specimens [female, mean (SD) age 79 ± 6 years] using pQCT (XCT 2000) at the 4, 6, 8, 10 and 30% site of the distal radius using four different acquisition resolutions (200, 300, 400, 500 μm). We evaluated (1) the accuracy of the Norland/Stratec XCT 2000 pQCT to assess low density bones by comparing pQCT outcomes to ashing and histomorphometry; (2) failure load in axial compression; and (3) the ability of structural parameters by pQCT and areal bone mineral density (aBMD) by DXA to predict bone strength at the distal radius. Using histomorphometry and ashing as criterion standards, we found that lower resolution (500 μm) pQCT scans underestimated total area and mineral content at the 30% site. In the failure studies, total bone mineral content at all sites was strongly associated with failure load. In particular, combined pQCT parameters accounted for up to 90% of the variance. The best DXA predictor was total density at the distal third site and it explained 83% of the variance. In summary, (1) low acquisition resolution can underestimate bone parameters at the radial midshaft and (2) pQCT can provide improved estimates of bone strength at the distal radius compared to DXA.

P400-Su

Bone Mineral Density in Hemodialysis Patients

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Ten male and eight female patients on maintenance hemodialysis (HD) treatment for more than 20 years were enrolled in this study from the outpatient clinic of our institution. The mean age was 56 years, and the duration of HD 286 months at the investigation. The radiographs of the hip joint were

evaluated for the presence of bone cysts and joint space narrowing. Body mass index (BMI) was calculated as the ratio of weight (kg)/height (m). Bone mineral density (BMD) was measured at the 1/3 distal radius by dual-energy X-ray absorptiometry. Serum levels of total calcium (Ca), phosphorus (P), alkaline phosphatase (Alp), intact-parathyroid hormone (PTH), and beta-2-microglobulin (BMG) were measured. Bone cysts were found in 12 patients (67%) and joint space narrowing was found in 4 patients (22%). The radiographic abnormalities were frequently bilateral in bone cysts (82%) and joint space narrowing (92%). Mean BMI was 19.9 kg/m. Mean BMD was 0.462 g/cm. The mean values of Ca, P, Alp, PTH, and BMG were 4.4 mEq/L, 6.7 mg/dl, 294.1 IU/L, 12.9 pg/ml, and 39.2 μg/ml, respectively. Hip arthroplasties were performed in 6 patients suffering from femoral neck fracture due to bone cysts (4 patients) and joint space narrowing (2 patients). All of 4 femoral neck fracture patients showed marked bone loss (mean 0.371 g/cm). The majority of long-term survivors (89%) showed hip joint alterations resulting in deterioration of activities of daily living. We should take care for preventive measures both of therapeutical and rehabilitative type.

P401-Mo

The Impact of Obvious Vertebral Degenerative Lesions on the Diagnostic Value of Bone Mineral Density Measurements

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The presence of degenerative lesions in the lumbar spine often hinders the precise evaluation of the DXA scans that measure the metabolically most active vertebral trabecular bone mineral density (BMD). The aim of this study was the evaluation of the alternative anatomic sites that might offer information about the status of trabecular bone. Ninety-five postmenopausal women [age 59.8 ± 5.5 years (mean value ± 1SD), age at menopause 50 ± 3.1 years, years since menopause (YSM) 9.8 ± 5.4, BMI 28.4 ± 4.6 kg/m^2] with obvious degenerative lesions in the DXA scans of their L1–L4 vertebrae were included. A BMD measurement was performed in all the women in areas of mixed bone [femoral neck (FN)] and trabecular bone [trochanter (TR)]. The BMD value of the least dense vertebra (LDV) was also evaluated. There were no significant differences between the age-adjusted (Z score) and T score values of L1–L4 vertebrae, FN and TR. The Z and T score values of the LDV were significantly lower than those observed in the 3 other anatomic areas ($P < 0.001$). The percentages of osteoporosis-osteopenia, based on the T score values of the 3 anatomic areas, (WHO criteria) did not significantly differ. The same percentages were significantly higher compared to all other sites, when based on the LDV T score values. A significant positive correlation was observed between the absolute and age-adjusted BMD values of L1–L4 and either FN ($r = 0.42$ and 0.56, $P < 0.001$) or TR ($r = 0.44$ and 0.57, $P < 0.001$), but the positive correlations between FN and TR respective BMD values were remarkably stronger ($r = 0.76$ and 0.77). In conclusion, the presence of obvious vertebral degenerative lesions in DXA scans, does not seem to lead to underestimation of the prevalence of osteoporosis-osteopenia. In such cases, the evaluation of the least dense vertebra significantly increases the same prevalence. In cases of obvious vertebral degenerative lesions, the association between the BMD values at other anatomic sites or at the least dense vertebra and the fracture risk, might reveal their diagnostic value for screening purposes.

P402-Tu

Discrepancies Between Individual Vertebral Bone Mineral Density Values in Healthy Males

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The presence of significant differences between the bone mineral density (BMD) values of individual vertebrae has not been extensively studied. The aim of this study was the evaluation of the prevalence of such differences amongst males. In 125 healthy men aged between 25 and 60 years, BMD measurements of trabecular bone [L1–L4 vertebrae, trochanter (TR)] and mixed trabecular-cortical bone [femoral neck (FN)] were performed by DXA. None of the men suffered from any disease or received any medication affecting bone metabolism. Men with obvious degenerative lesions in their vertebral DXA scans were excluded. In 31 (24,8%) of them (group A, age 45.8 ± 8.5 years, BMI 27, 06 ± 3.6 kg/m2), the BMD value of the significantly least dense vertebra (SLDV) was less than 90% than that of the immediate denser one. The remaining 94 men (age 41.8 ± 8.2 years, BMI 26.4 ± 3.1 kg/m^2) comprised group B. The men in group A were significantly older than those in group B ($P < 0.05$). Between 83 healthy men aged less than 50 years, 16 (19%) carried an SLDV. Among the L1–L4 vertebrae, L2 accounted for 39% of SLDV, L1 for 32%, L4 for 23% and L3 for 6%. The mean L1–L4 and TR BMD values were lower, but not significantly, in group A. The FN BMD values were significantly lower in group A ($P < 0.05$). The age-adjusted BMD values (Z scores) of all anatomic areas did not significantly differ between the two groups. In group A, a significant positive correlation was observed between the SLDV BMD values and the BMI ($P < 0.05$). In both groups, the correlation between the FN and TR BMD values was stronger than the one between the FN and mean L1–L4 respective values. In conclusion, major differences occur between the BMD values of individual vertebrae in a remarkable proportion of healthy men. L2 is the most

frequent and L3 the most uncommon significantly less dense vertebra. The mean L1–L4 BMD measurement by DXA may conceal the presence of a vulnerable vertebra, underestimating the vertebral fracture risk.

P403-Su

Assessment of Bone Mass Change by DXA vs. DXR in Postmenopausal Women Treated with Alendronate

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Purpose: The primary endpoint was to compare by two different methods, Digital X-ray Radiogrammetry (DXR-BMD) and Dual-energy X-ray Absorptiometry (DXA), the variation in bone mineral density (BMD) in a group of postmenopausal women. The secondary endpoint was to assess the BMD change in the same group of women following the treatment with alendronate (ALN) 70 mg once weekly by both these methods.
Materials and methods: Of the 2675 healthy women referred to our bone densitometry service using DXR-BMD (Pronosco X-posure SystemTM) 1830 were postmenopausal. Using the WHO criteria for osteoporosis diagnosis, 698 women were found to have osteopenia and 318 osteoporosis. In 53 women with osteoporosis BMD was also measured by DXA lumbar spine (Hologic). The mean age of osteoporotic patients was 68.2 years. This group of women received ALN 70 mg once weekly for 12 months. At the completion of treatment BMD was determined by both methods.
Results: DXA confirmed osteoporosis levels of BMD in all cases diagnosed by DXR. After 12 months of treatment with ALN 70 mg once weekly, the absolute mean levels of BMD by DXR showed an increase from 0.433 to 0.452 g/cm^2, that is 4.3% per year. With DXA lombar spine the absolute mean levels had increased from 0.693 to 0.726 g/cm^2, that is 4.7% per year. Mean BMD had increased significantly ($P < 0.001$) from baseline at the lombar spine and also at the metacarpal bones.
Conclusion: In the diagnosis of osteoporosis there is a good correlation between these two methods, DXR and DXA. In following up, the BMD change after the administration of ALN an almost similar yearly percentage increase by both methods was found. ALN 70 mg once weekly is safe. It produce increases in bone density. These results allow us to assert that DXR-BMD, largely used by us in the diagnosis and follow up of therapy in osteoporosis, is a reliable method, comparable with DXA.

P404-Mo

Effects of Technology and Operator Experience on Bone Densitometry Precision Error

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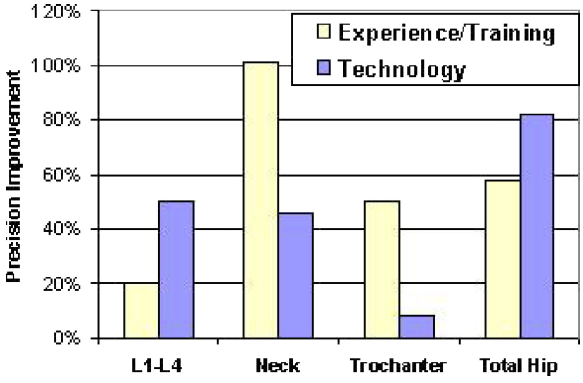
We examined the effect of operator experience and DXA technology on BMD precision by comparing precision (CV) at expert sites and non-research (clinical) centers. Expert CV was reported previously for 3 research sites using both GE and Hologic fan-beam systems [1]. Clinical CV was determined at 27 sites with fan-beam systems: 17 GE (2 Lunar Prodigy Advance, 15 Lunar Prodigy), 17 Hologic (3 Discovery, 6 Delphi, 8 4500). 7 clinical sites had GE and Hologic devices. Each center measured 30 subjects 2× or 15 subjects 3×, with 30-second scan modes and repositioning between measurements. Hologic BMD values were converted to Lunar equivalents [1,2] for comparison. Differences in average intra-manufacturer precision (RMS CV) between expert and clinical sites were attributed to operator experience. Differences in inter-manufacturer CV at expert and clinical sites were attributed to technology. Expert CV was superior to clinical CV: operator experience improved CV 20% at the spine and 50%–100% at the hip. There were significant inter-manufacturer CV differences based on technology. Use of GE technology improved CV at both expert and clinical sites by 50% at the spine, and 50%–80% at neck and total hip. Operator experience and DXA technology have significant independent impacts on spine and hip CV. Best precision is obtained with experienced operators and utilization of the most advanced DXA technology.
[1] Shepherd (2004) IOF/WCO, Brazil; P115SA.
[2] Osteoporos Int (2001) 12:438–444.



P405-Tu

Bone Mineral Density Measurement in Calcaneus with DXA: Prevalence of Osteoporosis in Female General Population

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Background: In recent years, several new devices based on X-ray have been introduced for measuring bone mass in peripheral locations. Moreover, the clinical use of these bone densitometry measurements raises a number of problems. Although forearm BMD was included in the

original WHO T-score definitions of osteoporosis, this criteria diagnostic cannot automatically be applied to diagnostic of osteoporosis.

Objectives: Evaluate different cut-points of T-score in calcaneus to establish the prevalence of osteoporosis in female general population.

Methods: We studied 727 women obtained from the cohort Hortega. Men and women between 18 and 84 years were selected from the population census of Valladolid. We sent a mail questionnaire. The response rate was 34.35%. A group of 727 women were selected randomly by age and sex in a second stage. The densitometric studies were carried out in the calcaneal region using a dual-energy X-ray densitometer (Pixi-Lunar, Lunar Radiation, Madison, WI, USA). The variation coefficient of the method was 1.5%. All patients gave their written informed consent.

Results: The table 1 show the prevalence of osteoporosis according to age and T-score.

The prevalence of osteoporosis with T-score <−1.6 is 12.4%. The same prevalence of osteoporosis (12.4%) was obtained in female population in Spain with a spine/hip DXA.

Conclusions: A cut-point of *T*-score <−1.6 in calcaneus densitometry (DXA) is useful in epidemiological studies de prevalence of osteoporosis in general population

Table

Age	−1.6 T (%)	95% CI (%)	−2 T (%)	95% CI (%)	−2.5 T (%)	95% CI (%)
20–29	2.2	0.8–5	1	0.5–3	0	0
30–39	6.2	2–10	1.5	0.5–4	0.8	0.7–2
40–49	1.5	0.6–4	1.5	0.5–4	0.8	0.7–2
50–59	11.5	4–19	7.7	2–14	3.8	0.4–8
60–69	15.9	7–25	9.5	2–17	3.2	1–8
70–79	32	25–39	19	13–25	10.3	6–15
>80	41	28–54	27	15–38	18	8–28

P406-Su

Identification of Women with Spine or Hip Osteoporosis Using Bone Mineral Density at the Calcaneus

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Background: Bone mineral density measurement at peripheral skeletal sites can be used to asses the risk of fracture in postmenopausal white women. Peripheral equipment cost less and takes up less space that conventional DXA systems, is simple operate and makes BMD investigations more accessible in the community.

Objective: Evaluate the clinical usefulness of bone mineral density at the calcaneus as a tool to identify patients with spine or hip osteoporosis.

Methods: 58 postmenopausal women with mean age of 66 ± 8 years attending in a osteoporosis clinic were measured by pixi-DEXA (Lunar Corp, Madison, Wisconsin, USA) and by DXA (Lunar DPX-L, Corp, Madison, Wisconsin, USA) in spine and femoral neck. We use the following T-score calculated from measurement at the calcaneus bone, <−1, <−1.6, <−2, <−2.5. The variation coefficient of the method was 1.5%. Osteoporosis was defined by T-score <−2.5 (spine or hip DXA). All patients gave their written informed consent.

Results: The results are showed in Table 1.

Conclusions: (1) Patients with T-score less than −2.5 must be treated. (2) Patients with T-score between −1 and −2.5 must be referred for spine and hip BMD measurement. (3) Patients with T-score greater than −1, without other risk factors of osteoporosis, are not osteoporotics.

Table

	<−1 T	<−1.6 T	<−2 T	<−2.5 T
Sensitivity	85	77	72	31
Specificity	46	72	66	91
PPV	56	69	82	73
NPV	79	79	52	62

P407-Mo

An Investigation of the Differences in Geometry of Proximal Femur According to Sexuality and its Relation to Femoral Bone Mineral Density

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108 male and 452 female subjects were studied in order to investigate the effects of sexual differences on femoral geometric measurements and their relation to femoral bone mineral density.

Hip axis length, femoral neck width and neck/shaft angle were measured on DEXA scan printouts.

The mean values of hip axis length, femoral neck width and neck/shaft angle were found as 7.33 cm, 2.18 cm, 128.86° in men and 6.33 cm, 1.85 cm, 127.68° in women respectively. The values of femoral and lomber BMD and also the measurements of proximal hip geometry were found higher in men ($P < 0.005$). Despite the age was found to have no effect on femoral geometric measurements ($P > 0.05$), the weight and height had significant effect on hip axis length and femoral neck length ($P < 0.05$) except for the femoral shaft angle ($P > 0.05$). After correction for weight and height, these differences persisted in both sexes. By using stepwise regression analysis, 96.2% of the changes in femoral BMD could be explained by femoral geometric measurements.

Subjects were classified into 3 groups according to femoral total *t*-score as normal, osteopenic and osteoporotic and femoral geometric measures were compared between the groups after correction for age. It was found that femoral geometric measures increased gradually from normal to

osteoporotic group in both sexes. In all three groups of men, differences were statistically significant in femoral axis length and femoral shaft angle ($P < 0.05$) except for the femoral neck width ($P > 0.05$). In women, only the difference in femoral shaft angle was statistically significant ($P < 0.05$).

As a result, after the correction for weight and height, femoral geometric measures were higher in men osteoporotic group compared to normal and osteopenic group. Furthermore the changes in femoral BMD might be explained by femoral geometric measurements with high probability (%96.2). Related to all our findings, we conclude that, higher incidence of hip fractures in Turkish men population might be linked to femoral geometric measurements, together with the other possible risk factors.

P408-Tu

Hip or Spine for DXA Measurement in a Czech Population Over Age 65—Is Hip BMD Really More Sensitive for Detection of Osteoporosis?

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Due to the common presence of degenerative changes in a population over age 65, which may increase lumbar spine bone mineral density (BMD) (1), it is recommended to base osteoporosis diagnosis on hip BMD measurements (2). We explored validity of this recommendation for the Czech patients who were measured at our densitometry center: we evaluated in this age group if hip BMD is more sensitive for diagnosis of osteoporosis compared to lumbar spine BMD. Spine (L1–L4) and left Total Femur BMD scan data were obtained from May 2000 through August 2004 on patients >65 years, irrespective of other medical conditions or therapy. Patients were routinely scanned on Lunar Prodigy scanner (GE Healthcare), software version 3.5 (5.6). The German reference database provided by the manufacturer was used for analysis.

Our data (see Table) suggest that the percentage of female patients diagnosed as being osteoporotic differs considerably depending on the region of interest (ROI). Diagnostic sensitivity of lumbar spine BMD appears to be almost twice as high as that of total hip. Surprisingly, we could not prove such finding in the male population where diagnostic sensitivity of spine and total hip BMD were equal. Our results do not support the hypothesis that lumbar spine BMD is less sensitive in terms of osteoporosis diagnosis compared to hip BMD. Our findings that hip T-score is somewhat higher compared to spine T-score are rather in line with some other published data (3). Potential causes for the observed discrepancy (reference population, bone edge detection algorithm, etc.) will have to be explored.

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[2] Arlot ME, Sornay-Rendu E, Garnero P, Vey-Marty B, Delmas P. J Bone Miner Res 1997;12:683–690.

[3] Miller PD, Calcif Tissue Int 2000; 66:317–319.

Table

Gender	ROI	No. of subjects	Age average	No. with T <−2.5	% with T <−2.5
Female	Total hip	11,225	72.3	1794	15.95
Female	L1-4	11,662	72.4	3698	30.87
Male	Total hip	1012	72.8	157	15.5
Male	L1-4	1033	72.8	176	17.06

P409-Su

Comparison Between Left and Right Hip DEXA Measurements

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Objective: To compare DEXA measurements between left and right hip. Also, to evaluate the difference in diagnosis of osteopenia (op) or osteoporosis (OP) and in administration of treatment if bone density was measured in only one of two hips.

Methods: Bone density at both hips was measured by DEXA (Lunar Prodigy) in women. According to WHO criteria, OP and op are defined as *T*-score below or equal to −2.5 and between −1 and −2.5, respectively. Based upon National Osteoporosis Foundation guidelines, individuals with *T*-score below −2.0 and without other risk factors for fracture are candidates for antiosteoporotic treatment. According to International Society for Clinical Densitometry recommendations for diagnosis of op or OP, the clinician takes the lowest *T*-score calculated from the following 3 sites: total hip, femoral neck, and total spine.

Results: Four hundred thirty-six women with mean age (±SD) 60.3 ± 11.3 (range: 25–94) years participated in the study. Hip OP had 83 (19%) women. The correlation between DEXA measurement of left and right femoral neck, and left and right total hip was 0.942 and 0.944, respectively. Mean *T*-score of femoral neck and total hip was −1.67 ± 1.08 and −0.88 ± 1.13 at left side, and −1.63 ± 1.14 and −0.90 ± 1.15 at right side, respectively. Comparing mean *T*-score between both sides, a statistically significant difference was found between left and right side only at femoral neck, but not at total hip (paired *t* test, $P < 0.05$).

In femoral neck, 48 (11%) women had discordance between both sides [normal (n)–op in 26 (6%) women and op–OP in 22 (5%)]. In total hip, 48 (11%) women had discordance between both sides [n–op in 33 (7.6%) women and op-OP in 15 (3.4%)]. So, 30 (6.9%) of the women [17 (3.9%) with op and 13 (3%) with OP] would have a mistaken diagnosis if only one hip was measured by DEXA.

Forty-eight (11%) and 25 (5.7%) of the women had *T*-score below −2.0 at only one of both femoral necks and total hips, respectively. Using a *T*-score threshold of −2.0, 18 (4.1%)

of the women would not received treatment if only one hip was measured by DEXA.
Conclusion: There was a very good correlation between left and right hip DEXA measurement. However, a significant number of women had different measurements at both sides that influenced diagnosis of op or OP and on decision for treatment. For this reason, DEXA measurement at both sides is suggested.

P410-Mo
The Lateral Distal Femur: An Alternative DXA Site For Children with Cerebral Palsy
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The lateral distal femur (LDF) is a site that is accessible in most children with cerebral palsy (CP) and at times is the only site that can be meaningfully measured by dual energy X-ray absorptiometry (DXA). The presence of metallic implants, positioning difficulties, and excessive movement often preclude the use of typical DXA measurement sites. Measuring this site is relevant to children with CP since the femur is the bone most commonly fractured. An overview of the LDF technique will be provided including how it is obtained, how scans are analyzed, and how we have used it clinically. We have used this technique in over 600 children with CP ranging in age from 2 to 21 years. Normative data have been published and the clinical utility of the scan site has been shown. The lateral distal femur is an accessible and meaningful site to measure by DXA in children with CP. Pediatric centers in the US are starting to use the LDF site for DXA on their children with CP.

P411-Tu
Do All Elderly Hip Fractures Have Osteoporosis?
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It is widely assumed that all elderly patients with hip fractures have osteoporosis and it is therefore advantageous to treat them, where possible, with bisphosphonates without prior DXA. This approach has been supported by The National Institute for Clinical Excellence (NICE) in the UK. Treating such individuals, however, adds to the burden of drug treatment, carries a significant cost, and in some patients will be associated with adverse effects. We investigated the notion that all elderly patients with hip fractures have osteoporosis by measuring spine (L1–L4) and femoral neck BMD on a Hologic 4500C system in all patients over the age of 75 years able to attend the Osteoporosis Unit. Patients who were admitted for hip fracture were assessed by osteoporosis nurses on the wards and if able to attend the Osteoporosis Unit were invited for a scan. The results of BMD measurements on consecutive individuals attending for BMD are presented. 127 patients have been investigated including 100 females, average age 80 years, and 27 males, average age 78 years. Among the females, the average T score was -2.07 (SD 1.46) lumbar spine and -2.45 (SD 1.79) femoral neck. 3 female patients could not have spine BMD and 15 hip BMD measured. Defining osteoporosis as $T < -2.5$, 41/85 (48%) patients were osteoporotic at the hip and 39/97 (40%) at the spine. Among 83 patients with measurements at both sites, 46/83 (55%) were osteoporotic at spine, hip, or both. Using a higher threshold of $T < -2.0$, 66/83 (80%) would fall below the threshold at one or both sites and for $T < -1.5$, 74/83 (89%) would fall below the threshold. The average T score at the spine for males was -1.08 (SD 1.74) and femoral neck -1.89 (SD 0.65). 24 patients had spine measurements and 25 hip measurements. 6/24 (25%) were osteoporotic at the spine and 5/25 (20%) at the hip. Among those with measurements at both spine and hip (Nos = 24), 8/24 (33%) were osteoporotic at one or other site. 13/24 (54%) had measurements below the $T < -2.0$ threshold and 19/24 (79%) below the $T < -1.5$ threshold. Using $T < -2.5$ threshold as defining osteoporosis, we conclude that many elderly patients with hip fractures are not osteoporotic. If treatment thresholds are set at higher levels than $T < 2.5$, it may be justified to treat without prior BMD although even at thresholds as high as $T < -1.5$ some patients would be inappropriately treated.

P412-Su
Comparison of the Lunar DPX-IQ and Prodigy Dual Energy X-RAY Absorptiometers in Assessment of Total Body Composition
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Aim: This study examined the Lunar® DPX-IQ (DPX) and the newer Prodigy (PRO) bone densitometers for total body measurement, especially total body fat mass (FM), percentage of fat mass (%FM), lean tissue mass (LM), and bone mineral density (BMD).
Methods: A total of 121 subjects (healthy volunteers or patients; 85 female and 36 male), aged 45.5 ± 13.5 years (17.9–74.3), participated in the study. After determining weight (kg) and height (m), subjects were scanned consecutively on the DPX (4.7d software version) and the PRO (enCORE v. 3.6 software).
Results: Paired t test showed significantly higher values with the PRO for FM (0.19 ± 1.00 kg, $P = 0.038$), %FM (0.51 ± 1.20%, $P < 0.0001$), and BMD (0.04 ± 0.11 g/cm^2, $P = 0.0002$). Conversely, LM measures were higher with the DPX (0.90 ± 1.15 kg, $P < 0.0001$). FM, %FM, LM, and BMD correlated strongly ($P < 0.0001$), but regression analysis determined that slopes for these four parameters

were significantly different from the line of identity. Bland Altman plots demonstrated no discrepancies for BMD between PRO and DPX (R^2 = 0.0001, P = NS), and slightly higher values by the PRO for the FM (R^2 = 0.06, P < 0.01) and the %FM (R^2 = 0.03, P < 0.05). After exclusion of three subjects with the greatest measurement differences of %FM (−4.59 to −3.52%), Bland Altman plots demonstrated no discrepancies (P = NS) between PRO and DPX for any of the four parameters (BMD, FM, %FM, and LM) in the remaining 118 subjects.

Conclusions: These data demonstrated that total BMD, FM, %FM, and LM values obtained with these two instruments were highly correlated. The slightly higher values for fat mass observed with the Prodigy were insignificant in clinical practice. Cross-calibration equations should make it possible to compare measures assessed with these two scanners.

P413-Mo

The Impact of Nice Guidance On An Osteoporosis Service

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The National Institute of Clinical Excellence has developed guidance for the treatment of post-menopausal women with a previous fragility fracture. We have reviewed these guidelines using data collected by an osteoporosis nurse working in a fracture clinic.

Method: 668 patients with low trauma fractures were seen by an osteoporosis nurse during a 2-year period (1999–2001). The majority had peripheral fractures and the study included both males and females. All these patients had an initial estimation of bone density by quantitative ultrasound (QUS) of the calcaneum. Those with a T score of −1.0 were offered DEXA of the lumbar spine and hip. The study involved patients between the ages of 40 and 80 years.

Results: Patients over 75: 38 of the 252 (15%) who had a QUS T score >−1.0 were over 75. 83 had a QUS <−1.0 and were offered DEXA and 73 attended. 3 had normal BMD, 21 were osteopenic but with a Z score >−1.0. 49 were osteoporotic with 25 having a Z score >−1.0.

Patients 65–74: 60 patients had a QUS T score >−1.0 and were not sent for DEXA. 7 of the 149 referred for DEXA had a normal BMD. 57 were osteopenic with a Z score >−1.0. 32 had a T score between −2.5 and −3.0, and 20 of these had a Z score >−1.0.

Patients <65: 154 had QUS >−1.0 and were not offered DEXA. 180 had a DEXA. 24 had normal BMD. 30 of the 89 osteopenic patients had a Z score of <−1.0. 31 had a T score between −2.5 and −3.0, and 24 <−3.0.

Discussion: Patients over 75: In those with peripheral fractures in this study, 38 (31%) of those over 75 had a QUS T score >−1.0. Under our existing guidelines, we may have treated between 32% and 67% of the 73 patients who had a DEXA. Patients 65–74: The new NICE guidance suggests treating 71 of the 135 who attended for DEXA. We may have considered treatment for some of the osteopenic patients, if they had risk factors.

Patients <65: In our study, we had not offered DEXA to 154 (46%) of the 334 patients in this group. Under existing guidelines, we would have considered treatment for 30 of the osteopenic patients with a Z score <−1.0 in addition to the 54 osteoporotic. NICE guidance would suggest that only 24 should definitely be offered treatment.

Conclusion: DEXA should be offered to patients over 75 with a peripheral low trauma fracture to prevent inappropriate treatment. NICE guidance is likely to reduce the number of patients under 65 being offered treatment but many may well require follow up.

P414-Tu

Young Normal Reference Values for DXA BMD in European Men and Women. Results from the Network for Male Osteoporosis (NEMO) Study

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It currently remains unclear whether young normal BMD reference values specific to USA population can be validly used for T-score calculation in European populations. As part of the Network for Male Osteoporosis (NEMO) study, we collected population-based BMD data from 263 men and 330 women aged 19–29 years (mean = 25 years, SD = 3) from 13 centers across Europe with a view to comparing mean and SD values for BMD in men and women with those published by the NHANES III study. BMD was measured at the femoral neck, trochanter, and/or L2–L4 spine using mainly pencil beam DXA densitometers manufactured by Hologic (n = 3), Lunar (n = 10), and Norland (n = 1). Cross-calibration was done with the European Spine Phantom (ESP). The only exclusions were for technically inadequate scans. A linear regression model was used to derive reference values. In men, the mean BMD values in g/cm^2 at age 25 years (95% CI) and standard deviations (SD) were: femoral neck 0.997 (0.978, 1.016) SD = 0.158; trochanter 0.827 (0.810, 0.843) SD = 0.135; and L2–L4 spine 1.102 (1.085, 1.119) SD = 0.138. The respective estimates in women at age 25 years were: femoral neck 0.871 (0.857, 0.884) SD = 0.123; trochanter 0.694 (0.683, 0.705) SD = 0.101; and L2–L4 spine 1.059 (1.046, 1.072) SD = 0.117. The hip results compared with the NHANES III results in ESP standardized units (no NHANES III data for L2–L4 spine were available). In conclusion, the BMD means and SDs in these European subjects differed little from the NHANES III results from the USA. These findings have implications

for the continued use of *T*-scores based on NHANES III in Europe.

P415-Su

Blood Pressure and BMD in a Spanish Early Postmenopausal Women Population

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Objective: The aim of this study was to investigate the relation between the bone mineral density (BMD) and the blood pressure (BP) in a Spanish early postmenopausal women population.
Methods: 183 women aged 50–55 years (mean 52.6 year, 95% CI: 53.4–52.9 year) were randomly selected in the province of Albacete (Spain). All women were postmenopausal, from 6 to 36 months, and they did not have diseases or taking drugs known to affect bone metabolism. BP and heart rate (HR) were measured by an Omrom 705CP device. BMD was measured 1 year later by DXA at the AP lumbar spine (L2–L4) by a Norland XR 26 Mark 2 densitometer. Statistical analyses were performed by Pearson's correlation coefficients and Student's test. A value <0.05 was accepted as a level of significance.
Results: The statistics (M, CI 95%) were: systolic blood pressure (SBP) 126 mm Hg (123.5–128.6); diastolic blood pressure (DBP) 80.5 (79.1–81.8); HR 71.3 b/m (70–72.6); 0.136 g/cm^2. There was no correlation between the BMD and±BMD 0.940 SBP (r = 0.111, P = 0.136), DBP (r = 0.143, P = 0.053), and heart rate (r = 0.014, P = 0.848). The Student's test did not show any difference between hypertensive and nonhypertensive women. These are the data of the BMD (g/cm^2): SBP-Hypertension 0.973 ± 0.158(n = 33), SBP-No hypertension 0.933 ± 0.130 (n = 150), P = 0.124. DBP-Hypertension 0.959 ± 0.144 (n = 30), DBP-No hypertension 0.936 ± 0.134 (n = 153), P = 0.395.
Conclusion: We did not found correlation of the BMD at the lumbar spine to the blood pressure nor the heart rate in early postmenopausal women.

P416-Mo

Volumetric Spatial Decomposition of Trabecular Bone—A New Method for Local Bone Morphometry

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Introduction: Bone microarchitecture is believed to play a key role in determining bone quality and the competence of bone. To improve the understanding of bone mechanical competence, it is therefore important to have a method to separately analyze individual elements of the bone microarchitecture on a trabecular level.
Materials and methods: A new algorithm for the volumetric spatial decomposition of trabecular bone samples into its basic elements (rods and plates) was developed and validated on computer-generated models. This new algorithm was applied to 328 human trabecular bone samples harvested from 70 donors at five different anatomical sites (calcaneus, femoral head, iliac crest, lumbar spine 2, and lumbar spine 4), which were previously scanned by micro-computed tomography. Standard three-dimensional morphometric algorithms were used to analyze the trabeculae on an individual basis with respect to their volume, surface, and thickness. The results were statistically compared for the five sites.
Results and conclusions: In this study, it was possible, for the first time, to spatially decompose trabecular bone structures in its volumetric elements; rods and plates. The size of the largest element in the structures showed significant differences for the five compared sites. In samples from femoral head, we found that basically one major element was spanning through the whole structure, whereas in lumbar spine and calcaneus, smaller elements dominate. From this we suggest that the strength of strong, dense plate-like structures is determined by the major elements whereas in looser rod-like structures the competence is given by the arrangement, quality, and shape of a whole set of elements. Furthermore, we found that globally determined structural indices such as the mean curvature of the bone surface or related to this the structure model index (SMI) are almost exclusively explained by the arrangement of the plates. This also suggests that rods hold independent information characterizing the mechanical competence of trabecular bone, especially in the spine. These findings may improve the understanding of the site-specific role of bone microarchitecture in determining bone quality and the competence of bone.
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P417-Tu

Dissociation Between Tibia Stress—Strain Indexes (RESISTOMETRY) with Bone Mineral Content (DENSITOMETRY). Example-Cases Revealed with a PQCT System

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Bone planar density (DXA) is being used as a surrogate of bone resistance in spite of having been observed for its imprecise correlation with fracture rates. Other bone strength indexes include independent components such as volumetric bone mineral density (vBMD by QCT) at cortex and structural variables such as sectional areas, cross-sectional

moment of inertia, and/or estimation of trabecular apparent volume. Hence, "resistometry" comprises geometric variables apart from material properties. In practice, we often find cases where bone resistometry is not associated with bone densitometry. Bone density (vBMD) and resistance (stress–strain index or SSI_{polar}) were calculated from peripheral QCT (XCT 3000, Stratec-Germany) scans at tibia mid-shaft. The individual dissociations were estimated as the rate of the respective percent deviation from reference values, showing how much deviated is resistance from density normal values. The table below describes example-cases from daily practice (arrows show status from limit-normal range).

Dissociation can be wider as limit-normal value has been considered, and its linearity has not been proven. This suggests that bone structure adaptation can be partially affected, besides bone material disturbances (osteopenia), and that true fragility occurs only when osteopenia and structural defects converge as with OI patient. It is here postulated that bone density and bone resistance index should be considered apart in practice, when DXA values do not match with the clinical picture of a given patient.

Table

Diagnosis	Patient features (gender/age)	$vBMD_{900}$ (mg/cm^3)	SSI_{polar} (mm^3)	Dissociation (SSI%/BMD%)
Parathyroid adenoma	male, 55 years	1118	3150↑↑	1.38
Marfan syndrome	female, 49 years	1167↑	1567	0.81
Aromatase deficiency	male, 33 years	949↓	3617↑↑	1.77
Osteogenesis Imperfecta	male, 19 years	998↓	368↓↓↓	0.26
Hypothyroidism	female, 61 years	1107	1200↓	0.94
Bifid spine	female, 25 years	1148↑	1105↓	0.88
Hip luxation	female, 64 years	1093	983↓↓	0.78
Hypoparathyroidism	female, 61 years	1099	1263	0.99
Early menopause	female, 45 years	1172↑	1733	0.90

P418-Su

Electron Microscopy for Investigation of Order/Disorder in Bone Structure

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Electron diffraction physics and phase-contrast imaging became an integral part of bio-medical examinations. Investigation of biomineralization mechanism in living organisms must include the composition (phase) identification, observation of morphology, and organization of the mineral units. Electron microscopy methods (scanning and high resolution transmission electron microscopy with image processing and simulation, X-ray energy dispersive spectroscopy) were applied for imaging the possible micro- and nanoarchitectural deterioration of bone tissue characterized by lowered mineral mass up to *T*-score = 3.7. Fragments of lumbar vertebrae (L1–L5) obtained from 10 patients of 45–62 years old after spine fractures were examined and compared with the structure of normal lumbar fragments extracted during the surgery because of a car accident. The goal of such investigation was to get the direct proofs of the widely accepted mechanism of elevated resorption activity of osteoclasts. The cells secreted the acid and erosion of bone occurred underneath. This can lead to the higher than normal dissolution rate of the main mineral component—hydroxyapatite. The dynamical equilibrium in calcium and phosphate ion metabolism is broken and therefore some changes in phase composition could occur and other stable calcium phosphate modifications aroused. For instance, the dicalcium phosphates and octacalcium phosphate phases can form at lower pH of aqueous medium. Also, the change in size of hydroxyapatite crystals and their orientation relatively to each other and the collagen fibers in osteoporotic bones might be expected. Chemical analyses of blood and urine of all patients demonstrated normal concentrations of calcium and phosphorus. Electron microscopy showed the tremendous overgrowth of organic tissue as non-mineralized fibers filled up the former paths for blood vessels while no changes in phase composition of mineral part, crystal sizes, and their arrangement on collagen fibers in bones were detected. All these facts require considerable attention for effective treatment of such kind of bone disorder.

P419-Mo

Bone Quality of the 3rd Phalanx Assessed by Two Different Ultrasound Devices—The Omnisense and the DBM Sonic 1200

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Introduction: Different types of quantitative ultrasound (QUS) devices are available for assessing parameters associated with qualitative bone characteristics. The DBM Sonic 1200 uses the transmission technique to measure amplitude-dependent speed of sound (ad-SOS). The Omnisense scanner works with access to the bone from only one side. It uses a single probe which is both a transmitter and a receiver. It measures the speed of an ultrasound wave

travelling through a few centimeters of bone parallel to its axis within the outer 2–6 mm.
Aim: To compare the results of phalanx measurements obtained by the DBM Sonic 1200 and the Omnisense scanner.
Methods: Sixty-two women were examined (age 67 ± 12 years, height 162 ± 7 cm, weight 63 ± 9 kg). Ad-SOS and SOS of the left 3rd phalanx were measured using the DBM Sonic 1200 and the Omnisense, respectively.
Statistics: Pearson's correlation analysis and multiple regression analysis with stepwise selection. Duplicate measurements of SOS and ad-SOS were made in 10 subjects. The precision error was expressed as the root mean square coefficient of variation (RMSCV).
Results: RMSCV of SOS and ad-SOS: <1%. SOS: 3743 ± 246 m/s, ad-SOS: 1860 ± 149 m/s. A significant correlation was found between SOS and ad-SOS (R-Pearson = 0.56, $P < 0.00001$). SOS and ad-SOS were also correlated with age and height, but not with body weight. In a multiple regression analysis with ad-SOS as the dependent variable and SOS, age, and height as independent variables, ad-SOS was weakly but significantly predicted by SOS (R-partial = 0.29, $P < 0.05$) and by age (R-partial = 0.28, $P < 0.05$).
Conclusion: A poor association was found between SOS and ad-SOS of the 3rd phalanx. This finding may reflect that different bone characteristics are measured by the DBM Sonic 1200 and the Omnisense scanner.

P420-Tu
"XTREMECT" A New Dimension in Bone Micro Architecture Evaluation in Vivo in Humans
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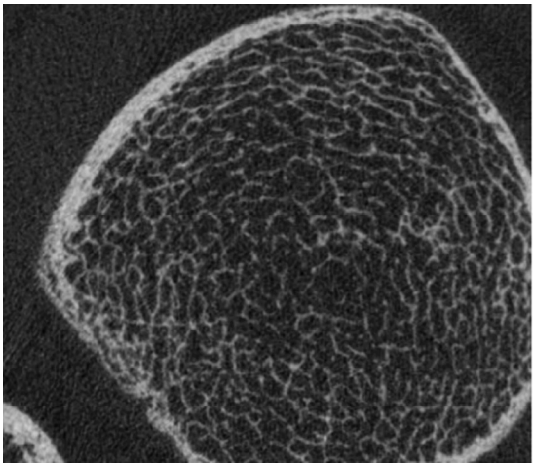
The quantitative and non-invasive 3D structure analysis in vivo became now available in humans with the help of high resolution pQCT (Densiscan-3D/XtremeCT, Scanco Medical Ltd). The new high resolution pQCT enables the simultaneous acquisition of a stack of 100 tomograms with a maximal diameter of 126 mm. The isotropic resolution is 120 μm (10% MTF, isotropic voxel size 82 μm). The tomograms are taken 7 mm proximal from the endplate of the distal radius and/or 20 mm of the distal tibia (see figure). The data acquisition time is about 3 min. The reproducibility is <1% for structure elements. In praxi, it is now routinely possible to quantify in vivo in humans the microarchitectural features as number of trabeculae, trabecular and cortical thickness, trabecular spacing, and endosteal surface in addition to the volumetric BMD of trabecular and cortical bone. Such quantitative characterization will give better insight in pathogenesis and treatment of osteoporosis.



P421-Su
Multi-Detector-Row CT Imaging of Vertebral Microstructure for Evaluation of Fracture Risk
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Bone mineral density (BMD) alone has limitations in predicting fracture, and techniques for clinical assessment of bone microstructure are awaited. We have developed a method to evaluate the axial trabecular microstructure in vivo using multi-detector-row CT (MDCT). We first determined the optimal scanning conditions of MDCT using excised human vertebrae and micro-CT images as reference. Each specimen was scanned by micro-CT at a spatial resolution of approximately 40 μm and by MDCT (SOMATOM plus 4 Volume Zoom) at a maximal spatial resolution of approximately 200 × 200 × 300 μm^3. Structural indices were calculated by using a 3D image analysis system (TRI/3D-BON). The following microstructure parameters were calculated: trabecular thickness (Tb.Th), trabecular number (Tb.N), and trabecular separation (Tb.Sp). Non-metric parameters were also calculated, including connectivity (Euler's number), structure model index (SMI), and degree of anisotropy (DA). To evaluate how this technique is useful in discriminating trabecular microstructure with and without recent (within 6 months) vertebral fracture, we examined

microstructural parameters obtained by MDCT from 64 postmenopausal women (60–80 years of age), including 33 without and 31 with spinal fracture who had not received drugs affecting bone mass for more than 6 months prior to examination. BMD values of the lumbar spine were determined by using dual X-ray absorptiometry (DXA and quantitative CT (QCT)). QCT values are also easily provided by MDCT scanning with a calibration phantom. There was no significant difference between the 2 groups in terms of age, age at menopause, body weight, or body height. Compared with postmenopausal women without fracture, those with fracture had a significantly lower BMD, fewer trabeculae (Tb.N.), more rod-like rather than plate-like structures (SMI), and lower connectivity (Euler's number). The correlation with the presence of fracture was much higher for microstructure indices, including SMI (odds ratio: 13.9), Euler's number (13.7) than for spinal DXA (odds ratio: 5.3 for DXA, and 9.1 for QCT). QCT and non-metric parameters showed higher odds ratios than DXA and metric parameters, respectively. Odds ratios of BMD were greatly improved by adding microstructural parameters. In conclusion, microstructural analysis by clinical CT is more useful than BMD for assessing fracture risk.

P422-Mo

Severity of Vertebral Fracture Reflects Deterioration of Trabecular Bone Microarchitecture

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Objective: This study describes the relationship at baseline between the severity of vertebral fractures defined by qualitative features and vertebral height reductions using visual semiquantitative (SQ) analysis of radiographs and trabecular microarchitecture analyses of iliac crest biopsy samples in a subset of patients from the teriparatide Fracture Prevention Trial (Neer et al., N Engl J Med 2001).
Materials and methods: Bone structure indices measured by 2D histomorphometry and 3D micro-CT in 74 and 51 patients, respectively, are shown for each baseline fracture SQ severity grade, classified by vertebral height loss.
Results: There were significant correlations observed among the 2D and 3D measurement groups ($r = 0.64$, $P < 0.01$ and $r = 0.82$, $P < 0.01$, respectively). Given the small sample size, consistent SQ vs. bone architecture relationship, and strong inter-dimensional measurement correlations, a multivariate ANOVA was performed for each set of 2D and 3D measurements. The results of both the 2D and 3D analyses suggest that a subject's maximum SQ grade is a strong predictor of the bone microarchitecture quality. An increase in vertebral fracture severity is characterized by a reduction in trabecular bone volume, impaired trabecular connectivity, and a transformation of trabeculae from plate-like to more rod-like morphology (2D, $P = 0.009$; 3D, $P = 0.07$, Wilks' Lambda multivariate test).
Conclusions: These results demonstrate that a deterioration of bone structure, with low trabecular bone volume, is a continuous and progressive process. Consistent with the suggestion from Parkinson and Fazzalari (J Bone Miner Res 2003), microarchitectural deterioration is exponential when bone volume falls below a critical value of ~15%. In conclusion, this correlation between baseline SQ vertebral fracture severity and histological features of bone quality deterioration explains the accelerated cascade of risk observed in patients with severe fracture.

Table
Bone quality and vertebral fracture severity, mean ± SD

	SQ0 (<20%)	SQ1 (20–25%)	SQ2 (25–40%)	SQ3 (>40%)
2D trabecular bone volume	0.25 ± 0.09 ($n = 6$)	0.17 ± 0.07 ($n = 23$)	0.14 ± 0.06 ($n = 35$)	0.12 ± 0.05 ($n = 10$)
2D marrow star volume	16.1 ± 24.0 ($n = 6$)	22.0 ± 30.9 ($n = 22$)	35.0 ± 36.1 ($n = 31$)	41.8 ± 33.6 ($n = 9$)
3D trabecular BV/TV	0.16 ± 0.05 ($n = 4$)	0.14 ± 0.07 ($n = 19$)	0.13 ± 0.05 ($n = 19$)	0.08 ± 0.03 ($n = 9$)
3D structure model index	1.41 ± 0.33 ($n = 4$)	1.79 ± 0.55 ($n = 19$)	1.96 ± 0.46 ($n = 19$)	2.12 ± 0.49 ($n = 9$)

P423-Tu

Age-Related Changes in Volumetric Bone Mineral Density and Femoral Neck Geometry

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Bone mineral density (BMD) is a well-recognized determinant of fracture risk. Although not so well known, several studies suggest that bone geometry parameters may also be important. Those results have been usually obtained from quantitative CT analysis or from DXA data analyzed with a special software to identify the narrow region of the femoral neck. Nevertheless, some of the parameters could be easily estimated from the usual output of clinical densitometers. Therefore, the objective of this study was to analyze the age-related changes in femoral neck geometry and volumetric BMD in normal subjects of both sexes, as estimated from the output of standard densitometry.

We studied 665 control subjects aged 22–87 years (390 women and 275 men). BMD was estimated by DXA (Hologic) at the spine (L2–L4) and the femoral neck. Volumetric BMD and bone geometry were calculated using published formulas.

In young subjects, areal BMD was higher in men than in women. However, the difference appeared to be related to the larger male skeleton; indeed, volumetric BMD was similar in both sexes. Hip areal BMD showed an age-related decrease in both sexes. However, only women showed a significant decrease at the spine. On the other hand, volumetric BMD decreased with advancing age at the spine and the hip both in males and in females.

There was an age-related increase in the average external diameter of the femoral neck in both sexes. On the other hand, the mean cortical thickness showed a significant reduction, particularly in women. Consequently, the section modulus decreased and the buckling ratio (an index of cortical instability) increased with advancing age in both sexes. Although the buckling ratio of young men and women was similar, the age-related increase was somewhat higher in women. Thus, the buckling ratio tended to be higher in old women than in old men.

Although these estimations may be less accurate than those obtained using special software, this study suggests that the standard DXA output may be used to estimate some parameters of femoral neck geometry related to bone biomechanics. Further studies are needed to establish their actual role as predictors of fracture risk.

P424-Su

Precise Alignment of Murine Femora is Mandatory for Accurate Assessment of Bone Strength

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Sensitive and precise methodologies to determine both genotype and phenotype are critical when evaluating genetic effects. Biomechanical testing is a straight-forward procedure to assess bone phenotype, but is challenging in view of the small size of murine bones, which are typically used in genetic studies; for genetically identical mice, large variations in strength have been reported, even within the same study. Therefore, the aim of this study was to assess the influence of femoral alignment on femoral neck strength as a possible cause for these variations.

We measured one murine femur with a muCT40 (Scanco Medical) using a 20-mm resolution. The digitally econstructed femur was rotated into a predefined, upright position using three computationally determined markers: (1) center of the femoral head; (2) center of a cross-section distal to the lesser trochanter; and (3) center of a cross-section proximal to the condyles. A microstructural finite element model was created for the proximal 70% of the femur. The model was solved using the element-by-element method for three orthogonal load cases; for all of them, a 10-N force was applied at the top of the femoral head. The results for arbitrary loading directions were determined by scaling and superpositioning of the results of the three initial load cases. Strength was estimated as the loading at which 2% of the elements in the femoral neck reached effective strain levels above 0.7%.

We found that the femoral neck strength depended strongly on the direction of loading; a small misalignment of 5° could already introduce a 13% change in strength; a 10° misalignment led to a 31% strength change; as an extreme case, strength was 198% higher when the loading was parallel to the femoral neck axis.

Two points for discussion. First, although bone failure is a non-linear event, experimental testing of similar femora showed that the force–displacement curve is nearly linear until abrupt failure occurs; hence, modeling of bone failure using a linear-elastic approach provides realistic results. Second, at present, the precise failure criterion is unknown. Therefore, we implemented different criteria; these resulted in different absolute strength values; however, relative changes were very minor.

In summary, we conclude that accurate alignment of murine femora is a critical step in the accurate assessment of bone mechanical properties; of course, this holds for computational as well as for experimental testing.

P425-Mo

Detection of Trabecular Discontinuities in the CT Images of Trabecular Bone

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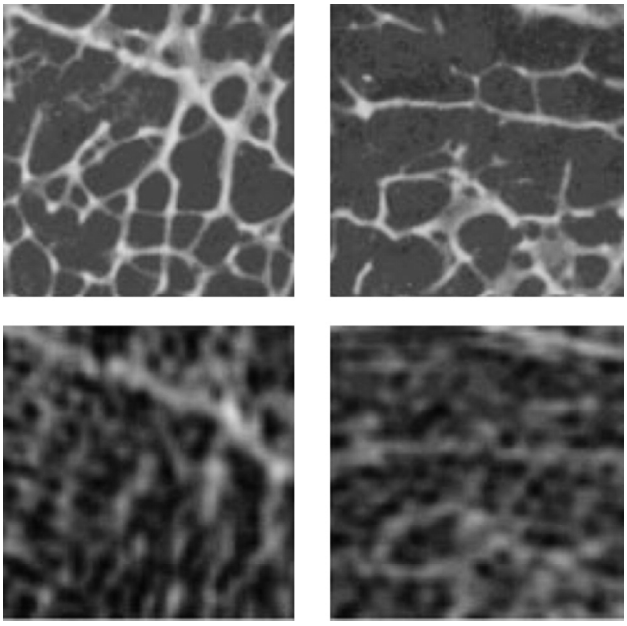
The density of free-end trabeculae has been shown to discriminate in vitro between fracture and non-fracture subjects matched for BMD. In this study, a method is proposed as a surrogate of the above-mentioned quantity. The method is designed, however, for application to the analysis of low-resolution images of trabecular bone, obtained in vivo. A phantom, built of 500-μm-thick sections of trabecular bone, was imaged with CT (low–resolution data) and mammographic X-ray system (high–resolution data). 20 discontinuous and 20 continuous trabecular regions of size 8×8 mm^2 were found in high-resolution images and then identified in low-resolution images (Fig. 1, top-high-resolution images, bottom–low-resolution images, left–continuous region, right–discontinuous region). The proposed method to distinguish between continuous and discontinuous regions utilizes the background of the percolation theory. The method is based on finding an optimal path, i.e., specially defined sequence of pixels, crossing brightest parts of the low-resolution image. It was confirmed that the parameters of the optimal path allow discriminating between continuous and discontinuous

regions, while the mean intensity as well as bone volume fraction is the same for both kinds of regions. It was also shown that segmentation destroys the information about the presence of discontinuity. The application of the method in the diagnosis of bone fracture is also discussed.



P426-Tu
An Efficient Finite Element Model for Accurate Prediction of Trabecular Bone Mechanical Properties in Man

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A quantitative assessment of bone mechanical properties is essential for the understanding of failure mechanisms associated with osteoporosis. As direct assessment of bone competence in vivo is not possible, it is inevitable to predict it using appropriate simulation techniques. Although accurate estimates of bone competence can be obtained from micro-finite element models (muFE), it is at the expense of large computer efforts. In this study, we investigated the application of structural idealizations to represent individual trabeculae by single elements. The objective was to implement and validate this technique.

We scanned 42 human vertebral bone samples (10-mm height, 7-mm diameter) with a muCT 40 (Scanco Medical) using a 20-mm resolution. After scanning, direct mechanical testing was performed. Topological classification and dilation-based algorithms were used to identify individual rods and plates. Two FE models were created for each specimen. In the first one, each rod-like trabecula was modeled with one thickness-matched beam; each plate-like trabecula was modeled with several beams. The models were solved using MARC (MSC Software, USA), assuming one isotropic tissue modulus for all elements. After reducing the voxel size to 40 mm, a second FE model was created using a standard voxel conversion technique and solved using the element-by-element method. Again, one tissue modulus was assumed for all elements in all models. Bone volume ranged from 3.7% to 19.5%; Young's moduli from 43 MPa to 649 MPa. Both models predicted measured apparent moduli equally well (R2 = 0.85) and were in excellent agreement with each other (R2 = 0.97). Tissue modulus was estimated at 9.0 GPa and 10.7 GPa for the beam and voxel models, respectively. On average, the beam models were solved in 250 s, reducing CPU usage up to 500 times. Relative to 20-mm voxel models, 8000-fold reductions can be expected.

The presented beam FE model is an abstraction of the intricate real trabecular structure using simple cylindrical beam elements. Nevertheless, it enabled an accurate prediction of global mechanical properties of microstructural bone. The strong reduction in CPU time opens up ways for research that was not possible before, such as the routine assessment of mechanical properties of (large) bone specimens. With upcoming in vivo high-resolution imaging systems, this model has the potential to become a standard in bone failure prediction.

P427-Su
Combining Bone Shape, Density, and Trabecular Structure to Improve Identification of Hip Fracture: A Pilot Study

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Hip fracture is one of the most serious consequences of osteoporosis; however, bone mineral density (BMD) only explains part of the difference between patients who do and do not fracture. Many other factors are also involved, including the risk of falling, bone turnover, and the structural properties of the femur. This study investigated the improvement in separation of the two groups gained by combining three different measures related to bone quality: the BMD, the shape of the proximal femur, and the trabecular structure. The shape of the femur and its trabecular structure were selected because they are known to effect fracture risk and can be measured automatically from radiographs.

This pilot study used automated measures of trabecular shape and structure which were at least as good as BMD for separating the fracture and control groups (area under the receiver-operating characteristic curve (Az) = 0.79 for femoral neck BMD, 0.81 for shape, and 0.84–0.93 for trabecular structure). Fifty standard pelvic radiographs were

used from age-matched fracture (26 subjects) and control (24 subjects) groups of postmenopausal women who also had BMD measurements from a DXA scan. The trabecular structure was analyzed at 5 sites perpendicular to its preferred orientation using a Fourier analysis, while the shape was measured using a statistical model called an active shape model. Neither the shape nor the texture measures were significantly correlated with BMD or with each other, indicating that in combination they may be more powerful to discriminate between the fracture and control subjects. Stepwise discriminant analysis was used to identify the best combination of variables to separate the subjects. This process selected one of the trabecular structure measures, the shape measure, and femoral neck BMD. The discriminant score from this combination was able to achieve near-perfect separation of the fracture and control groups (Az = 0.99). These results need to be confirmed in larger studies; however, they show that the combination of these uncorrelated variables can improve our ability to separate fracture and control subjects.

P428-Mo

Comparison of Measures of Complexity Derived from Peripheral Quantitative Computed Tomography Images with Static Histomorphometry and with Bone Strength Performed on Human Lumbar Vertebral Bodies

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When the voxel size of a pQCT scan is larger than the trabecular size, the density of each voxel represents contributions from several structural elements. Consequently, the density of each voxel carries structural information which is lost by binary threshold filtering. Symbolic dynamics is applied to the trabecular and to the entire (trabecular plus cortical) regions in order to extract structural information by encoding the voxels with 5 different symbols according to local differences in attenuation. A series of measures of complexity is then applied to the symbol-encoded data. The material comprised human vertebral bodies L3 and L4 from each of 13 women (75–98 years) and 10 men (57–88 years). Horizontal pQCT images were obtained with a Stratec XCT 3000 scanner at the transaxial center of the vertebrae with a slice thickness of 2 mm and a pixel size of 250 μm × 250 μm. The determination of measures of complexity was conducted using proprietary software. From one-half of each L4 a 9-mm-thick horizontal slice was obtained corresponding to the location of the pQCT scan. These specimens were embedded in methylmetacrylate, cut in 10-μm-thick sections, and stained with aniline blue. The sections were scanned into a computer with a pixel size of 10 μm × 10 μm and static histomorphometry was conducted using a computerized method. The entire L3 was compression-tested in Instron 5566 materials testing machine until failure. The bone strength was determined as the load at failure divided by the average cross-sectional area of the vertebrae. High correlations were established between different measures of complexity based on symbolic dynamics calculated from the trabecular bone region, and, in particular, trabecular bone volume, trabecular separation, and trabecular number obtained by static histomorphometry. Furthermore, high correlations were established between some measures of complexity and bone strength of the entire vertebral bodies obtained by compression testing. In conclusion, the proposed non-invasive technique may help to quantify alterations in bone architecture and may thus help to diagnose and monitor changes in the bone structure. This study was made possible in part by grants from the Microgravity Application Program/ Biotechnology from the Manned Spaceflight Program of the European Space Agency (ESA).

P429-Tu

Biomechanical Analysis of pQCT Indicators of Bone Health in Forearms and Legs of 200 Normal Men and Pre- and Post-Menopausal Women

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Aim: To analyze the normal values of pQCT indicators of bone mass, volumetric density, geometry, and strength, and muscle mass in forearms (sites 4% and 66% proximal to the wrist joint) and legs (sites 4%, 14%, 38%, and 66% proximal to the ankle joint) of normal men and pre- and post-MP women ($n = 40, 60, 100$) aged 25–85 years, beyond the DEXA scope. **Results:** Values of all indicators were generally congruent for the different sites studied. Bone mass (total/cortical BMC or cross-sectional area (CSA), trabecular vBMD), geometry (endo- and periosteal perimeters, cortical thickness, bending, and torsional moments of inertia (xMI, pMI)), and stiffness/strength (Bone Strength Index (BSI), Stress–Strain Index (SSI)) were significantly higher in men than women at any age. However, bone tissue mineralization (apparent density, as assessed by the cortical vBMD) was significantly higher in women than men until MP. All indicators declined after MP (especially the cortical vBMD), excepting only the MIs. The cortical bone distribution (MI, y)/mass (CSA, x) positive relationships showed similar slopes but higher intercepts for men than pre-MP women. Muscle CSA was significantly larger in men than women and decreased slightly after MP. **Interpretation:** Three different patterns of evolution and gender-related differences were detected for bone mass, tissue mineralization, and design, respectively. (1) Men have bigger and stronger bones (more massive, better architectural design, higher stiffness/strength indicators) than women at

any age. (2) Until MP, women have a denser (less porous) cortical bone than men. After MP, they loose bone mass and especially cortical density (enhanced porosity) rapidly. (3) However, post-MP women tend to maintain the architectural design of the cortical shell despite the cortical mass loss (suggesting the maintenance of an efficient homeostatic control of cortical bone distribution by bone mechanostat). Nevertheless, bone strength balance after MP is generally negative. Gender-related differences in muscle CSA paralleled those in most bone indicators. The post-MP decline of most indicators suggests changes in metabolic (bone mass and mineralization) rather than mechanical (bone design) factors. Congruent results in forearms and legs suggest little influence of gravity on these relationships. Data support a large influence of muscles, rather than body weight, in the physiologic control of bone structure and strength in both genders.

P430-Su

Interrelationships Between pQCT-Assessed Indicators of Bone Mass, Geometry, or Strength, and Muscle Strength in Forearms and Legs of Normal, Healthy Adults

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Aims: To analyze the biomechanical interrelationships between pQCT indicators of cortical bone mass (BMC, area (CSA)), mineralization (volumetric cortical BMD, vBMD), cross-sectional design (bending and torsional moments of inertia, xCSMI, pCSMI), or strength (bending and torsional Bone Strength Indices, xBSI or pBSI = xCSMI or pCSMI vBMD), and muscle strength (muscle CSA (MCSA)) in radial and tibial scans taken at sites 66% of the forearm or leg length proximal to the wrist and ankle joints of healthy men and pre-MP and post-women (n = 40, 60, 100).
Results: Indicators of bone mass, diaphyseal design, and strength (y) correlated positively with MCSA (x) following always single, nonlinear saturation functions for grouped men and pre-MP women data. Post-MP women showed less significant relationships, with lower slopes than those shown by the other groups, and no relationships between the CSMIs and MCSA. Suitably Z-scored, reference graphs of these relationships allowed to show a lower-right shift of post-MP women data, less evident for the CSMIs than for the other indicators. Calculated Z-scores of those relationships for the post-MP women decreased significantly with time since MP, excepting for the correlations between CSMIs and MCSA. No relationship was observed between cortical vBMD and MCSA.
Interpretation: Results reflect the significant impact of regional muscle strength on cortical bone mass, architectural design, and strength in normal adults. This impact tend to vanish after MP, more dramatically concerning bone mass and strength than diaphyseal design. The bone mechanostat system (a mechanism adapting cortical bone density and distribution to the history of strains derived from mechanical usage of the skeleton) would optimize the modeling-dependent cortical design in post-MP women, but not enough as to compensate for the negative impact of the remodeling-dependent decrease in vBMD. The Z-scored versions of the curves for men and pre-MP women provide reference charts for a non-invasive evaluation of muscle influences on bone mass, design, and strength, and the functional status of bone mechanostat specifically for men, pre-MP, and post-MP women, regardless of age and anthropometric traits, beyond the DEXA scope.

P431-Mo

Gender-Related Differences and Post-Menopausal Changes in the Cross-Sectional Distribution of pQCT-Assessed vBMD of Radial and Tibial Diaphyseal Bone in Healthy Adults

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Aim: To describe the gender-related differences and the influence of menopause (MP) on the distribution of previously defined regions of interest (ROIs) with high, medium, or low values of diaphyseal vBMD (pixels with attenuation coefficient values >1.0 cm^{-1}, HD; 0.4–1.0 cm^{-1}, MD; and <0.4 cm^{-1}, LD, respectively) in healthy men and pre- and post-MP women (n = 40, 60, 100) in pQCT scans of the sites 66% proximal to the wrist and ankle joints in the left radii and tibiae.
Results: Results were generally coherent for radii and tibiae. The percentage distribution of HD, MD, and LD pixel areas was similar in men and pre-MP women. The HD area was lower and the MD area was higher in post-MP than pre-MP women. The LD area showed little inter-group and post-MP variation. The HD area decreased proportionally to the years elapsed since MP (YSMP). A single, negative-exponential relationship between the percentual HD (y) and MD (x) areas of all the studied bones showed characteristic distribution zones, showing decaying values of the HD/MD relationship in the order: men > pre-MP women > post-MP women with up to 7 (forearm) or 9 (leg) YSMP > post-MP women with more YSMP. The post-MP loss of HD area determined also a loss of geometrical continuity of the HD ROI.
Interpretation: pQCT allows for an interesting, qualitative, and quantitative structural–biomechanical analysis of the volumetric density distribution of diaphyseal bone in men and women. Quantitatively, the proportion between the percentual HD and MD areas, similar in males and fertile females, decayed in women with time since MP. Qualitatively, the post-MP loss of HD area (representing the relative amount of the stiffest and strongest cortical tissue)

determined also a geometrical discontinuity of the respective ROI in the cross-section, which may have severe mechanical consequences. Congruence of results in forearm and leg suggests little or no influence of gravity on this aspect of skeletal physiology. The interdependence between the percentual HD and MD areas was reflected by the negative relationships observed between those variables in both forearms and legs when all bones were studied together. These curves provide reference charts suitable for evaluating the relative deterioration of the structure and mechanical ability of cortical bone (shifts toward the lower-right region of the graphs) in men and women in clinical studies, beyond the DEXA scope.

P432-Tu

Interrelationships Between pQCT Indicators of Cortical Bone Volumetric Density (vBMD) and Cross-Sectional Geometry (Distribution/Quality Curves) in Radii and Tibiae of Normal Adults

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Aims: To analyze the interrelationships between pQCT-assessed indicators of geometric properties (bending and torsional cross-sectional moments of inertia, xCSMI, pCSMI) and vBMD of cortical tissue in forearms (radial scans taken 4% and 66% proximal to the wrist joint) and legs (tibial scans taken 4%, 14%, 38%, and 66% proximal to the ankle joint) of normal, healthy men and pre- and post-MP women (n = 40, 60, 100) concerning the homeostatic control of bone structure. The CSMIs represent the architectural efficiency of cortical design concerning bending (xCSMI) and torsion (pCSMI), while the cortical vBMD can be regarded as a partial indicator of bone material's stiffness. Bending and torsional Bone Strength Indices (xBSI, pBSI = xCSMI or pCSMI vBMD) were also calculated.
Results: Men showed higher CSMIs and lower vBMD values than pre-MP women. Negative relationships between any of the CSMIs (y) and vBMD (x; distribution/quality curves) showed higher ordinates for men than pre-MP women. Post-MP women had lower cortical vBMD values for the same CSMI values than pre-MP women. Reference, Z-scored versions of the curves for pre-MP women allowed to show a shift to the left ("catabolic" region of the graph) of post-MP values. The calculated Z-scores of that relationship for post-MP women decreased significantly with time since MP. Multiple regression analyses showed a higher independent impact of bending or torsional CSMIs in men than women, and a higher influence of vBMD than CSMIs in women than men, on the determination of the respective BSIs.
Interpretation: Distribution/quality curves would reflect the optimization of diaphyseal design as a function of cortical tissue stiffness (known to vary linearly with vBMD). The "catabolic" shifts of post-MP women data in those graphs suggest a displacement of the mechanostat threshold for triggering negative-balance remodeling. In other words, bone mechanostat would still contribute to optimize MI despite of the bone mass loss, but not enough as to compensate for the remodeling-induced vBMD impairment. Bone strength (BSIs), sensitive to CSMIs rather than vBMD in men, is affected by vBMD rather than CSMIs in women, especially after MP. The developed Z-scored distribution/quality curves provide separate, specific reference charts for an original quantitative evaluation of the bone mechanostat status in men and pre- and post-MP women, independently of age and anthropometric characteristics, beyond the DEXA scope.

P433-Su

Assessment of the Microstructure of Human Vertebrae Using Volume CT: A Feasibility Study

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Purpose: Established methods to diagnose osteoporosis rely on bone mineral density (BMD). BMD alone is considered an insufficient predictor of bone strength. Structural information can be acquired at peripheral sites like the forearm, but since the correlation with central sites is rather low, a method measuring structural parameters, e.g., directly at the spine would be of interest. Volume CT (VCT) on interventional C-arm systems provides an isotropic spatial resolution on the order of 30 lp/cm. In this feasibility study, structural parameters of human vertebrae are measured and compared to the results of a μCT scan.
Methods: A total of 7 excised human vertebrae were imaged with a conventional C-arm X-ray system (Integris Allura, Philips Medical Systems, Best, The Netherlands). For precision measurements, 5 consecutive scans of each vertebra with interim repositioning were taken. Accuracy was assessed by comparison with a μCT (FanBeam μ-Scope, Stratec, Pforzheim, Germany). All scans had an isotropic resolution of 156 μm. Standard structural parameters were calculated after binarization. The threshold had a fixed value for all μCT scans but due to varying grey levels in the VCT images an adapted threshold had to be manually assigned to each vertebra. Variables measured include bone volume fraction BV/TV, trabecular number Tb.N, anisotropy, and connectivity density, all calculated in 3 dimensions using our in-house software Structural Insight.
Results: Structural variables are summarized in Table 1 together with accuracy and precision errors. Both anisotropy and connectivity density are systematically under-respectively overestimated.
Conclusion: The precision error of structural variables measured by VCT is acceptable. It is affected by the manual setting of the thresholds; a more objective method would be

desirable. The accuracy measurements show an overestimation of connectivity density that might result from the increased noise level. The feasibility of VCT for in vivo application requires further studies.

Table 1
Structural variables

Variable	BV/TV	Tb.N [1/mm]	Anisotropy	Conn. Density
Range VCT	0.085–0.206	0.585–1.062	1.106–1.203	0.739–1.955
Range μCT	0.099–0.199	0.592–0.920	1.237–1.344	0.398–1.380
Accuracy error (%)	6.0	10.6	12.9	33.6
Precision error (%)	4.6	4.1	2.2	6.8

P434-Mo

Physicochemical Properties of Trabecular Bone in Intracapsular Fractured Hips: Analysis of the Different Types of Alterations and their Distribution by Raman Spectroscopy

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Bone mass reductions and bone quality damage are the most significant parameters which provoke an intracapsular hip fracture. The physicochemical properties of bone constituents (organic matrix and mineral crystals) are an important aspect of bone tissue quality. For this reason, information about the status of both constituents is important for a better assessment of fracture risk. Nowadays, an emergent technique, Raman spectroscopy, allows the determination of physicochemical composition of biological tissues in a hydrated state without any sample preparation. This work deals with the analysis of the different types of alterations in the physicochemical properties of human femoral necks with intracapsular hip fracture (ICHFx) and their distribution by Raman spectroscopy.

Materials and methods: Femoral head–neck biopsies were taken during standard hip arthroplasty in patients with ICHFx. To analyze the samples, cross-sections of each femoral neck were divided into 4 subregions and 3 equidistant points were analyzed in each subregion by FT-Raman spectrometer. From the experimental analysis, mineralization degree of the collagen matrix, mineral crystallinity, type-B carbonate substitution, and monohydrogen phosphate to phosphate ratio (MP/P) were determined.

Results and discussion: All the physicochemical properties determined were found to be heterogeneous for all the anatomical quadrants considered. The mineralization degree was the parameter with more variations between inner and subcortical localizations (mean ± standard deviation: 1.60 ± 1.41; 1.15 ± 0.55, respectively). A certain tendency to a higher mineralization degree and type-B carbonate substitution is appreciated in the anterior quadrants of the femoral neck (1.76 ± 1.55–1.07 ± 0.39; 0.87 ± 0.70–0.53 ± 0.33, respectively) compared to posterior ones. Moreover, the MP/P was found to be great in the anteroinferior quadrant which could mean an increase in the mineral phase dissolution rate.

Conclusions: Raman spectroscopy is a suitable technique to determine the physicochemical properties of bone. The alterations found in the samples were in good agreement with the pathological described in osteoporosis.

P435-Tu

Significance of Osteoprotegerin, Ob-Re, and Trap for Prediction of Bone Density

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Background: RANKL and its inhibitor Osteoprotegerin (OPG) are decisive for osteoclast differentiation and osteoresorption function so that they became the aim of an intensive research. Recently, there are some information about the importance of Ob-Re (leptin receptor short form) and TRAP5b (tartrate resistant acid phosphatase 5b) assays for BMD prediction.

Objectives: (1) To determine OPG, Ob-Re, and TRAP5b concentrations in persons with various degree of reduced bone density and to find correlations between BMD and listed markers. (2) To assess correlation between OPG, Ob-Re, and TRAP5b concentrations and other routinely used laboratory bone remodeling markers.

Methods: We examined 199 patients who were under the follow-up for osteopathy or recurrent urolithiasis. All individuals were examined for OPG, Ob-Re, TRAP5b, b-ALP, osteocalcin, urine DPD/creatinine, Ca, P, Mg, creatinine concentrations in the serum, and urine. We performed DEXa of the skeleton. Patients were divided into the subgroup of persons with normal bone density (T score >-1 "N") and individuals with reduced bone density (osteopenia or osteoporosis; T score <-1, "OP").

Results: 31 patients were normal (bone density) and 168 were osteopenic (or osteoporotic). OPG: individuals with normal T score had median OPG value 60.8 ng/ml, probands "OP" had median OPG values 73 ng/ml ($P < 0.05$). Ob-Re: values did not differ significantly in "OP" vs. "N" patients (22 vs. 25 ng/l) even after correction with BMI. TRAP5b: values did not differ significantly in "OP" vs. "N" patients (17.8 vs. 21 nkat/l). The OPG cut-off for "OP" individuals was 128 ng/ml. All patients with higher

OPG values had significantly reduced BMD. We proved a negative correlation between BMD and OPG concentration (−0.31, $P = 0.02$). No significant correlations with other bone markers were detected in Ob-Re and TRAP5b. Discrimination analysis revealed that the known OPG concentration allows a correct prediction into given groups in 79.5% of cases; a positive predictive value of OPG for BMD reduction was 100% in our group under study.

Conclusion: The negative correlation between OPG and BMD was detected. OPG concentration >128 ng/l discriminates probands with significant BMD decrease. TRAP5b and Ob-Re (either alone or in combination with other markers of bone remodelation) cannot be used for mathematical assessment of bone density.

P436-Su

Osteoprotegerin Relates to Bone Markers During Osteoporosis Treatment

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The discovery of the osteoprotegerin/RANKL system enabled better understanding of bone metabolism and many skeletal disorders. Determination of osteoprotegerin was made available after development of commercial assay, but its clinical significance is still under investigation. In order to study the relationship of osteoprotegerin and standard bone markers during osteoporosis treatment, we have performed measurements in a sample of 54 women with postmenopausal osteoporosis. Total and bone alkaline phosphatase, telopeptide, and osteoprotegerin were measured in sera by standard methods or commercial kits before and once or twice during treatment with either bisphosphonates ($N = 41$) or calcium/vitamin D combination ($n = 13$). No difference between therapy groups existed for age, duration of menopause, or hip *T*-score, although those treated with bisphosphonates had lower spine *T*-score. Introduction of treatment resulted after 6 months on average in statistically significant decrease of total alkaline phosphatase ($P = 0.02$) and telopeptide ($P = 0.006$) and an increase of osteoprotegerin ($P = 0.03$). Follow-up of bone markers during treatment (on average 13 months) showed statistically significant decrease of telopeptide ($P = 0.001$) and an increase of osteoprotegerin ($P = 0.003$). Percentage of change did not differ between parameters after introduction of treatment, but during treatment continuation the highest percentage was observed for telopeptide ($P < 0.01$) and osteoprotegerin ($P < 0.02$) in comparison to total and bone alkaline phosphatase. Choice of therapy did not have any effect on the results. In conclusion, osteoprotegerin changes during osteoporosis treatment were similar in trend and magnitude to telopeptide, but with different direction. Both parameters were found in this population sample to be superior to bone formation markers (total and bone alkaline phosphatase). Further investigations are necessary to determine whether osteoprotegerin might serve as an additional bone marker.

P437-Mo

Immobility is Associated with High Bone Turnover in the Frail Elderly

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Background: Rapid bone loss with negative calcium balance due to acute immobilization is well recognized, but the effect of reduction in mobility with ageing on bone turnover has been less well studied. We assessed the associations between bone turnover and measures of mobility in a prospective cohort of elderly subjects.

Methods: We measured serum levels of the aminoterminal propeptide of type I collagen (PINP), a marker of bone formation, and serum concentrations of the carboxyterminal telopeptide of type I collagen (CTX-I), a marker of bone resorption, as well as serum intact parathyroid hormone (PTH), serum 25 hydroxyvitamin D (25OHD), serum creatinine, and calcaneal bone ultrasound attenuation (BUA) in 1283 elderly men and women living in residential care.

Results: The mean age of subjects was 86 years (range 65–101) years and 69% used a walking aid. Vitamin D deficiency was common (78% has a serum 25OHD level <39 nmol/L). Age and gender-adjusted serum CTX and PINP were significantly higher in those with poorer mobility and worse static balance. There were similar trends for lower serum 25OHD and serum albumin with worsening grade of these two measures. After controlling for other potential confounders, serum PINP remained significantly higher in subjects with poorer mobility and worse static balance. In a multiple regression model, both mobility ($P < 0.001$) and serum PINP ($P < 0.05$) were significantly independently associated with BUA.

Interpretation: In the frail elderly, reduced mobility is associated with increased bone turnover markers.

P438-Tu

Random Calcium/Creatinine Ratio vs. 24-h Urinary Calcium Output in the Assessment of Patients with Metabolic Bone Disease (MBD)

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MBD is often caused by an imbalance in vitamin D, calcium, or phosphorus homeostasis. Management may require patients to receive calcium and/or vitamin D supplements.
Aim: To investigate the correlation between random urine (Ca/Cr ratio) and 24-h urinary calcium output (24-hrUCa) and their relation to MBD and markers reflecting calcium homeostasis.
Results: Between January 2002 and July 2004, 12,604 urinary samples were analyzed. A significant correlation ($r = 0.747$, $P < 0.0001$) with an odds ratio of 5.26 (95 CI, 5.15–5.37) existed between Ca/Cr ratio and 24-hrUCa. Cross tabulations with 24-h urinary creatinine output showed majority of the discordance was explained by the inappropriateness of urine collection.
Relationship to MBD revealed some disparity, 24-hrUCa was high in subjects with celiac disease and liver cirrhosis while Ca/Cr ratio was low.
Females had significantly higher Ca/Cr ratio compared to males (0.56 ± 031 vs. 0.40 ± 0.22), a significant trend with advancing age only seen in female. However, 24-hrUCa was similar for both genders (Female 3.67 ± 2.0 vs. Male 3.75 ± 2.32) with no trend with age.
PTH positively correlated with CTX ($r = 0.4$, $P < 0.0001$), ALP ($r = 0.24$, $P < 0.0001$), negatively correlated with 24-hr UCa ($r = -0.2$, $P < 0.0001$), Ca/Cr ratio ($r = -0.1$, $P < 0.0001$), PO4 ($r = -0.18$, $P < 0.0001$), and total vitamin-D ($r = 0.23$, $P < 0.0001$).
Conclusion: Ca/Cr ratio increased with advancing age highlighting the role of the renal tubules in the negative calcium balance of women with osteoporosis. Ca/Cr ratio permitted an easy, rapid, and inexpensive estimation of the daily urinary calcium excretion but also a potential marker of renal tubular calcium handling.

P439-Su
NTX in 500 Patients in Clinical Practice
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Bone mineral densitometry (BMD) carries significant limitations in patient assessment due to confounding spinal disease and slow changes in response to treatment. We investigated the resorption marker, urine NTX expressed as SMV/Creat ratio collected as a second morning sample, in clinical practice to supplement BMD information. Studies were performed on 497 patients average age 61 years (109 male, 388 female). 256 patients on treatment for more than 6 months were investigated to detect low bone resorption and had an average NTX of 32 (SD20), of whom 201/256 had an NTX below 40 and 11/256 above 60. Of these 11, 4 were on Alendronate, 1 Risedronate, 2 Didronel PMO, 2 HRT, 1 Calcium and Vit D, and 1 Pamidronate (Short Bowel). Of the patients on treatment, 99 were on Alendronate with an NTX of 28, 48 on Risedronate with NTX of 32, 24 on calcium and or Vit D alone with NTX of 35, 18 HRT with NTX of 40, and 12 Didronel PMO with NTX of 45. A change in treatment was made in 51 patients following the NTX result. Follow-up measurements obtained on 83 patients show an average pre-treatment NTX of 58 with a post-treatment decline of 36%. 41/84 had a more than 40% decline in NTX. 5 patients had no decline. 23 patients had initial NTX results over 100 of whom 7 had hyperparathyroidism, 2 Coeliac disease, 2 steroids, 1 short bowel, 2 RA, 1 Crohns Disease, 1 Depoprovera user, 1 Epileptic medication, and 6 postmenopausal osteoporosis. In 39 patients, NTX was performed because of a decline in BMD. The mean NTX was 32 and after excluding 5 patients not on treatment 29. In 12 patients, a change in therapy was made. Results from this study show most patients on antiresorptive therapy have a low NTX, but a change in therapy is made in a significant number of patients following the NTX result. In the context of a declining BMD, a change in therapy was made in 1/3. Unusually high bone turnover should prompt a search for secondary causes of osteoporosis. Following treatment, a decline in NTX can be observed in most patients, providing early evidence of a early response to therapy.

P440-Mo
Reference Interval of Serum Tartrate-Resistant Acid Phosphatase Type 5b Activity with a Novel Assay of Japanese Subjects
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Tartrate-resistant acid phosphatase is a well-known marker for bone resorption, but it is not enough for clinical use in specificity of bone and instability in serum causes less usefulness in screening and monitoring the treatment for osteoporosis.
Upon analysis with electrophoresis, it was found that Tartrate-resistant acid phosphatase had isotypes and the type 5b of them (TRAP5b) is derived from osteoclasts. Recently, Igarashi and Nitto Boseki Co., Ltd. group developed the novel immunoassay for serum TRAP5b activity and reported its usefulness in monitoring hormone replacement therapy. Employing this assay, we studied to establish the reference interval of serum TRAP5b activity in Japanese subjects. We confirmed this immunoassay had a satisfactory performance with intra-assay variance of 2.25–2.89%, inter-assay var-

iance of 2.56–4.47%, and also TRAP5b activity in serum was so stable as to be acceptable for routine use.
815 subjects were enrolled in this study under informed consent. When subjects that had the diseases and took the medicines that affect bone metabolism (e.g., osteoporosis, thyroid disease, fracture, rheumatoid arthritis, osteoarthritis, oophorectomy, hysterectomy, taking steroid, receiving fertility treatment) were excluded, the measured values of TRAP5b activity showed logarithmic normal distribution. After outliers were rejected by rejection test, 366 women and 316 men aged from 18 to 82 years old qualified for the study.In females, TRAP5b values in postmenopausal women were significantly higher than those in premenopausal women ($P < 0.05$) and maintain at higher level after menopause persisted for the long term. In males, the activities did not become higher with age. From these observations, we calculated the reference intervals (logarithmic mean ± 1.96 SD) for premenopausal women and men, and the calculated intervals of serum TRAP5b activity were as follows.

Table

Group	Age	Number of subjects	Reference interval (U/L)
Men	20–82	316	1.70–5.85
Premenopausal women (All)	18–55	217	1.20–4.81
Premenopausal women	33–44	99	1.16–4.85
Postmenopausal women	45–77	148	2.20–7.99

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Is There Any Relation Between Osteoporosis and C-Reactive Protein in Elderly Patients?

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The role of inflammation has been investigated in the pathogenesis of some chronic diseases. The aim of this study is to determine the relationship between osteoporosis and low-grade inflammation as assessed by C-reactive protein (CRP) levels among geriatric patients admitted to Division of Geriatric Medicine of Internal Medicine Department in Hacettepe University Hospital between February 2002 and December 2004. A total of 2422 patients–1534 females and 888 males–aged 65 years and over were included in this cross-sectional study. All patients had a complete comprehensive geriatric assessment. Osteoporosis was defined as T score value below −2.5 and low grade inflammation as CRP levels 1 mg/dl and over. Acutely ill patients were excluded. The number and percentage of patients with high CRP and normal CRP level within 1424 osteoporotic patients were 256 (%18.0) and 1168 (82.0%), respectively. Among 998 non-osteoporotic patients, these percentages were 182 (%18.2) and 816 (81.8%), respectively. In our study, CRP level did not differ between patients with or without osteoporosis ($P = 0.871$). In univariate analysis, high CRP level was related with diabetes mellitus and chronic obstructive pulmonary disease, P values were 0.009 and 0.014, respectively. In multivariate analysis, none of these diseases were related with high CRP level. We did not find a significant statistical relation between osteoporosis and low grade inflammation as assessed by CRP.

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Day-to-day and Diurnal Variations of Serum Tartrate-Resistant Acid Phosphatase Type 5b with Newly Developed TRAP5b Kit and Their Comparison to Other Bone Resorption Markers

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Objective: Bone resorption markers are useful in monitoring the treatment for osteoporosis, but most resorption markers may have considerable physiological and analytical variations, so we need to adopt those data carefully. Tartrate-resistant acid phosphatase type 5b (TRAP5b) is an osteoclast-produced enzyme and was reported to be a promising marker for bone resorption. The aim of this study was to evaluate the variation of serum TRAP5b with a newly developed TRAP5b kit which uses high specific monoclonal antibody against TRAP bone isozyme (Nitto Boseki Co., Ltd., Japan), and compare to other biochemical markers of bone turnover.
Methods: We evaluated the day-to-day and the diurnal variations of serum TRAP5b, urinary N-terminal cross-linked telopeptide (u-NTX), C-terminal cross-linked telopeptide (u-CTX), free deoxy-pyridinoline (f-DPD), and serum bone specific alkaline phosphatase (BAP). For day-to-day variation, ten healthy postmenopausal women were enrolled. Blood and urine samples were collected at morning (0900–1100) for 8 days with at least 4-day interval. For diurnal variation, six healthy postmenopausal women were enrolled. Blood and urine samples were collected at morning (0900–1100), afternoon (1300–1400), and evening (1600–1800) of the same day.
Results: Serum markers, TRAP5b, and BAP showed smaller day-to-day and diurnal variations than urinary markers. From the results of day-to-day and diurnal variations, we calculated the least significant changes

(LSC, $P < 0.05$) and the minimum significant change (MSC, $P < 0.08$) in case of collecting samples during the morning, and those were LSC 22.4%/MSC 16.2% for TRAP5b, 90.6%/65.4% for u-NTX, 82.9%/59.9% for u-CTX, 48.3%/34.8% for f-DPD, 17.8%/12.9% for BAP.

Conclusions: Totally, TRAP5b had smaller variation than those of urinary bone resorption markers. We reported the percentage change of TRAP5b in hormone replacement therapy was equivalent to u-NTX, and therefore these results suggest TRAP5b is the most effective bone resorption marker in monitoring treatment for osteoporosis.

P443-Mo

Dynamic of Bone Turnover Markers in Chronic Diabetic Charcot Neuroarthropathy

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Background and aims: The dynamic of bone turnover markers in chronic Charcot osteoarthropathy (CO) patients is not fully known. Lack of osteoblast activity is believed to be responsible for the bone changes associated with chronic CO in diabetes mellitus.

Materials and methods: We observed 28 patients (64% Type 2 diabetes mellitus) with chronic CO (18 male, 10 female, mean age 42.7 ± 8.9 years, duration of diabetes 25.7 ± 12.6 years) during 12 months. In 3, 6, and 12 months, all patients reviewed by means of clinical assessment, skin temperature, bone turnover markers (osteocalcin, b-CrossLaps), and X-ray (on necessity).

Results: In 3 months, the averages were: serum osteocalcin was 32.53 + 8.91ng/ml in male and 16.62 + 6.92 ng/ml in female; serum b-CrossLaps was 420.5 + 34.9 pg/ml in male and 366.6 + 26.9 pg/ml in female. In 6 months, serum osteocalcin was 10.5 + 4.9 ng/ml in male and 6.4 + 3.2 ng/ml in female; serum b-CrossLaps was 489.6 + 28.7 pg/ml in male and 359.7 + 22.5 pg/ml in female. In 12 months, serum osteocalcin was 11.43 + 3.89 ng/ml in male and 5.89 + 3.82 ng/ml in female. Serum b-CrossLaps was 487.66 + 26.75 pg/ml in male and 345.77 + 27.58 pg/ml in female. There was no temperature difference during the observation. The osteocalcin levels were statistically significantly decreased in 6 months (male: 32.5 ng/ml vs. 10.5 ng/ml $P = 0.01$; female: 16.6 + 6.9 ng/ml vs. 6.4 + 3.2 ng/ml $P = 0.01$) and there was no statistical difference in 12 months (487.66 + 26.75 pg/ml in male and 345.77 + 27.58 pg/ml in female. Serum b-CrossLaps, a marker of osteoclastic bone resorption, was not significantly different during the hole period of observation.

Conclusion: These results suggest that the chronic Charcot foot demonstrates depress of osteoblastic activity.

P444-Tu

Biochemical Markers of Bone Turnover and Response of Bone Mineral Density to Menopause in Saudi Women

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Objectives: To assess the ability of some biochemical bone turnover markers to monitor the increased bone resorption associated with menopause.

Subject and methods: A total of 892 Saudi women were studied and stratified for menopause, age, and bone mineral density (BMD). The following biochemical bone markers were measured: uNTX, uDPYR, sCTX, and uCTX. Women were classified with osteopenia or osteoporosis and compared with healthy pre-menopausal and postmenopausal with normal BMD values according to WHO criteria. Regression analysis was used to calculate Pearson's coefficient for correlation between each of the biochemical markers in the whole population. The significance of differences between groups was calculated by non-parametric Mann–Whitney testing.

Results: All biochemical markers were significantly increased in the postmenopausal group. The difference between the two groups was most pronounced for sCTX ($P < 0.001$) in postmenopausal as compared to premenopausal group. Correlation between the levels of biochemical markers and age of the studied groups showed significant results: the highest was observed for uCTX. The markers exhibited a correlation to age ranging from weak ($P < 0.05$) for uCTX to a strong correlation ($P < 0.001$) for sCTX, uDPYR, and uNTX values, respectively. When postmenopausal women were classified according to BMD values, all biochemical marker values were increased in osteopenic and osteoporotic as compared to both pre- and postmenopausal counterparts. Compared with premenopausal values, the marked increase in both osteopenic and osteoporotic women was evident for mean sCTX. sCTX was increased in normal postmenopausal group as compared with pre-menopausal women. Urinary levels of CTX, NTX, and DPYR were increased by 41%, 65%, and 39% in osteopenic and osteoporotic women as compared with normal postmenopausal women, respectively. Mean serum levels of CTX were increased by 23% and 37% in these two groups, respectively.

Conclusions: Postmenopausal Saudi women exhibited increased turnover with all biochemical bone resorption

markers studied, but with great individual variation. Biochemical bone resorption markers were markedly increased in osteopenic and osteoporotic women as compared with that obtained for pre- and post-menopausal healthy women.

P445-Su

The Newly-Developed Bone-Specific Tartrate-Resistant Acid Phosphatase (TRAP5b) Kit is Useful for the Monitoring of Bone Metabolic Changes by Osteoporosis Treatment with Risedronate

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Background: The metabolic markers of bone are expected as tools to identify the effectiveness and keep compliance on osteoporosis treatment, but most markers are not satisfactory due to wide variation.
Purpose: To investigate the clinical usefulness of the newly-developed measurement kit utilizing the new specific antibody for TRAP5b (reported by Igarashi IBMS 2003) for the treatment of osteoporosis.
Methods: The subjects consist of 53 osteoporotic postmenopausal women (Age: Mean 67.9, Mean L2-4BMD: 0.646 ± 0.059 g/cm^2). During follow up, the treatment by risedronate, the metabolic marker of bone (BAP, TRAP5b, NTX (sNTX and uNTX), urinary etaC-TX (uCTX), and urinary DPD (uDPD)), was measured.
Results: TRAP5b of patients before treatment was 4.70 ± 1.97 U/L (normal range of premenopausal women 30–44 years old: 1.16–4.85 U/L[#1]), BAP was 34.5 ± 12.0 U/L. The ratio of patients whose marker level was over young adult mean ± 1.96 SD was 58.5% (BAP), 37.3% (TRAP5b), 52.5% (sNTx), 69.8% (uNTx), 71.7% (uCTx), 58.5% (uDPD), respectively. The correlation of TRAP5b with BAP was 0.63, with sNTX was 0.77, with uNTX was 0.73, with uCTX was 0.72, with uDPD was 0.30. Serum TRAP5b was reduced by risedronate from 5.14 ± 2.43 U/L to 3.17 ± 1.60 U/L ($P < 0.01$) on 4th week (35.6% reduction), and the reduction rate was 40.9% on 12th week. The suppressions of other markers on the 4th and 12th month were 2.7% and 22.1% (BAP), 8.9% and 12.2% (sNTX), 37.8% and 51.3% (uNTX), 37.0% and 55.5% (uCTX), and 10.4% and 17.1%(uDPD), respectively. The suppression rate of TRAP5b on the 4th week was not different from those of uNTX and uCTX. The percentages of patients showing greater suppression of TRAP5b than MSC (16.16%) were 77.8%, and LSC (22.38%)[#2] was 70.4% on 4th week, and on 12th week were 87.0% and 82.6%. Those of uNTX and uCTX on 4th week were 17.9% and 28.6% (>MSC), and on 12t^h week were 45.8% and 54.2%.
Conclusion: The similar suppression of TRAP5b by risedronate to uNTX or uCTX was found in the early course of treatment, but the new TRAP5b measurement kit can identify more patients with risedronate effect (>MSC or >LSC) compared with other measurements. This method is expected to identify patients who respond to raloxifene treatment. ([#1]Nishizawa et al. will present data at IBMS 2005. [#2]Mochizuki et al. will present data at IBMS 2005.)

P446-Mo

Bonetrap® Assay: A Routine Diagnostic Imunoassay for the Quantitative Determination of Tartrate-Resistant Acid Phosphatase 5b (TRACP 5b) Activity in Human Sera

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Tartrate-resistant acid phosphatase 5b (TRACP 5b) is a marker of osteoclastic activity and bone resorption. The TRACP enzyme exists as two isoforms, 5a and 5b. Both isoforms are present in serum, whereas exclusively the active isoform 5b is specifically associated with osteoclasts. We validated the commercial BoneTRAP® assay detecting specifically the activity of TRACP 5b in serum samples. The results reflect the rate of bone resorption at the time of sample taking. With this immunoassay, we determined the activity range of TRACP 5b in sera of healthy blood donors, patients with renal disorders, and tumor patients with bone metastases. Furthermore, intra-assay and interassay variation, person-to-person variation, dilution linearity, and limit of quantitation (LOQ) were investigated. In addition, the real-time stability was determined.
Our investigations revealed a normal TRACP 5b activity (mean ± 2-fold standard deviation) of 2.5 ± 1.4 U/l for women <40 years (n = 46), 2.8 ± 1.4 U/l for women >50 years (n = 42), and 3.1 ± 1.6 U/l for men (n = 62). The activity of all these blood donor sera ranges from 1.1 to 5.2 U/l. A large proportion of patients' sera shows a TRACP 5b activity above 5.2 U/l (34.8% of 28 sera from patients with renal disorders and 40.9% of 122 sera from the tumor patients), reflecting an increased bone resorption depending on the status of disease. Coefficient of variation (CV) for each sample in the reactivity range was below 14% for intra-assay measurements. For interassay variation, the CVs of six sera were below 8%, and for person-to-person variation the CVs were below 13%. LOQ determination (0.88 U/l) documented the validity of the measuring range of 1–10 U/l. The linearity of the assay was very good. The real-time stability study with three different batches over a period of 21 months demonstrates no changes in TRACP 5b activity.
The BoneTRAP® Assay is easy to perform. Due to its technical and diagnostic performance, it is well suited for routine use. The assay is designed to be used for small as well as large sample size and provides reliable results within 2 h.

P447-Tu

Four Molecular Markers for Assessment of Bone Metabolism

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Aims of the study: (i) To compare the reference limits of the bone markers in an Estonian population sample with those recommended by the manufacturers of the reagents (kits), (ii) to examine the behavior of two bone resorption markers and two formation markers in patients with postmenopausal osteoporosis.

Material and methods: 23 healthy women, aged 31–48, mean 37 with normal lumbar DXA finding served as the controls. The study group consisted of 17 patients with postmenopausal osteoporosis (48–65, mean 56 years). Eight of them had received risedronate therapy for 6 months. Bone resorption was assessed by collagen type I C-terminal telopeptide in serum, S-CTx-I (Roche, Elecsys), and by urinary free Dpd (Pyrilinks-D, DPC, Immulite), expressed per mmol creatinine. Bone formation was assessed by serum procollagen type 1 amino-terminal propeptide, S-P1NP, and osteocalcin, Oc, levels (Roche, Elecsys). Spearman's rank correlation and regression analysis were used for data processing.

Results: Several differences were revealed between the upper limits of the normal (ULN) levels in the controls and those recommended by the manufacturers were observed: observed ULN for CTx-I was 48% and Oc 14% lower, Dpd/crea 35%, and P1NP 22% higher of that given by manufacturers.

In most (3/4) cases, women with osteoporosis had elevated resorption as well as increased (2/3 of cases) formation of bone collagen. Nevertheless, in an appreciable proportion of osteoporosis cases (1/4), bone resorption did not differ from normal. Before risedronate therapy, the two resorption markers showed weak correlation (rho = 0.370). Much stronger was the correlation between the formation markers (rho = 0.574, $P = 0.02$). Surprisingly strong correlation was found between S-CTx-1 and S-P1NP before (rho = 0.541, $P = 0.03$) and during the therapy (0.726, $P = 0.000$). Regression models including S-P1NP or Oc allowed predicting 25% or more of the variability of S-CTx-1.

Conclusions: (1) Check-up of the reference limits for a population might be helpful in finding valid diagnostic levels. (2) Bone metabolism in women with postmenopausal osteoporosis is heterogeneous, including cases with normal resorption levels. (3) Changes of S-CTx-1 are accompanied by synthetic processes in bones.

P448-Su

Bone Markers and Blood Pressure Relationship in Early Postmenopausal Women

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Objective: The aim of this study was to investigate the relation between the biochemical markers of bone turnover (BM) and the blood pressure (BP) in a Spanish early postmenopausal women population.

Methods: 183 women aged 50–55 years (mean 52.6 years, 95% CI: 53.4–52.9 years) were randomly selected in the province of Albacete (Spain). All women were postmenopausal, from 6 to 36 months, and they did not have diseases or taking drugs known to affect bone metabolism. Systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) were measured by an Omrom 705CP device. To assess bone turnover, bone phosphatase alkaline (BAP), ostecalcin (OC), deoxypyridinoline (DPD), urinary NTX, serum CTX, and urinary calcium (Ca/Cr) were measured. Statistical analyses were performed by the Pearson's test and the Student's test. A P value < 0.05 was accepted as a level of significance.

Results: There was no correlation between the SBP, DBP, and any markers. The Student's test did not show differences in urine calcium between hypertensive and non-hypertensive women. These are the data of Ca/Cr (mg/mg). SBP-Hypertension 0.19 ± 0.10 ($N = 33$), SBP-No hypertension 0.17 ± 0.08 ($N = 150$), $P = 0.234$. DBP-Hypertension = 0.19 ± 0.08 ($N = 30$), DBP-No hypertension 0.17 ± 0.09 ($N = 153$), $P = 0.348$. We found correlation of heart rate to NTX ($r = 0.158$ $P = 0.035$).

Conclusion: We found no correlation of blood pressure to the bone markers in early postmenopausal women. There was correlation between heart rate and NTX in the same population.

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Bone ALP Level is Associated with Aortic Calcification

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Osteoporosis and atherosclerosis are two major chronic causes of morbidity. Although they tend to be regarded as two independent, age-related processes, in women the prevalence of both disorders increases dramatically after menopause. It has been suggested that there were several possible linkages between vascular calcification and osteoporosis. In addition, the processes of vascular calcification might have a common etiology with bone. According to these observations, we hypothesized that the serum levels of bone metabolic markers were different between osteoporosis patients with and without vascular calcification. In the present study, we examined in a group of postmenopausal women with osteoporosis whether the presence of calcifications in the abdominal aorta was

associated with the serum or urine levels of bone metabolic markers. We showed that the serum level of bone-specific alkaline phosphatase activity in osteoporosis patients with abdominal aortic calcification was of higher value than those without the calcification. On the other hand, there was no significant difference of urine level of NTX and serum level of OC, Ca, and P. Bone-specific alkaline phosphatase was the most important maker for osteoblast differentiation; furthermore, the serum level of its activity might reflect the process of calcification of aorta in osteoporosis patients.

P450-Tu

Urinary Gamma-Glutamyl Transpeptidase Activity Measurement is Useful as A Novel Biochemical Marker of Bone Turnover

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The purpose of this study is to investigate if the measurement of urinary gamma-glutamyl transpeptidase (GGT) activity is useful as a biochemical marker of bone turnover in postmenopausal women.

A total of 80 postmenopausal Japanese women, aged 48–74 years, were admitted to the study. All participants were followed up without any lifestyle modification. A total of 55 women (mean age 53.4 years) had received conjugated equine estrogen, 0.625 mg daily each morning for 12 months (HRT group). HRT group subjects had experienced natural menopause for at least 1 year but not longer than 5 years. A total of 25 women (mean age 64.2 year) had received alendronate sodium 35 mg daily each morning for 3 months (alendronate group). Urinary cross-linked N-telopeptides (NTX), GGT activity, and serum GGT activity were measured on all HRT group subjects before the start of therapy and for 3, 6, and 12 months after therapy. Urinary NTX and GGT activity were measured on all alendronate group subjects before the start of therapy and 3 months after therapy. Lumber spine (L2–L4) bone mineral density (BMD) and femoral neck BMD were measured using a dual X-ray absorptiometer (DPX-alpha, Lunar Co). These parameters were measured on all HRT group subjects before the start of therapy and for 6 and 12 months after therapy.

Correlations between urinary GGT activity and NTX were significant before the start of therapy, and those relations were being maintained within the period of therapy (HRT group and alendronate group).

L2–L4 BMD was increased after the start of the HRT, averaging +5.12% and +6.21%, at the 6 months and 12 months, respectively. Femoral neck BMD was slightly increased compared with baseline value, averaging +1.24% and +1.23%, at the 6 months and 12 months after HRT.

High GGT activity before the start of therapy was the prospective factor of the high % change rate of L2–4 BMD at 12 months after HRT.

In conclusion, we now report that urinary GGT activity measurement is useful as a novel biochemical marker of bone turnover.

P451-Su

Relation of Homocysteine, Biochemical Bone Markers, and Bone Mineral Density in Peri- and Postmenopausal Women

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Background: Prevention of osteoporosis by identifying risk factors is a major public health issue. Recently, increased plasma homocysteine (HCY) has been suggested as an independent risk factor for osteoporotic fractures. It is tempting to speculate that HCY adversely affects bone metabolism. This study aimed to analyze the relation between HCY, biochemical markers of bone metabolism.

Materials and methods: We investigated 143 peri- and postmenopausal women (mean age: 66 ± 12 years). All subjects underwent a detailed medical examination, measurement of bone mineral density (BMD) at lumbar spine (BMD-LS) and total hip (BMD-HIP), fastening venous blood, and urine sampling. Osteocalcin (OC), serum β-crosslaps (CTx), alkaline phosphatase (ALP), osteoportegerin (OPG), soluble TNF-alpha receptor antagonist ligand (sRANKL), and urinary desoxypyridinoline crosslinks (DPD) were studied.

Results: According to BMD, subjects were classified as normal ($n = 24$), osteopenic ($n = 51$), and osteoporotic ($n = 68$). Median HCY did not change with increasing quartiles of BMD. Contrary, DPD (bone resorption marker) increased with increasing quartiles of HCY ($P = 0.043$), while OC ($P = 0.425$), ALP ($P = 0.872$), CTx ($P = 0.262$), OPG ($P = 0.129$), and sRANKL ($P = 0.426$) did not. Partial correlation analysis (correction for creatinine and age) confirmed an association between HCY and DPD ($r = 0.175$, $P = 0.038$). No relations were seen between Hcy and OC ($r = -0.009$, $P = 0.912$), CTx ($r = -0.067$, $P = 0.443$), ALP ($r = 0.075$, $r = 0.378$), OPG ($r = -0.067$, $r = 0.539$), sRANKL ($r = -0.108$, $r = 0.326$), BMD-LS ($r = 0.166$, $P = 0.134$), and BMD-HIP ($r = 0.075$, $P = 0.493$).

Conclusion: Our results suggest an increased bone resorption in the presence of elevated HCY. The relation between HCY and bone resorption was independent from OPG and sRANKL. Future studies have to clarify the mechanistic role of HCY in osteoporosis.

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The Assessment of Process Factors Involved in Conducting the Canadian Quality Circle (CQC) Pilot Project for the Care of Patients at Risk for Osteoporosis (OP)

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The CQC project was planned in two phases, the pilot and national study, to improve family physicians' adherence with the Osteoporosis Society of Canada 2002 clinical practice guidelines for osteoporosis (OP). One specific aim of the pilot project was to examine the process factors involved to determine if a planned national study was possible. Current analyses evaluated the success of the processes for completion of participant recruitment, data collection, and CQC meetings. During recruitment, 52 physicians were recruited as members in 7 Quality Circles (QC). Members collected baseline (BL) and follow-up (FU) data at random on separate patients via chart reviews and the completion of the CQC form that captures data on OP. Physicians were responsible to assess 30 patients at BL and 30 at FU using the CQC form. Individual and QC data were collated in profiles and provided to the members. The QC then met to discuss the profiles and participate in an OP workshop. The primary focus of the workshop was to assess postmenopausal OP and risk factor identification. FU data collection occurred after the intervention and was used to provide feedback and to gauge overall progress. The Ontario College of Family Physicians Standard Evaluation Criteria Form consisting of nine items was used to assess the physicians' satisfaction with the workshop. High scores indicate high satisfaction.
Results showed that of the 52 physicians who started the project, only 2 dropped out (4%) prior to FU. At BL and FU, 96% (n = 1505) and 91% (n = 1359) of the required patient evaluations were completed and entered in the database, respectively. The percent satisfaction ratings of the workshop from the CQC participants were as follows: method used in the workshop (86%), new knowledge acquired (88.8%), presentation was clear and effective (87.8%), facilitator created an interactive environment (91.6%), high attention level (87.8%), linkage between learning and practice (85.6%), training met educational needs (85.6%), would register to a similar program (85.6%), excellent overall organization (88.8%). The overall program obtained an 88% satisfaction rating.
In conclusion, physicians appeared motivated and evaluated the required number of patients. The educational intervention strategy was well received. The pilot provides evidence that the processes that have been developed for the study have been successful and should be implemented for the national study.

P453-Tu

Contribution of the New Method of Lateral Vertebral Assessment (LVA) by Dual X-Ray Absorptiometry (DXA) in the Detection of Vertebral Fractures

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Vertebral fractures are not frequently suspected. Ascertainment of them is important, since it is a hallmark of established and severe osteoporosis, associated with increased morbidity and mortality risk. For this purpose, LVA has been developing recently, a technique that uses DXA scanners to acquire a lateral image of the spine at point of care unit.
Aims: To determine the specificity and sensibility of LVA in diagnosing vertebral fractures and the correlation coefficient between LVA and conventional lateral radiographs (X-ray).
Methods: Fifty ambulatory women aged 50 and over with osteopenia or osteoporosis were submitted to both LVA and X-ray. Vertebral deformities were graded according to a combination of semiquantitative method of Genant and quantitative morphometry. LVA was performed through lateral spine imaging of T6-L4 using a Lunar Prodigy. All the images of LVA were analyzed by a same examiner, independently of another examiner that evaluated all images of X-ray. The agreement between LVA and X-ray was determined according to kappa statistics (κ).
Results: Seven patients (14%) with vertebral fracture were detected by X-ray; LVA was normal in one of them and in the other two it was uninterpretable. LVA detected 6 patients (12%) with fractures; X-ray was normal in one of them and it could not be performed in another because she had scoliosis. The 3 individuals that could not have both tests analyzed were excluded. Agreement about the presence of fracture in the 47 remaining patients had a κ-score of 0.80. X-ray identified 13 vertebra fractured, but six of them were not detected by LVA. LVA identified 8 fractures and X-ray failed to notice one of them—agreement about which vertebra were fractured had a κ-score of 0.74. The agreement about fractures' severity was poor (κ-score: 0.44), because the densitometer does not classify fracture as grade 1. The sensibility and specificity of LVA to detect patients with vertebral fracture, when X-

ray was considered as gold standard, were 80.0% and 97.6%, respectively.
Conclusion: LVA is a useful test to exclude the presence of vertebral fracture. It had a good agreement with X-ray in detecting patients with vertebra deformities, except about fracture severity.

P454-Su
History of Fractures and Mechanism of Fracture: An Easy Way to Identify Patients with Possible Osteoporosis
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Background: Osteoporosis is a well-known disease. However, in many patients who come to medical attention, osteoporosis goes unrecognized and no treatment is prescribed.
Objective: The aim of this study was to recognize, by means of anamnesis, patients with osteoporosis who would deserve a diagnostic procedure and treatment. Another objective was to evaluate in how many patients the diagnosis of osteopenia and osteoporosis was known.
Method: Patients, who were admitted to the Internal Medicine Department of 20 hospitals in Spain, during November 2003 to June 2004, were evaluated. They were included if they were women, 55–80 years, admitted to the hospital for any reason not related to osteoporosis, and had a lateral chest radiograph performed as part of the routine evaluation. Exclusion criteria were a known bone disease apart of osteoporosis or being participant of another trial. Both localization and mechanism of previous fractures were registered. Recalled mechanisms of fractures were: non-intense movement, falling from standing, no associated trauma, intense trauma, and others. Fractures associated to the first three were considered fragility fractures. Known diagnosis of osteopenia and osteoporosis was also registered.
Results: A total of 689 women (age, 71.26 + 6.54) met the study criteria. An 18.3% (126 patients) presented 227 previous fractures. Of these patients with previous fractures, 78 presented one fracture and 48 with multiple fractures. Fracture location was vertebra (125), wrist (39), humerus (16), leg (14), hip (13), and others (20). Total number of fragility fractures was 208 and occurred in 113 patients. Among these patients, 35% had a previous known diagnosis of osteopenia and/or osteoporosis and were currently on treatment for osteoporosis 32.7%.
Conclusions: (1) Osteoporosis is a well known, but underdiagnosed disease. (2) A good way to identify possible osteoporosis is to ask about previous fractures and the mechanism of production. (3) Only a small amount of patients with fractures, probably due to fragility, receive therapy.

P455-Mo
Instant Vertebral Assessment (IVA) in Case-Finding for Osteoporosis in Patients of 50 Years and Older with A Recent Fracture
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The Dutch Guidelines for Osteoporosis (April 2002) recommend a case-finding strategy based on risk factors. A fracture in a woman above the age of 50 years is one of the risk factors, which should lead to bone mineral density assessment. Instant Vertebral Assessment (IVA) is a method that allows a fast identification of persons with prevalent vertebral fractures. The aim of this project is to determine the prevalence of vertebral fractures in elderly with a fracture and to explore whether IVA has additive value in the diagnostics of osteoporosis in our group of subjects.
All patients of 50 years and older who came to the Surgery Department of VU University Medical Centre with a fracture were invited to participate. Polytrauma patients and patients with skull fractures were excluded. From June 2004 to December 2004, 357 patients were invited by mail for a DXA scan and were screened for risk factors for osteoporosis with the use of a questionnaire. IVA images were obtained using the DXA machine, IVA showed the vertebral bodies from T4 to L4.
The results of the case-finding were used to evaluate the incidence of osteoporosis in patients with fractures and in patients with high risk scores. An advice was prepared for the general practitioner based on risk score and BMD. The advice included the diagnosis and the proposed treatment (no treatment, calcium and vitamin D, bisphosphonates or raloxifene, referral to a specialist).
From the 357 invited patients, in 63 DXA and IVA were made. In 15 patients (23.8%), a prevalent vertebral deformity was observed. Of these 15 patients, 1 had a normal BMD and 6 had a BMD diagnosed as osteopenic by DXA. We classified in patients with a vertebral fracture a ("clinical") osteoporosis. As a result of the IVA, the diagnosis changed to osteoporosis for 7 of the 15 patients. For 4 persons, the advice altered from "expectative" to "treatment with bisphosphonates" and these 4 persons would have been diagnosed "no osteoporosis" and "no treatment" if IVA had not been made. In conclusion, IVA provides important additional information concerning future

fracture risk in patients of 50 years and older with a recent fracture and has important consequences for how patients should be treated.

P456-Tu

Evaluation of CA-41 as A New Isotopic Tool to Assess the Impact of Interventions on Bone Health

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Bone research is limited by available methods to detect changes in bone metabolism. While dual X-ray absorptiometry is rather insensitive, biochemical markers are subject to significant intra-individual variation and cannot be combined to assess bone balance. Within the presented studies, we evaluated a new isotopic labeling of bone using ^{41}Ca, a long living radiotracer.

A minute amount of ^{41}Ca (100 nCi) was administered orally to 22 postmenopausal women. For following the labeling process, urinary ^{41}Ca/^{40}Ca isotope ratios were monitored by accelerator mass spectrometry and resonance ionization mass spectrometry up to 700 days. After that, subjects were treated with interventions known to positively affect bone health in order to evaluate the potential of the ^{41}Ca technique and compare outcomes against changes in BMD and conventional biomarkers of bone metabolism. Six women with diagnosed osteopenia received a bisphosphonate (risedronate) over 6 months, while the remaining 16 subjects received Ca supplements (750 mg Ca/d) for 3 months in a randomized, placebo-controlled trial using a cross-over design.

Both interventions were effective but demonstrated the limitations of conventional techniques. Ca supplementation resulted in a significant lowering in D-Pyr (bone resorption marker; −19.4%, $P = 0.04$) and BAP (bone formation marker; −7.2%, $P = 0.04$) while findings were less conclusive for the bisphosphonate intervention. A positive effect on BMD was found for spine (+3%, $P = 0.01$) but not for radius. In contrast to that, BAP was significantly reduced (−35.7%, $P = 0.001$) and changes in D-Pyr could not be observed in response to the intervention. Population pharmacokinetic analysis (NONMEM) of ^{41}Ca data demonstrated the power of the new technique. Labeling data were fitted best by a linear three-compartment model with a central compartment and two sequential peripheral compartments. Bisphosphonate treatment resulted in a decrease in Ca transfer rate between the slow exchanging and the fast exchanging pool by 56% ($P < 0.0005$). Ca supplementation did not influence the transfer rate from the slow exchanging pool but decreased transfer rate of Ca from the fast exchanging to the central pool by 31% ($P < 0.0005$).

In conclusion, the new technique can be used to assess the impact of interventions on bone health directly at high sensitivity.

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P457-Su

LDL-Cholesterol, Triglycerides, HDL-Cholesterol, and Bone Density (QUS) in Postmenopausal Women

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Study was designed as cross-sectional one in the group of 100 women in the period not longer than 5 years after menopause. The object was the possible relationship between osteoporosis and risk of atherosclerosis of lipid origin.

Results: Postmenopausal women with high-risk levels of LDL-cholesterol have statistically significant smaller bone mass according to all parameters when compared to women who do not have high risk levels of LDL-cholesterol. Postmenopausal women with border and high risk levels of triglycerides have statistically significant smaller bone mass when compared to women who have normal levels of triglycerides. Bone mass parameters in postmenopausal women compared to the HDL-cholesterol do not differ among themselves significantly.

Conclusion: LDL-cholesterol and triglycerides but not HDL-cholesterol levels are accompanied with bone mass reduction. One common treatment of both disorders is reasonable.

P458-Mo

Are Plasma Leptin Levels Predictive for the Bone Mineral Density in Postmenopausal Women?

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Leptin is the product of the obese (ob) gene and is positively correlated with the total amount of body adipose tissue.

Obese postmenopausal women are usually protected against osteoporosis and have a tendency to an increased bone mineral density in comparison with lean women. The total fat body mass is considered to be one of the major determinants of bone mass. The aim of our study was to assess whether leptin is a predictor for bone mineral density (BMD) in postmenopausal women and to evaluate the impact of body composition on BMD. We studied 150 postmenopausal Caucasian women aged between 44 and 78 years and in addition a control group of 57 premenopausal women aged between 19 and 48 years. All women were assessed for serum leptin, lipid profile, and total and ionized calcium. Body composition was performed by a Body Composition Analyzer TBF 310 GS (Tanita Corporation) measuring body mass index (BMI; kg/m^2), Fat %, Fat Mass (kg), and Fat Free Mass (kg). BMD (g/cm^2) was measured by dual-energy X-ray absorptiometry (DEXA) at the lumbar spine. Positive strong correlation ($P = 0.0001$) was found between circulating leptin levels and the indices of adiposity in both pre- and postmenopausal women. We found a moderate positive correlation also between plasma leptin levels and BMD, $P = 0.017$ (Spearman) in all studied women. The postmenopausal women were divided according to BMD *T*-scores in osteoporosis ($n = 76$), osteopenia ($n = 41$), and normal BMD ($n = 33$). We assessed the leptin levels in these three subgroups, matched according to their BMI. The circulating leptin levels were significantly lower ($P = 0.003$) in osteoporotic postmenopausal women in comparison with osteopenic or normal BMD, but only in normal weighted women. We conclude that plasma leptin levels can be predictive for bone mass, but mainly in postmenopausal women with normal BMI.

P459-Tu

Quantitative Ultrasound at the Hand Phalanges in 2850 Females Aged 7–77 Years: A Cross-Sectional Study

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In the study, skeletal status was evaluated in 2850 females aged 7–77 years by the use of quantitative ultrasound. Skeletal status was assessed by device DBM Sonic 1200 (IGEA, Carpi, Italy) which measures amplitude-dependent speed of sound, Ad-SoS, m/s at hand proximal phalanges II–V, and the end result is a mean value of four measurements. All measurements were performed by the same operator and CV% was 0.7%. In subjects studied were not present factors known to affect bone metabolism (chronic diseases or prolonged medications). Ad-SoS ranged from 1923 ± 30 m/s to 1876 ± 81 m/s, and the peak value (2121 m/s) was achieved in 19-year-old females. Ad-SoS increased significantly between subgroups aged 11 and 12 years, 12 and 13 years, 13 and 14 years, 14 and 15 years, and 15 and 16 years. After the age of 19 years, the only significant drop was noted between age groups 47 and 48 years.

Multiple stepwise regression analyses of Ad-SoS on age, weight, and height were performed separately for age ranges: 7–11 years (before an increase in Ad-SoS, $n = 454$), 12–19 years (from the onset of the fast increase in Ad-SoS to the peak value, $n = 329$), and for subjects older than 19 years (after an achievement of peak value of Ad-SoS to menopause, $n = 732$). In females after menopause ($n = 1335$), YSMs were also taken into consideration. The following equations were obtained, respectively: Ad-SoS [m/s] = 1663 + 5.4 × age [years] − 2.16 × weight [kg] + 2.2 × height [cm], $r = 0.47$, $P < 0.00001$, SEE = 33.0; Ad-SoS [m/s] = 1345 + 21.4 × age [years] + 2.95 × height [cm] − 1.68 × weight [kg], $r = 0.78$, $P < 0.00001$, SEE = 44.3; Ad-SoS [m/s] = 1934–1.43 × age [years] − 1.82 × weight [kg] + 1.94 × height [cm], $r = 0.56$, $P < 0.00001$, SEE = 45.2; Ad-SoS [m/s] = 2178–4.99 × age [years] − 0.44 × weight [kg] + 0.64 × height [cm], $r = 0.6$, $P < 0.00001$, SEE = 51.8. Multiple stepwise regression analysis of Ad-SoS with age, weight, and height was also performed in the whole group and the following equation was obtained: Ad-SoS [m/s] = 1370 − 2.2 × age [years] − 0.3 × weight [kg] + 4.66 × height [cm], $r = 0.6$, $P < 0.00001$, SEE = 64.1.

The best fit of Ad-SoS with age was expressed by polynomial function (regression equation Ad-SoS [m/s] = 1647.3 + 40.6 × age [years] − 1.059 × age [years]2 + 0.0077 × age [years] 3 + 0.0000017 × age [years]4 − 0.000000027 × age [years]5, $r = 0.47$; SEE = 33.0; $P < 0.00001$.

Concluding, QUS measurements at the hand phalanges are a useful tool in the assessment of skeletal status in female population.

P460-Su

Bone Mineral Density Loss and Back Pain Symptoms in Pregnancy

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Objective: To evaluate whether back pain symptoms in pregnancy are associated with bone mineral density changes using non-invasive ultrasound measurements of the os calcis.
Methods: Consecutive patients were prospectively recruited from a low risk obstetric clinic over a period of 12 months. Basic anthropometric data were recorded, and bone mineral density measurements were performed at the os calcis

bilaterally between 14–20 weeks and 36–38 weeks using a Hologic Sahara Clinical Bone Sonometer system. A computer-derived bone mineral density value (BMD) was obtained with each measurement. Body fat composition was also measured using a Tanita 501 bio-impedance assay system. These patients were then surveyed for back pain symptoms during pregnancy by means of a standard questionnaire in the early postpartum period.

Results: Of a total 463 patients, 231 (49.8%) reported one or more episodes of significant back pain during pregnancy. A mean fall in BMD of 0.034 gm/cm^2 (around 5.5%) was demonstrable across the two measurements from early to late gestation. There were no significant differences between the age, parity, and occupational status of those who had back pain symptoms or those without. Those with back pain symptoms have a higher early pregnancy body mass index (23.6 kg/cm^2 vs. 22.7, $P = 0.004$), higher weight gain between the two measurements (10.9 kg vs. 10.3 kg; $P = 0.031$) than those with no pain, but the mean increase in body fat percentage did not differ (7.58% vs. 7.50%). The mean BMD loss at the os calcis across the two serial measurements was significantly greater in the back pain group compared to those without back pain (0.0348 g/cm^2 vs. 0.0232 g/cm^2; $P = 0.012$). The pregnancy outcome did not differ between the two groups. A logistic regression model using presence or absence of back pain as the dependent variable showed weight gain ($P = 0.001$) and BMD loss in pregnancy ($P = 0.007$) remained significant factors related to back pain symptoms in pregnancy.

Conclusion: A significant fall in BMD was demonstrable using ultrasound measurement of the os calcis from early to late pregnancy. Those with back pain symptoms had a greater fall in BMD values at the os calcis together with higher pregnancy weight gain compared to those without pain. This observed fall in BMD could be a reflection of true general loss of bone mineral content or an alteration of bone architecture.

P461-Mo

Estimation of Femoral Bone Density Using Site Specific Quantitative Ultrasound—Results on Excised Specimens

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Quantitative ultrasound (QUS) measurements at peripheral sites can be used to estimate osteoporotic fracture risk. However, measurements at these sites are less suitable to predict bone mineral density (BMD) or fracture risk at the central skeleton. In a European collaboration, we investigated the ability of QUS velocity (SOS) measured at the proximal femur to predict BMD at the human femur.

10 femora (6 females, 4 males, age: 55–90) were used in Kiel for the evaluation of the methodology (evaluation set) and 38 femora (12 females, 26 males, age: 45–91) were measured in Paris to test the methodology in an independent data set (test set). The femora were scanned in transverse transmission mode using focussed US transducers of 500 kHz center frequency. The specimens were degassed prior to the measurements and positioned between the transducers in a water bath at room temperature. The SOS values were averaged over a region similar to the total hip region of dual X-ray absorptiometry (DXA) measurements. Especially at the fringes of the bone, some signals could not be analyzed and were excluded. BMD was measured using DXA (QDR 4500A, Hologic).

SOS and BMD correlated significantly ($P < 0.0001$) in both data sets (evaluation set: $R^2 = 0.93$, test set: $R^2 = 0.86$). Residual errors for the estimation of BMD were 8.2% in the evaluation set and 9% in the test set.

In our study, the residual error of 8–9% is comparable with the residual error of 8.9% for the estimation of femur neck BMD from trochanteric BMD and smaller than the error of 13% for the estimation of total femur BMD from calcaneus SOS (data from the Opus study). The results of the study show that SOS is able to predict total BMD with high accuracy. At the moment, we are producing an in vivo applicable device which should be tested within this year. If femoral BMD could be obtained in vivo with comparable accuracy, new opportunities of ultrasound measurements at the human femur would be opened.

P462-Tu

Multisite Ultrasound and Vertebral Deformity: Findings from the Canadian Multicentre Osteoporosis Study (CaMoS)

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The use of multisite ultrasound to assess fracture risk is an attractive technology due to its simplicity of use, lack of ionizing radiation, low capital cost, and portability. It has been hypothesized that the qualitative ultrasound assesses the architectural properties of bone, such as stiffness, trabecular connectivity, and cortical porosity, rather than density. This investigation used a subset of the Canadian Multicentre Osteoporosis Study (CaMoS) dataset to cross-sectionally assess the association between ultrasound (speed of sound) at the distal radius, phalanx, or tibia and vertebral deformity (>3 SD lower than population mean in anterior, middle, or posterior vertebral height) in women aged 50 years or greater. Data from Saskatoon, Quebec, St. John's, Calgary and Hamilton, were used in this analysis. A Sunlight

OmniSense Multisite Ultrasound (Israel) was used for all ultrasound assessments and all ultrasound data were collected at year 5 of CaMoS. A general linear model analysis was used to assess the association between ultrasound measures at all sites and vertebral deformity risk with correction for height, weight, age, and CaMoS center. Following a radiograph of the spine at year 5 of CaMoS, 744 women (74.4%) were found to have no vertebral deformity, whereas 256 women had a vertebral deformity (25.6%). Women without a vertebral deformity had higher ultrasound measurements at the distal radius by 30.2 points (95% CI: 5.5, 55.1), at the tibia by 32.3 points (95% CI: 9.6, 55.0), and at the phalanx by 10.6 points (95% CI: −20.8, 42.0; NS). This analysis has shown that the Sunlight OmniSense Multisite Ultrasound has the ability to discriminate between women with or without vertebral deformity whether used at the distal radius or tibia, but not the phalanx, site. Since the data analysis for vertebral deformities and non-vertebral fractures in CaMoS is ongoing and soon to be completed, updated analyses investigating the use of Sunlight OmniSense Multisite Ultrasound for discriminating between those with a risk for both vertebral deformity and non-vertebral fracture will be reported at the meeting.

P463-Su

Ability of Ultrasound Calcaneal Bone Density Measurement to Diagnose Spine Osteoporosis in Women

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Introduction: Ultrasound calcaneal bone density measurement is an easy and cheap method. The recommendation of International Society for Clinical Densitometry requires the use of a device-specific cutpoint on the peripheral device that detects 90% of individuals with osteoporosis at either spine or hip.
Objective: To determine the 90% sensitivity cutpoint of Achilles Lunar plus ultrasonometer for identifying spine osteoporosis in women using ROC analysis.
Methods: Ultrasound bone density at the right heel was measured in women using the Achilles Lunar plus device. The result of measurement was given as Stiffness Index (SI) that is determined by broadband ultrasound attenuation (BUA) and speed of sound (SOS), and *T*-score that is the number of SDs of SI from mean SI of normal young adult women. Also, spine bone density was measured by DEXA (Lunar DPX). The result of DEXA measurement was given as bone mineral density (BMD) and *T*-score. Spine osteoporosis was diagnosed if DEXA *T*-score (L1–L4) was below or equal to −2.5.
Results: Fifty-three women with mean age 59 ± 8.5 years participated in the study. Fourteen had spine osteoporosis. By ROC analysis, at a heel *T*-score of −1.0, the sensitivity was 92.9% and specificity was 25.6% for identifying spine osteoporosis. Higher specificity of nearly 50% could be obtained with a *T*-score cutpoint of −1.52, which had a sensitivity of 64.3%. The table below depicts the sensitivity and specificity of different heel *T*-score values for detecting spine osteoporosis in women.
Conclusion: At a heel *T*-score of −1.0, the sensitivity was 92.9% with low specificity for detecting spine osteoporosis in women using the Achilles Lunar plus ultrasonometer. The results of ROC analysis may be better in a larger statistical sample, in a statistical sample consisted only of postmenopausal women, or for detecting hip osteoporosis.

Table
Sensitivity and specificity of heel *T*-score values

Heel *T*-score	−1.0	−1.38	−1.52	−2.21	−2.4	−2.7
Sensitivity (%)	92.9	71.4	64.3	64.3	50	35.7
Specificity (%)	25.6	38.5	51.3	71.8	79.5	87.2

P464-Mo

Ultrasound Measurements at the Proximal Phalanges Related to Metacarpal Radiogrammetry in Women with Low-Trauma Hip Fracture

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Hip fracture is a common, morbid, and costly health problem. For a 50-year-old Caucasian woman today, the risk of incurring a hip fracture during her remaining lifetime is about 17%. The aim of this study was to evaluate the capacity of metacarpal bone mass morphometry and phalangeal bone ultrasound measurements to discriminate between women with and without low-trauma hip fracture.
Patients and methods: Metacarpal radiogrammetry and phalangeal bone ultrasound were performed on a group of 84 female patients aged 50–94 years who had suffered recent low-trauma hip fractures, and the results were compared with 63 healthy women matched for age and body mass index. Bone mass was calculated as a metacarpal cortical area/total area (CA/TA) index, measured by radiogrammetry. The ultrasound device used was a model DBM Sonic 1200R® that measures the amplitude-dependent speed of sound (m/s) transmitted through the phalanges.
Results: In the patients group, 35.7% had a documented history of previous fractures, and 21 were diabetic. Amplitude-dependent speed of sound declined with age in the control group ($P < 0.0001$) but not in the patients group ($P > 0.05$). There was a significant positive correlation of amplitude-dependent speed of sound with CA/TA, and negative with weight, body mass index, and endostal diameter ($P < 0.03$ in all). The amplitude-dependent speed of sound of the fracture group was lower than the control group ($P < 0.01$). The endosteal diameter, indicative of bone

resorption, differed between the two groups ($P < 0.05$). Only weight and the endosteal diameter were found to be significant and negative in a stepwise regression of the amplitude-dependent speed of sound as dependent variable against age, and the anthoprometic, biological, and radiometric parameters as independent variables ($P = 0.0021$). In conclusion, these findings concerning metacarpal measurements reflect the importance of bone resorption, as opposed to deficient bone formation, in the etiology of hip fracture in women. Radiogrammetry is closely correlated with amplitude-dependent speed of sound measurements, and both may be well-suited to the study of women with hip fracture, providing simple, inexpensive, and sufficiently precise methods to evaluate bone mineral status. This may be particularly useful in emergency services, where investigation for osteoporosis is often overlooked.

P465-Tu

How Does Estrogen Use in Early Postmenopausal Women Affect the Diagnostic Performance of the Osteoporosis Self-Assessment Tool and Quantitative Ultrasonography?

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Early postmenopausal women are increasingly referred for a BMD status due to cessation of hormone replacement therapy (HRT). We aimed at exploring how HRT affects the diagnostic performance of the Osteoporosis Self-Assessment Tool (OST) and calcaneal quantitative ultrasonography (cQUS). We used data collected at the 5-year follow-up in two centers in the Danish Osteoporosis Prevention Study. The analysis included 821 women, mean[range] age 55[48–64] years. OST was calculated as: $0.2 \times$ (weight $-$ age) truncated to an integer. The cQUS variable stiffness (STF) was measured using a Lunar Achilles device. BMD of the spine, femoral neck, and total hip was measured by Hologic 2000 densitometers. *T*-scores (*T*) were calculated using NHANES-III (hip) and Hologic (spine) reference values. The outcome was defined as $T \leq -2.5$ in any region. Cut-offs for OST and STF generating closest to 90% sensitivity (SN) were determined on ROC curves. The cut-off generating 90% SN for STF varied little between groups (see table). Applying the cut-off (<81) generated for all 821 women to never and ever users resulted in (SN, SP) = (90%, 53%) and (SN, SP) = (86%, 69%), respectively, the difference in specificity (SP) was 17, 95% CI (10–23). For OST, the cut-offs were constant between groups, the difference in SP was 6, 95% CI (1–10). STF and OST had the same ability to rule out $T \leq -2.5$ (NPV 97–100%) in never and in ever users, but the SP of STF was significantly higher than the SP of OST in both groups (McNemar's test: $P < 0.0001$, both groups).

Conclusions: The accuracy of OST appears to differ only marginally between early postmenopausal never and ever HRT users. Conversely, the performance of STF is slightly better in ever HRT users. In addition, whereas OST and STF seem equally effective in ruling out $T \leq -2.5$ in never and in ever HRT users, STF reduces the number of referrals of women with $T > -2.5$ appreciably in both groups due to its higher PPV.

Table
Performance of OST and cQUS in never and ever HRT users

	All (821)	Never HRT (461)	Ever HRT (360)
Prevalence of $T = <-2.5$ n(%)	65 (7.9)	51 (11.1)	14 (3.9)
Stiffness AUC/Cut-off (+)	0.86/<81	0.84/<80	0.87/<82
SN/SP/PPV/NPV (%)	89/60/16/98	90/58/21/98	93/65/10/100
OST AUC/Cut-off (+)	0.75/<4	0.75/<4	0.77/<4
SN/SP/PPV/NPV (%)	91/32/10/98	90/34/15/97	93/29/5/99

P466-Su

Multisite Quantitative Ultrasound as First Parameter for the Diagnostic Screening of Postmenopausal Osteoporosis

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Studies in vitro and in vivo have shown that quantitative ultrasound (QUS) is a valid tool for the assessment of bone status. Current QUS methods using the transmission technique are limited to one peripheral bone site only. Sunlight (OMNISENSETM 7000S) (OMNI) is a system measuring speed of sound (SOS, in m/s) along the surface of the bone based on an axial transmission technique. It measures SOS at the distal 1/3 of the radius (RAD), mid-shaft tibia (TIB), proximal phalanx III (PLX), and metatarsal V (MTR). To define the cutoff limits for OMNI predicting reduced bone density as evaluated by DXA in postmenopausal SD age 63.3 ± 0.64 years; age ± women, we studied 157 postmenopausal women (mean at menopause 47.8 ± 0.3 years; BMI 24.6 ± 0.3 kg/m^2) without treatments or diseases known to negative or positive affect bone metabolism. They underwent both OMNI multisite (RAD, TIB, PLX, MTR) and DXA (Hologic QDR4500A). A positive correlation between DXA (BMD) and OMNI (SOS) was found at the levels of RAD, TIB, and PLX (*r*: 0.36, 0.44, and 0.26, respectively, $P < 0.005$). A positive correlation between DXA and OMNI *T*-score was found at RAD, TIB, PLX, and

MTR (r: 0.22, 0.34, 0.19, and 0.18, respectively, $P < 0.05$). A positive correlation between DXA and OMNI Z-score was found at the level of TIB only ($r = 0.22$, $P < 0.01$) while showed a trend toward association at RAD. By assuming DXA as golden standard and −2.5 T-score for OMNI, specificity (SP) and sensitivity (SE) of the method were 73% and 32% at RAD, 84% and 29% at TIB, 81% and 20% at PLX, and 92% and 3% at MTR. OMNI SP and SE by grouping 2 sites of evaluation (RAD and TIB) were 66% and 40%. The value of OMNI as first parameter for the diagnostic screening of osteoporosis was evaluated again in this population by using receiver operating characteristic (ROC) plot analysis and assuming as reference normal DXA T-score for normal subjects. The T-score cutoff limits for OMNI calculated using the ROC curve analysis were −1.6 at RAD (SP and SE: 59% and 62%); −2.2 at TIB (78.6% and 43%); −0.5 at PLX (32.5% and 78.2%); −0.8 at MTR (53% and 59.4%). The SE would obviously be further increased by loosing SP. For instance, a SE of 95% was associated to a SP of 11.8% for a T-score of 0.7 at RAD level. In conclusion, we defined by ROC analysis the cutoff limits of QUS by OMNI as first parameter for the diagnostic screening of osteoporosis. The results show that the reliability of this method to this purpose depends on appropriate cutoffs. The best bone sites for QUS by OMNI seem RAD and PLX that couple acceptable SE and SP.

P467-Mo

Association of Ultrasounds and Fractures in Adult Men and Women in Polish Population—The Epolos Study

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Introduction: Increased risk of fractures and decreased values of bone mineral density (BMD) that characterize osteoporosis refer to women as well as to men. Ability to predict fractures, hallmark of osteoporosis, with the use of non-invasive, safe, and effective method is a real need, with ultrasounds as potential candidate.

Objective: The aim of this study was to analyze the potential association between ultrasound bone parameters and fractures in men and women from the Polish population.

Methods: The study group comprised 724 subjects, adult males ($n = 320$) and females ($n = 404$), in age range 20–80 years, randomly selected in 7 centers from the Polish population. Fracture data were received retrospectively from questionnaire filled by the physician. Vertebral and non-vertebral fracture cases were analyzed. Fractures due to accidents were excluded. Control groups were selected by center, sex, and age. Ultrasound parameters utilized Stiffness values, measured for heel bone with Achilles GE Lunar.

Results: Levels of Stiffness values in female group with fractures ($n = 140$) compared to female control group ($n = 264$) are significantly lower ($P < 0.0012$). Stiffness was normalized on age. Also, levels of Stiffness values in male group with fractures ($n = 113$) were significantly lower ($P < 0.001$) compared to male control group ($n = 207$). Difference in Stiffness between fracture cases and control group was 4.58% for females and 6.04% for males.

Conclusions: Presented results show, both in males and females, significant differences in ultrasound parameters when fracture was utilized as discriminating parameter.

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P468-Tu

Development of Direct Assay of Serum Phytoestrogens with ELISA; Association of Phytoestrogens Levels with Bone Metabolism in Japanese Postmenopausal Women

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Objectives: Phytoestrogens are attracting much attention for their anticancer effects, their reduction of menopausal symptoms, and their preventive effects against osteoporosis in the human body at high concentrations. We developed direct assay system by Enzyme Linked Immunosorbent Assay (ELISA) for the measurement of serum genistein and daidzein levels in postmenopausal women. The first antibody to genistein and daidzein and the second antibody to the first antibody were raised in the rabbits. We analyzed the association of serum phytoestrogens levels with bone metabolism in postmenopausal Japanese women.

Subjects and methods: Sixty postmenopausal Japanese women were enrolled to measure bone mineral density, bone metabolic biochemical markers, and serum phytoestrogens levels. Twenty-nine women were osteoporotic under the diagnosis of WHO criteria. Daily soy protein intakes in all subjects were calculated after the questionnaires for protein

intake were completed. As bone metabolic markers, type I collagen cross-linked N-telopeptide (NTx), Urinary deoxypyridinoline (DPD), bone alkaline phosphatase (BAP), and osteocalcin (OC) were measured in all subjects. Serum phytoestrogen concentrations were compared between non-osteoporotic group and osteoporotic group. Statistical analyses were done with ANOVA and with Pearson's method.

Results: Average age and BMI in postmenopausal women were 73 ± 7.7 years old and 21.6 ± 2.7 kg/m^2, respectively. The lowest values to detect genistein and daidzein were 1 ng/ml in ELISA. Intra-assay variances for genistein and for daidzein were 5.6% and 4.2%, respectively. Dilutional reliability demonstrated a linear curve. Soy protein intake was significantly associated with serum genistein ($r = 0.29$, $P = 0.05$) and daidzein levels ($r = 0.30$, $P = 0.48$). There were no positive associations of phytoestrogen levels with bone metabolic markers. However, genistein ($P = 0.06$) and daidzein levels ($P = 0.04$) in non-osteoporotic group were significantly higher than those in osteoporotic group.

Conclusions: ELISAs for phytoestrogen assay were reproducible and sensitive to measure and reliable to monitor soy protein intake in postmenopausal Japanese women. In order to confirm the role of phytoestrogens in bone metabolism, we should furthermore measure phytoestrogen levels in cohort by ELISA.

P469-Su

Weight-Bearing Exercise Overrides Impact of Dietary Protein on Bone Growth—Results from A 3-Year Longitudinal Study

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Adequate dietary intake and regular physical activity are required for optimization of skeletal health within the individual's genetic potential. Physical activity is suggested to be quantitatively a more important factor affecting bone than nutrition. We have shown that physically active young females exhibit significantly higher bone mass than controls (Nurmi-Lawton et al., 2004). The dietary influences on bone growth are now examined.

A total of 45 female competitive gymnasts (G) and 52 sedentary controls (C), aged 8–17, were recruited. Dietary intake was measured at baseline, 6, 9, 12, 24, and 36 months using estimated 3- or 7-day food records. BMC and BMD were measured by DXA. Calcaneal BUA and VOS were assessed. Maturity status was assessed by secondary sex characteristics and by estimation of age from peak height velocity. A multilevel regression model was fitted and the independent effects of body size, maturity, physical activity, and diet were identified across the study.

The gymnasts were shorter, lighter, had higher BMC, BMD, BUA, and VOS compared with controls. Protein intake was greater in gymnasts when adjusted for weight or lean mass, but crude intake showed no differences. Longitudinal analysis of the determinants of total body BMC showed that after adjusting for body size, maturity status, and exercise, protein intake had a significant independent positive effect on total body BMC (1 g of dietary protein predicted an average increase of 1.8 g of bone mineral; the effects of the other determinants were: 1 cm in height 11 g, 1 kg in weight 22 g, exercise group (G/C) 172 g). Total energy intake had a small negative effect. When the gymnasts were analyzed separately, the dietary effects disappeared, whereas when sedentary girls alone were examined, dietary effects were still significant.

These results suggest that high-impact weight-bearing exercise has a stronger effect on bone growth than nutritional factors, specifically protein intake. These findings support the suggestion that dietary intake has a permissive effect on bone mineral accrual whereas physical activity has a modifying effect, although further research investigating the synergistic effects of these exogenous factors is required.

Nurmi-Lawton JA et al. Evidence of sustained skeletal benefits from impact-loading exercise in young females: a 3-year longitudinal study. JBMR 2004;19:314–322.

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P470-Mo

Lycopene Consumption Significantly Decreases Oxidative Stress and Bone Resorption Markers in Postmenopausal Women at Risk for Osteoporosis

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Oxidative stress induced by reactive oxygen species (ROS) is now causally associated with the risk of osteoporosis, and certain antioxidants were shown epidemiologically to counteract this risk. Lycopene, a carotenoid found in tomatoes and tomato products, is a potent antioxidant known to decrease the risk of cancer and cardiovascular disease. However, the role of lycopene in osteoporosis has not yet been investigated. Our objective is to examine the relationship between lycopene intake and antioxidant capacity, oxidative stress parameters, and bone turnover markers in postmenopausal women who are at elevated risk for osteoporosis.

33 women aged 50–60 were recruited and asked to complete a 7-day food intake record prior to giving fasting blood samples. The following parameters were measured in serum: total antioxidant potential, serum lycopene, lipid peroxidation and protein thiols (oxidative stress markers), and the turnover markers bone alkaline phosphatase and cross-linked N-telopeptides of type I collagen (NTx).
The serum lycopene per kilogram body weight of the participants was grouped into quartiles and analyzed statistically using one-way ANOVA and the Newman–Keuls post test to correlate with the above serum parameters. The average serum lycopene for each group was as follows (nM/kg): group 1, 1.1 ± 0.2; group 2, 2.6 ± 0.1; group 3, 4.0 ± 0.2; and group 4, 8.1 ± 0.6. The groups did not differ significantly in age, height, or weight. All the groups differed significantly in average NTx (nM Bone Collagen Equivalents) (ANOVA, $P < 0.005$); group 4 (17.1 ± 1.3) was significantly lower than group 1 (22.5 ± 2.3) ($P < 0.05$). All the groups differed (ANOVA) significantly in average protein thiols (<0.05); group 4 (592.2 ± 31.1) had significantly higher protein thiols than group 3 (457.4 ± 50.1) ($P < 0.05$). Thus, as serum lycopene increases, protein oxidation and NTx decrease. All the groups differed significantly in lycopene intake (mg/day) as determined from the food records (ANOVA, $P < 0.02$); group 4 (6.5 ± 1.0) was significantly higher than group 1 (1.8 ± 0.8) ($P < 0.01$). In conclusion, these results support our hypothesis that dietary lycopene is readily absorbed and acts as an effective antioxidant in reducing oxidative stress and bone turnover markers, thus reducing the risk of osteoporosis. Future dietary intervention studies are needed to demonstrate the beneficial effects of lycopene in the prevention and management of osteoporosis.

P471-Tu

Femur Fracture Incidence in Venice (Italy) in Year 2003: An Attempt of Historical Comparison with Year 1857

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Aim of the study: To calculate incidence of femur fracture in >60 women living in the historical center of Venice, Northern Italy, in year 2003 and compare current population data with those (limited) of year 1857 to estimate the number of femur fractures possibly occurring in that year in >60 women. The historical center of Venice has remained quite the same in hundreds of years, motor vehicles are not allowed and people are forced to walk for most of their common daily activities. There is only one general hospital in the city and virtually all acute patients are referred there. From the Orthopaedics Department files, data on femur-fractured patients in year 2003 were obtained, and from the Statistics Service of Venice City Hall, data about the population in year 2003 were retrieved, for males and females (M and F), subdivided into 5- and 10-year age intervals (a.i.). Population data for year 1857 were obtained by a historical source (1). In 2003, 95,617 people lived in Venice (44,506 males–51,111 females), with a mean age of 48 years (50.4 females–45.3 males). The percentage of people >60 was 34.5% (13.8% M-20.7% F) and that of people >65 was 26.5% (10.0 males–16.5% females). The incidence of femur fracture for women >60 was 7.6 × 1000 and for women >65 was 9.1 × 1000 (Italy 2002:10.6). In spite of a mean age higher than the Italian one (Italy 2001:42.2), 2003 femur fracture incidence in Venice was a little lower than the Italian one (decreased risk of car accident? beneficial effects of forced exercising?). In 1857, 124,548 people lived in Venice (60,278 males–64,270 females) with a mean age of 30.6 years (31.4 females–29.8 males). The percentage of people >60 was 9.4% (3.9% males–5.5% females). Assuming the 2003 fracture rate, in 1857 the number of femur fractures would have been 28. These data further support the well-known fact that femur fractures are growing as a social problem mainly because of the increase in the mean age and in the relative percentage of elderly people among the general population, mostly in the female one.
(1) C. Cantù Il Lombardo-Veneto Vol.II Milano 1858.

Table
Fracture incidence comparison 2003–1857

2003 Population	95,617	>60	>65	60–69	70–79	80–89	90+
Females	51,111	19,771	15,767	7617	6697	4378	1079
Fracture number		151	144	11	31	76	33
$n \times 1000$		7.6	9.1	1.4	4.6	17.4	30.6
1857	64,720	6792		4371	1665	700	56
Estimate		28		6	8	12	2

P472-Su

A Reanalysis of Two Studies that Reported Contrasting Results on the Association Between Statin Use and Risk of Fracture in the Same Datasource

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Background: In 2000 and 2001, two case-control studies were independently conducted to evaluate the association between use of statins and risk of fracture. Both studies used the same study data source, the UK General Practice Research Database (GPRD), but found opposite results. The objective of this study was to evaluate the methodological reason for this.

Methods: We repeated both study designs in GPRD: a "nested" case-control dataset, with fracture cases matched to controls nested within a selected cohort, and a "population-based" case-control dataset, with both cases and controls sampled from the total GPRD population. The cases and controls were matched by gender, age (5-year band), and general practice.
Results: The study included 131,855 fracture cases. In the "nested" case-control dataset, only 37% of the cases were matched by year of birth, while this was 99% in the "population-based" dataset. The crude OR for hip fracture in statin users differed between the two designs (0.37 [95% CI 0.27–0.52] in the "nested" and 0.54 [95% CI 0.39–0.74] in the "population-based" dataset). But this difference reduced when matching by year of birth, rather than by 5-year band (crude ORs 0.58 [95% CI 0.43–0.79] and 0.61 [95% CI 0.44–0.88], respectively). Other factors that introduced differences included the exposure time-window, confounder selection, and exclusion of high-risk patients. It was found that the risk of hip fracture was reduced after already one statin prescription (30 days of treatment) and remained stable with increased use.
Conclusion: Choices in study design may lead to contrasting results. A detailed sensitivity analysis may be important in the study of health care database.

P473-Mo
No Decreasing Hip Fracture Incidence in Older Women
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It is an epidemiologic task to evaluate if population screening and treatment effects are effective.
Method: In Switzerland for 2002, all public and private hospitals provided the collected information about discharged patients to the Federal Office of Statistics. For this study, transfers were excluded, defined as either admission from another hospital of discharge to another hospital. Any within-hospital move was also considered to be a transfer. The length of stay was calculated in days only for Swiss patients with a hip fracture as main and principal diagnosis. For 1988, the hip fracture rates were calculated applying the same conditions.
Results: The risk for osteoporotic hip fracture increases with age in postmenopausal women older than 60 years of age and in men older than 65 years of age, their annual incidences of proximal femur fractures exceeding clearly 100 per 100,000 persons and nearly doubling in every next 5-year age period. Comparing the hip fracture incidences of 1988 and 2002, the rates increased significantly for men, also for women aged 80+ years, but decreased modestly for women aged below 80 years. The age-standardized annual rate per 100,000 men aged 45+ years is 30% higher in 2002 than being in 1988. The female/male ratio of rates for the age group 55–79 years declined in the last 14 years from 2.3 to 1.8. The hip fractures are a major healthcare cost factor. On one side, the femur fracture cases increase in a growing (older) population, on the other side, the average length of hospital stay, calculated for people of 45+ years of age, decreased between 1988 and 2002 from about 35 days to 18 days. In 2002, the sum of hospital days for proximal femur fractures was 146,616 for women and 47,445 for men, corresponding at all to the annual cost of a hospital with 530 beds (780 beds in 1988). Neutralizing the population effect and the surgical progress, the true growth of hospital days is 29% for men and only 2.5% for females since 1988.
Conclusions: The hip fracture incidences decreased significantly for postmenopausal women aged between 55 and 79 years, being in agreement with the successful estrogen therapy of a minority in this female cohort. Men and older women with osteoporosis receiving by preference alendronate (available since 1996) beside calcium and vitamin D, it seems–reflecting the clinical practice–that hip fractures cannot be prevented in time by bisphosphonate treatment.

P474-Tu
Biomechanical Properties of 10 Different Hip Protectors, and the Influence of Soft Tissue
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A promising intervention in the prevention of hip fractures is the external hip protector. However, in a previous patient study that was performed by our department, hip protectors were not effective in preventing hip fractures, probably due to moderate compliance, and lower effectiveness than expected. Since the start of our study, many new hip protectors were developed. It is not clear which hip protector is the most effective in reducing the impact of a fall. Therefore, a biomechanical study was performed to examine which hip protector has the best force attenuation capacity. Both hip protectors of the energy-shunting and of the energy-absorbing type were included. In addition, the influence of soft tissue was examined.
Using a drop weight impact testing system and a surrogate femur, a weight of 25 kg was dropped from a height of 8 cm causing a force of almost 8000 N on the femur, which simulates a severe fall. After this calibration test, soft tissue and the different hip protectors in combination with the soft tissue were tested. Each test was repeated six times. To examine the difference in impact between more obese and normal-weight elderly persons, soft tissue was simulated by a 1-in. and a 1/2-in. thick layer of foam, absorbing 49% and 18% of energy, respectively.
In the 1-in. soft tissue test, all hip protectors were capable in reducing the impact to below the average fracture

threshold of elderly people (3100 N), although the hard types performed significantly better than the soft ones ($P < 0.001$). In the 1/2-in. soft tissue test, only the hard, energy-shunting hip protectors were capable of attenuating the energy to below the fracture threshold ($P < 0.001$).
In conclusion, in this study it was found that the hard, energy-shunting hip protectors were superior to the soft, energy-absorbing ones, especially in normal-weight elderly persons. In more obese elderly people, the energy-absorbing hip protectors might also be sufficient.

P475-Su
Recognizing Osteoporosis and Its Consequences in Québec (ROCQ): Fragility Fracture Rate Among Women Who Sustained a Fracture
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Objectives: To evaluate the proportion of fragility and traumatic fracture in women 50 years and over participating in a patient health-management program (ROCQ) aimed at improving diagnosis and treatment of osteoporosis.
Material and methods: ROCQ is prospective cohort study involving 23 centers in three socio-sanitary regions in the province of Quebec (Canada). Women with fragility and traumatic fractures were recruited during their visit at a cast or outpatient clinic and contacted by phone to answer a questionnaire aimed at identifying the specific circumstances of their fracture. Based on this questionnaire, patients were classified as having a fragility or traumatic fracture.
Results: After 9 months, 646 women (mean age: 65.3 years) have been recruited of which 514 sustained a fragility fracture (83.7%) and 105 a traumatic fracture (16.3%). 78% of women recruited with a fragility fracture were 75 years of age or less. The age distribution between fragility and traumatic fractures was similar.
Conclusion: In ROCQ, 83.7% of fractures were related to osteoporosis while the literature estimates that 70% of the fractures in those over age 45 are attributable to osteoporosis ($P < 0.0001$) (1).
ROCQ is the first prospective study evaluating fragility vs. traumatic fracture among women 50 years and over. It is an ongoing study and updated data on a larger population will be presented at the meeting after further collection has been completed.
(1) Iskrant AP, Smith RW Jr. Osteoporosis in women 45 years and over related to subsequent fracture. Public health Rep 1969;84:33–8.
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Table
Fragility and traumatic fractures by site

Site	Fragility number	Fracture (%)	Traumatic number	Fracture (%)
Wrist	210	36.8	36	29.5
Forearm	37	6.5	8	6.5
Humerus	47	8.2	17	13.9
Foot	39	6.8	14	11.5
Ankle	100	17.5	14	11.5
Tibia/Fibula	51	8.9	15	12.3
Femur	10	1.8	3	2.5
Hip	25	4.4	2	1.6
Other	52	9.1	13	10.7

P476-Mo
Prevalence and Cumulative Incidence of Vertebral Fractures in a Rural Japanese Community: The Miyama Study
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The objective of this study was to clarify the prevalence and cumulative incidence of vertebral fractures among general inhabitants of Miyama, a rural Japanese community. A cohort of 1543 inhabitants aged 40–79 years was established using resident registration in 1989. Four hundred participants were selected and divided into four age strata of 50 men and 50 women each. Participants completed a self-administered questionnaire and anthropometric measurements were taken. In 1990, the baseline bone mineral density (BMD) of the lumbar spine and proximal femur was measured using dual energy X-ray absorptiometry (DXA), and X-ray examinations of the thoracolumbar spine in the anteroposterior and lateral views were performed on the subjects. X-ray examinations of the same sites were carried out on the same participants in 2000. Vertebral fractures were diagnosed by an experienced orthopedic surgeon. The baseline prevalences of vertebral fractures for men in their 40s, 50s, 60s, and 70s were 2.1%, 8.3%, 8.0%, and 6.1%, respectively, and those in women were 0%, 8.2%, 8.0%, and 40.8%, respectively. After the exclusion of cases with prevalent vertebral fractures, new cases of vertebral fractures during a 10-year period were identified. The cumulative incidences of vertebral fractures following 10 years in men in their 40s, 50s, 60s, and 70s were 2.9%, 2.8%, 8.6%, and 21.1%, respectively, and those in women were 2.1%, 7.0%, 18.9%, and 31.3%, respectively. These findings show the sex and age difference of vertebral fractures in a rural Japanese population. The present study links questionnaire items regarding life-style factors to baseline BMD values and assesses whether or not they can predict future vertebral fractures.

P477-Tu

Italian Preliminary Reference Data of Normal Vertebral Dimensions for Morphometric X-ray Absorptiometry (MXA): Normal Morphometric Dexa (NORMODEXA) Study

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Accurate identification of vertebral fractures both for epidemiological studies and clinical practice depends on comparison to dimensions among normal (non-fractured) vertebrae. Aim of this multicentric study is to define Italian reference values of normal vertebral dimensions for morphometric X-ray absorptiometry.

Design of the study: According to Black method[1], we included both women with and without vertebral fractures. Assuming that values of height ratios of no fractured vertebrae follow a Gaussian distribution and that almost all the values of fractured vertebrae are in the tail of the distribution, we trimmed the highest and lowest 10% of the values. Each center recruited a sample of women within eight age ranges of 5 years: fertile women aged <44 and postmenopausal women aged 45–49, 50–54, 55–59, 60–64, 65–69, 70–74, >74 years. Up till now, six centers located across Italy are participating in this study (Ancona, Catania (2), Imola, Roma, Verona) involving a total of 1000 women. All participating women had MXA of vertebrae T4-L4, two centers used the Hologic QDR4500A, and four centers used the GE Lunar Prodigy. Both systems acquired spine images with dual-energy (high-definition). MXA images were sent to Rome for centralized analysis by an experienced radiologist (D.D.).

Results: Intra-operator precision was found to be similar on the two systems, Hologic and GE Lunar: the coefficient of variation (CV%) was 2.5% for the posterior height and 3% for the anterior height, resulting relatively higher in T4 and below in L4. The age-adjusted mean and standard deviation of the three vertebral heights (anterior, middle, and posterior) and their ratios for all vertebral levels were calculated for both systems. No statistically significant relationship was found between vertebral heights and age in premenopausal women. However, in postmenopausal women, a significant inverse relationship with age (r = 0.6, $P < 0.001$) was observed between age and mean vertebral heights and their ratios. Vertebral heights significantly correlated with years since menopause (r = 0.5, $P < 0.001$) decreasing much more in the first 5 years after the menopause.

Conclusions: These preliminary results indicate the usefulness also for MXA of normal values adjusted for age and for years since menopause.

[1]DM Black et al. A new approach to defining normal vertebral dimensions. JBMR 1991;6:883–892.

P478-Su

Cost Effectiveness Analysis of Systematic Prophylaxis of Fractures in Patients with Recent hip Fracture and Osteoporosis—Results of a Markov Analysis

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Randomized double-blinded studies have showed that pharmacological treatment of osteoporosis reduces the incidence of fractures significantly. Patients with hip fractures have an increased risk of future fractures but are often not offered examination or treatment for osteoporosis on a routine basis[a].

Objective: To analyze the cost effectiveness of treatment for osteoporosis in patients with a recent hip fracture.

Methods: A recently developed Markov model (Danish Osteoporosis Outcome Model[b]) was used to compare costs and effects of (a) no treatment and (b) calcium plus vitamin-D plus alendronate. The model simulated a cohort of 10,000 women followed from 50 years of age until "death" or age 100 years. The model has 9 different states of health including "well", "second hip fracture", and "dead". Duration of each cycle was 1 year. Our base case assumed an 81-year-old woman (mean age of Danish hip fracture patient) with osteoporosis diagnosed by DEXA (T-score <−2.5) discharged following her first hip fracture and living in her private home. Her increased fracture rate was set to 2.4[c]. Treatment was started immediately, continued for 3 years, and the relative risk reduction (RR) was set to 0.50. Furthermore, sensitivity analysis was made according to age and effect of treatment.

Results: The intervention cost for the cohort was estimated 16.5 Mio EUR. In comparison with (a) no treatment, the intervention (b) avoided 1245 fractures (including 570 hip fractures) and gained 1274 life years/1075 quality adjusted life years (QALY). The intervention provided substantial health benefit and saved healthcare resources (net 2.4 Mio EUR). In younger patients, the benefit and net saving were

reduced. Assuming a less efficacious treatment (RR = 0.75), the cost per QALY increased to 13.272 EUR in the base case (30.119 EUR and 6.220 EUR age 71 and 86, respectively).
Conclusions: In the base case treatment with alendronate, calcium and vitamin-D provided additional health benefits and net cost-savings. This was more pronounced in elderly patients; however, the cost per QALY was acceptable even in younger patients. A systematic treatment of osteoporosis in hip fracture patients would be cost effective in Denmark as judged from these assumptions. [a]Port et al. Osteoporosis Int 2003;14(9):780–4. [b]www.sam.sdu.dk/healtheco/pub/20032.pdf. [c]Klotzbuecher et al. J Bone Miner Res. 2000 Apr;15(4):721–39. Supported by MSD and Danish Centre for Evaluation and Health Technology Assessment.

P479-Mo
ICARO Study (Incidence and Characterization of "Inadequate Treatment Responder Patients" in Osteoporosis): Baseline Characteristics of Study Population, Final Cross-Sectional Report
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ICARO is a multicenter observational project that aims to identify, among patients with severe osteoporosis, those with "inadequate response to antiresorptive treatment", defined as patients prescribed with antiresorptive drugs (alendronate, risedronate, and raloxifene, as reported by Note 79) for at least 1 year, presenting a new fragility fracture (vertebral or non-vertebral) or discontinuing antiresorptive therapy for lack of compliance and/or side effects. The study consists of two phases: a cross-sectional and 12 months longitudinal one. In the cross-sectional phase, postmenopausal women with established osteoporosis, prescribed for at least 1 year with antiresorptive treatment, were enrolled in the study and were submitted to evaluation of clinical history, osteoporotic disease status, and concomitant therapy; X-rays and DXA result registration (if available); drug compliance to previous antiresorptive therapy; quality of life, measured by QUALEFFO-41. One thousand four hundred twenty-one women with severe osteoporosis, mean age 67.6 (±7.7) years, were studied. Patient with "inadequate clinical response" was defined as a subject presenting a new clinical fragility fracture (with following radiological documentation) after at least 6 months of antiresorptive therapy prescription.

During cross-sectional phase, 231 subjects with "inadequate clinical response" were identified (25.6% on 904 patients valuable with the above described criteria). The mean age at 1st fracture and mean treatment duration were, respectively, 64.97 and 2.14 years for responders, and 65.53 and 2.79 years for the "non-responders". Among Note 79 drugs, the most used one in "inadequate clinical responders" was alendronate, either in association with calcium and vitamin D or alone. Further data are expected from the longitudinal phase to establish the incidence of new "inadequate clinical responders" during 1-year antiresorptive treatment. Nevertheless, the present preliminary results show a proportion of "inadequate clinical responders" higher than expected on the basis of the scientific literature, with a greater prevalence of multiple clinica-vertebral fractures in these subjects.

Table
Site of fracture (% of total osteoporotic fractures)

	Multiple vertebral fractures	Single vertebral fractures	Hip fractures	Other sites
Adequate responders	44.3	45.1	4.5	6.1
Inadequate responders	53.5	32.1	4.6	9.9

P480-Tu
Is EBM-Based Antiresorptive Treatment of Bone Enough to Prevent Fractures in Elderly Women?
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Objectives: To examine the incidence of falls and clinical fractures and their association with parameters of neuromuscular function and osteodensitometry in a German cohort of elderly women.
Patients and methods: 1197 postmenopausal women aged 60–95 years were examined in the "Osteoporosis Risk Factor Survey" in summer 2002. This survey (including medical history, osteodensitometry by DXA, and measurements of neuromuscular function) was conducted in order to develop a high-risk score for osteoporosis based on clinical tests. Participants were contacted for a follow-up (by questionnaire and telephone) 24 months later to evaluate their actual health and medical supply status with special consideration of falls, clinical fractures, and drug treatment of osteoporosis.
Results: Complete follow-up data sets were evaluated for 786 women (mean age 70.9 years ± 7.2 SD) including 251 women

with osteoporosis, 341 with osteopenia, and 194 with normal BMD (according to WHO criteria). A total of 352 women (43.8%) experienced at least one fall during the 24 months follow-up period, 283 of them (80.4%) had at least one locomotoric fall. Some tests and parameters of neuromuscular function obtained at baseline were associated with the risk to fall, in particular a chair rising test >7.6 s (RR = 1.90; CI 1.30–2.77). 89 fractures occurred in 83 patients. 78 fractures (87.6% of all fractures) in 72 patients were related to a locomotoric fall. There was an age-related increase in the number of fractures ($P < 0.05$), but not in the number of falls. Every third faller in osteoporotic women (33.7%) sustained a fracture, every fourth in osteopenic women (24.8%), and even every fifth in women with normal BMD (20.3%). Non-fall related fractures occurred in those groups in just 3.8%, 1.3%, and 1.5%, respectively. In the subset of osteoporotic women, the percentage of fallers who sustained a fracture was not different between patients with or without EBM-based antiresorptive treatment of bone.

Conclusions: Falls as well as fractures are common in elderly women. Four-fifths of clinical fractures in the age group of 60 years and older are fall related. Whether EBM-based antiresorptive treatment of bone was given or not, one-third of osteoporotic patients who fell sustained also a fracture. These findings emphasize on the importance of fall prevention to reduce fracture incidence, in addition to drug treatment of bone metabolism.

P481-Su
The Femur Strength Index Predicts Hip Fractures Independent of Bone Density and Hip Axis Length

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Accurate fracture risk assessment is key in preventing hip fracture, the most incapacitating and costly of all osteoporotic fractures. The risk of fracturing a hip is related to several factors such as bone mineral density (BMD), age, height, weight, bone structure, and distribution. Femoral structural parameters, including hip axis length (HAL), cross-sectional moment of inertia (CSMI), and cross-sectional area (CSA), can be measured with newer bone densitometers. Femur Strength Index (FSI), also known as fall index, combines density, structure (CSMI and CSA), age, height, and weight to estimate the ability of a hip to withstand a fall on the greater trochanter (Yoshikawa et al., JBMR 9:1053–1064). The FSI is the ratio of a patient's estimated femoral strength and the expected force of a fall on the greater trochanter (based on height and weight). Larger FSI values indicate greater femoral strength, corresponding to lower fracture risk. We compared femur BMD with CSMI, CSA, and FSI for assessing hip fracture risk. A total of 286 women (62 had a prior hip fracture; 224 controls) had a DXA scan using the Lunar Prodigy (GE Healthcare). The non-fractured femur was measured in fracture subjects. BMD of the femoral neck was determined, as well as HAL, CSMI, CSA, and FSI using the Lunar Hip Strength Analysis program. Results for fracture cases and controls were compared using an unpaired *t* test.

Femoral neck BMD was significantly lower in the fracture group compared to controls. HAL was not significantly different between groups. However, FSI, even after adjustment for BMD and HAL, was significantly lower in the fracture group consistent with a reduced capacity to withstand a fall. We conclude that femoral neck BMD is an important predictor of femoral fracture. The Femur Strength Index, which combines BMD, geometry, age, height, and weight into a single risk factor, is also a significant predictor of hip fracture, even after adjustment for BMD and HAL.

Table

	Fracture group	Control group
Age (years)	80*	71
Height (cm)	162	161
Weight (kg)	66	70
Femur neck BMD (g/cm^2)	0.715*	0.820
HAL (mm)	106	106
CSMI	9162	9675
CSA (mm^2)	111*	127
FSI	1.40*	1.81

* Significantly different than controls ($P < 0.0001$).

P482-Mo
Characteristics of the Proximal Femur in Fractured Lebanese Subjects: Hip Axis Length Predicts Hip Fracture Risk

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The risk of fracturing a hip is related to several factors such as bone mineral density (BMD), bone distribution, age, and structural parameters. Modern bone densitometers can measure structural parameters beyond BMD including hip axis length (HAL). Studies have shown that certain structural parameters like HAL are independent predictors of hip fracture risk [1,2]. In this study, we wished to evaluate some of the geometric hip parameters in a Lebanese female population and their correlation with an increased risk of hip fracture.

We obtained Dual-Energy X-ray Absorptiometry (DXA) scans (Lunar Prodigy, GE Healthcare) of the proximal femur of 97 Lebanese women aged 51 to 89 years from three different university centers. Measurements of HAL and femoral neck BMD of 30 women with prior hip fracture and 67 non-fractured controls were obtained. For the fracture

subjects, DXA measurements were performed on the non-fractured femur. Results for fracture cases and controls were compared using an unpaired *t* test.
Femoral neck BMD was significantly lower in the fracture group compared to controls but most of the women were osteopenic and not osteoporotic according to the WHO criteria. The hip axis length was significantly longer in the fracture group, consistent with findings in other studies [1,2] From the preliminary results of this first study on risk factors for femur fracture in a Middle East population, we conclude that also for the Lebanese women femoral neck BMD and Hip Axis Length are important predictors of femoral fracture. These preliminary findings need to be further validated and compared to findings for other populations.
1. Faulkner et al., J Bone Miner Res 2002, 17: S152.
2. Faulkner et al., J Bone Miner Res 2004, 19: S68.

Table

	Age (years)	Height (cm)	Weight (kg)	Femur neck BMD (g/cm^2)	HAL (mm)
Fracture group	73	152.6	62*	0.615*	102.5*
Control group	72	151.3	67	0.729	100.0

* Significantly different than controls ($P < 0.05$).

P483-Tu
Osteoporosis and Vertebral Fracture in Patients with Colles' Fracture
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Background: Colles' fractures (CFs) have been shown in the past to be associated with an increased risk of hip fracture (Owen, 1982), with low bone mineral density, and changes in bone biochemistry (Earnshaw, 1998). This pilot study reports on the relationship of vertebral fracture (VF), parathyroid hormone (PTH), serum c-telopeptide (CTx), and serum osteocalcin (Oc) in a small population of patients with CF.
Methods: We recruited consecutive patients with CF from orthopedic outpatient clinics. All patients had bone mineral densitometry and lateral morphometry performed on a Lunar Prodigy scanner. Blood was taken for CTx (reference range 0.1–1.0 ng/ml), Oc (11–50 μg/ml), and PTH (15–65 pg/ml). Osteoporosis was defined by WHO criteria. Calcaneal ultrasound was performed on an Achilles Insight.
Results: We recruited 38 patients (7 males, 31 females) with an average age of 70 years (SD ± 11.9). 52% had osteoporosis, 39% were osteopenic. Only 9% had normal bone mineral density (BMD). VFs were present in 13%, of whom 4 were osteoporotic and 1 had normal BMD. Comparing those with VF to those without showed significant differences in mean age, PTH, and Oc (Table 1). There was a trend towards lower BMD at all regions on DEXA but differences were insignificant. Mean values of all bone markers were within reference ranges. Total hip BMD was positively correlated with qualitative ultrasound index (QUI) ($r = 0.45$, $P = 0.021$) and negative correlations were noted between BMD at the total hip and CTx ($r = -0.45$, $P = 0.008$). A weaker correlation was observed between BMD at the AP spine and CTx ($r = -0.393$, $P = 0.024$). No correlation was seen between bone markers and heel ultrasound results.
Conclusion: The prevalence of VF in CF patients is similar to that in the population as a whole (O'Neill, 1996). Despite the size of this pilot study, we have found significant differences between patients with and without VF. Serum PTH levels are significantly lower in patients with VF. In addition, heel ultrasound detected differences in bone quality between the groups. We have also shown extremely high rates of osteoporosis and osteopenia, highlighting the need for osteoporosis screening in this vulnerable group.

Table 1
Vertebral fracture vs. no vertebral fracture

	Vertebral fracture	No vertebral fracture	*P* value
PTH	34 ± 5.4	45 ± 19.3	0.02
Oc	17 ± 16.8	34 ± 15.4	0.056
QUI	77 ± 11.4	69 ± 17.0	0.04

P484-Su
Retrospective Study of Hip and Distal Forearm Fractures Incidence in Moscow Region
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The purpose of our study was to assess incidence of hip and distal forearm fractures in the Moscow Region in the period of 1998–2002.
Methods: Moscow Region population was about 6 618 000 persons at 1 January 2002 (not including Moscow City). For performing a retrospective study among the population aged 50 years, Kolomensky area was chosen which is located at the South-East of Moscow Region and had a total population of about 150 000 persons at 1 January 2002. Source documents were obtained from traumatologic hospital and traumatologic station of Kolomensky area.
Results: 527 hip fractures (142 in men and 385 in women) and 2420 distal forearm fractures (325 in men and 2095 women) were registered during 1998–2002 years. Hip fracture incidence varied from 77.1/100 000 in 1998 to 156.6/100 000 in 2001 among males ($P < 0.05$) and from 166.1/100 000 in 1998 to 216.0/100 000 in 2001 among females ($P > 0.05$). Distal forearm fracture incidence changed from 247.1/100 000 in 1999 to 309.3/100 000 in 2002 among males ($P > 0.05$) and from 1213.0/100 000 in 1999 to 1025.0/100 000 in 2000 among females ($P > 0.05$).

Incidence of both fractures was significantly higher ($P < 0.01$) in women than in men at all years of the study. Hip fracture incidence significantly grew with age with maximal values in age group >70 years old and there was no any dependence of distal forearm fracture incidence on age. We consider the provisional amount of hip fractures may be about 23 500 and the amount of distal forearm fractures may be about 105 300 during 1998–2002 in Moscow Region.
Conclusion: A retrospective study among the Moscow Region population aged 50 years revealed a high incidence of hip and distal forearm fractures both in males and females in the period of 1998–2002.

P485-Mo
The Evaluation of Quality and Cost of Early Medical Care for Hip and Distal Forearm Fractures in Moscow Region
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The aim of the study was to assess quality of the early medical aid and direct financial expenses for treatment of hip and distal forearm fractures in Moscow Region in the period of 1998–2002.
Methods: Source documents about patients with hip and distal forearm fractures were obtained from traumatologic hospitals, traumatologic stations, and municipal polyclinics of Moscow Region. Cost of medical aid was calculated on the basis of the prices of the State Obligatory Medical Insurance of Russian Federation and not included the treatment of late complications, additional rehabilitation, and social payments.
Results: In the Moscow Region whose population is 6 618 000 persons were registered about 23 500 hip fractures and about 105 300 distal forearm fractures in the period of 1998–2002. 33% of patients with hip fractures were hospitalized and 67% of ones were treated in outpatient settings. The average duration of hospitalization was 40 days. Only 4% of hip fractures were surgically operated at first 2 months after fracture. Direct financial expenses for treatment of the patient with hip fracture for first 2 months after fracture have made $582 in average. Early medical care cost for all hip fractures was about $13 677 000 in the period of 1998–2002. 99% of distal forearm fractures were treated in outpatient settings. The medical aid to the patient with distal forearm fracture cost $10 in average. Financial expenses for treatment of all distal forearm fractures have made $1 053 000 for 5 years.
Conclusions: The investigation revealed that patients with hip fractures did not receive an adequate qualified medical aid in Moscow Region. Very high financial assets were spent for early medical care to patients with hip and distal forearm fractures, so urgent introduction of preventive actions for decrease of incidence of osteoporotic fractures is necessary in Moscow Region.

P486-Tu
Pyrmonter Assessment of Complaints in Osteoporosis
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Objectives: The power of vertebral fractures to predict complaints ranges between 20% and 30%. Either the questionnaires applied do not yet target the complaints, or the patients downplay their symptoms, when being interviewed. Thus, we developed a new questionnaire in order to get better insight into the issue.
Materials and methods: 103 questions of a structured interview concern limitations, caused by vertebral fractures, but also social burden and willingness of patients to report them. 54 patients with at least 3 vertebral fractures and 20 sex- and age-matched controls were interviewed. The odds ratio of agreeing to statements by patients in comparison to controls was calculated in order to weigh the questions.
Results: An odds ratio (OR) >10 was found in 24 out of 103 questions. They dealt with peculiarities of self-care, household, and social life. The majority of patients try to hide symptoms and grade of being crippled. OR of positive answers out of these 24 questions as a sum gives a score that separates patients and controls (number of points 10 times as high in patients as compared to controls, $P < 0.0001$).
Discussion: This new questionnaire (PACO) promises high specificity and sensitivity, thereby giving new insight into the burden caused by vertebral fractures.

P487-Su
Factors Affecting Ambulatory Status and Survival of Osteoporosis Patients 90 Years and Older with Hip Fractures
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This study was conducted to assess the long-term functional outcome of very elderly osteoporosis patients with new 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 new hip fractures were analyzed and followed up until death or at least 4 years. Influences of various factors including age, gender, American Society of Anesthesiologists (ASA) score, background diseases, prevalent vertebral fractures, BMD, degree of dementia, walking ability, type of fracture, interval from the onset until admission, interval from the onset until surgery, type of surgery, timing of loading after

surgery, days of hospitalization, and place of residence were investigated. The mean age was 92.8 years and all patients were treated surgically. All patients had osteoporosis (mean BMD at distal third of the radius, 0.33 ± 0.07 mg/cm^2; *T* score, 48.0 ± 7.3%) 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 predictors of survival were the preoperative ASA score, walking ability, fracture type, type of surgery, and the number of prevalent vertebral fractures on admission. Dementia and the number of prevalent vertebral fractures were predictors of the recovery of walking ability. Thus, increasing the number of prevalent vertebral fractures on admission was correlated with poor recovery of walking and high mortality rate after surgery in patients with hip fractures, whereas BMD was not related to neither of them. These data suggest that it may be possibly more important for osteoporosis treatment to prevent vertebral fractures than improving BMD. Type of surgery and fracture type are collinear variables and it is difficult to separate out the effects of one vs. the other, therefore additional well-designed randomized study on the effect of type of surgery and fracture type on outcome is needed.

P488-Mo

Relationship Between Renal Function and Bone Mineral Density in Postmenopausal Women That Were Evaluated for Bone Mass

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In a recent study using NHANES III data, a high prevalence of significant compromise in renal function was found in patients with osteopenia/osteoporosis. In this study, we estimated renal function using Cockroft–Gault formula in 300 postmenopausal women between 50 and 80 years old that came to our institute for bone mass evaluation. We chose to use total femur BMD (TFBMD) for defining osteopenia osteoporosis according to WHO criteria. Renal functional compromise was defined as an estimated creatinine clearance (ECCr) <60 ml/min. Mean age of the sample was 66.9 ± 6.8 years. We found a significantly positive correlation between ECcr and TFBMD ($r = 0.389$) as with the weight ($r = 0.422$) and a negative correlation between age and ECcr ($r = -0.51$) and with TFBMD ($r = -0.22$). Sixty-one patients (20.3%) had osteoporosis of which 81.9% had renal functional compromise, against 54% of 239 women who had normal BMD or osteopenia (Chi2 <0.001); 4/239 (1.6%) women with normal TFBMD or osteopenia had severe renal insufficiency (CcrE ≤36 ml/min) compared with 6/61 (9.8%) of those with osteoporosis (Chi2 = 0.001). The ECcr of women with osteoporosis was significantly lower than that of patients without osteoporosis (52 ± 11 ml/min vs. 59 ± 12 ml/min; $P < 0.0001$). Our data confirm that there is a substantial prevalence of renal insufficiency, even severe, among patients who are candidates for osteoporosis treatment.

P489-Tu

Fracture Risk Associated with Oral and Topical Corticosteroids

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Objectives: To study the fracture risk associated with the use of corticosteroids in any formulation and administration.
Design: Case-control study.
Setting: Community based study in Denmark.
Subjects: Cases were all subjects with any fracture sustained during the year 2000 (n = 124,655). For each case, three controls (n = 373,962) matched on age and gender were randomly drawn from the background population. Adjustments were made for concurrent diseases (lung diseases, rheumatic disorders, etc.), use of other drugs (inhaled bronchodilators, etc.), contacts to hospitals and general practitioners, and social variables.
Results: An increased risk of any fracture, hip, spine, and forearm fractures was present with use of more than 2.5 mg of prednisolone or equivalent orally per day. For inhaled corticosteroids, a limited increase in the risk of any fracture was present in users of more than 7.5 mg prednisolone equivalents per day (equivalent to 1875 μg of budesonide per day). However, no increase in the risk of hip, spine, or forearm fractures was present in users of inhaled corticosteroids. For other topical corticosteroids (dermal, rectal, nasal, local application in the mouth, the eyes, or the ears), no increase in fracture risk could be demonstrated even at high doses after adjustment for confounders.
Conclusions: Ingestion of more than 2.5 mg of oral prednisolone equivalents per day is associated with an increase in fracture risk. No increase is associated with inhaled corticosteroids except at daily dosages above 7.5 mg of prednisolone equivalents. No increase in fracture risk is associated with other forms of topical corticosteroids.

P490-Su

Diabetes and Fracture Risk-Effects of Antidiabetic Medication

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Aim: To study the association between fractures and type 1 (T1D) and type 2 diabetes mellitus (T2D).

Methods: Population-based case-control study. All subjects diagnosed with a fracture (n = 124,655) in Denmark served as cases, and from the general population three controls (n = 373,962) matched for gender and age were retrieved for each case. Presence of T1D and T2D, and use of insulin and oral antidiabetic drugs, were exposure variables.
Results: T1D and T2D were associated with an increased risk of any fracture (OR = 1.3, 95% CI: 1.2–1.5 for T1D, and 1.2, 95% CI: 1.1–1.3 for T2D after adjustment for confounders) and hip fractures (OR = 1.7, 95% CI: 1.3–2.2 for T1D, and 1.4, 95% CI: 1.2–1.6 for T2D). Furthermore, T2D was associated with a significant increase in forearm fractures (OR = 1.2 (1.0–1.5)), and T1D was associated with an increased risk of spine fractures (OR = 2.5, 95% CI: 1.3–4.6), whereas T2D was not. Use of metformin and sulphonylureas was associated with a decreased risk of any fracture, whereas there was a non-significant trend associated with the use of insulin for any fracture. Except for a decrease in hip fractures with use of sulphonylureas, no change in fracture risk was associated with the use of insulin or oral antidiabetic drugs in the hip, spine, or forearm. In conclusion, T1D and T2D are associated with an increased risk of any fracture and hip fractures. The use of drugs to control diabetes seems to counter the increased fracture risk.

P491-Mo

Family Physicians' Awareness of Osteoporosis Risk Factors and Utilization of Bone Mineral Density (BMD) Screening Following a Multifaceted Osteoporosis Intervention Strategy: Canadian Quality Circle (CQC) Pilot Project

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The CQC project was developed to improve family physicians' (FP) adherence with the Osteoporosis Society of Canada 2002 clinical practice guidelines for osteoporosis (OP). 52 FPs were recruited as members in 7 Quality circles (QC). Members collected baseline (BL) and follow-up (FU) data on separate patients via chart reviews and the completion of the data capture form on OP risk factors, diagnosis, and treatment. 1505 BL and 1359 FU patient charts were selected at random. All patients were women 55 years of age and older. Individual and QC data were collated in profiles and provided to the members. The QCs then met to discuss the profiles, identify and analyze barriers, recommend solutions, and participate in an OP problem-based workshop. The primary focus of the workshop was to assess postmenopausal OP and risk factor identification. FU chart audit occurred after the intervention and was used to provide feedback on practice patterns. The current analyses examined the percent difference in physicians' awareness of 9 major and 4 minor risk factors for OP and the appropriate use of BMD assessment in high-risk patients in accordance with the guidelines. The guidelines state that high-risk patients (with at least one major or two or more minor risk factors) should have a BMD assessment.
The differences (BL%, FU%) in awareness of the major risk factors were as follows: age (>65 years), 0% (100%, 100%); prior hip, 2% (98%, 100%), wrist, 4% (96%, 100%); and vertebral fracture, 2% (96%, 98%); family history of fracture, 22% (46%, 68%); a fall in the last 12 months, 9% (89%, 98%); oral prednisone therapy (>3 months), 1% (99%, 100%); menopause before age 45, 17% (72%, 89%); and height loss (2–4 cm), 20% (57%, 77%). For minor risk factors, the differences were: caffeine intake (>4/day), 29% (57%, 86%); alcohol intake (>9/week), 8% (89%, 97% F-UP); current smoking, 0% (99%, 99%); and weight, 4% (95%, 99%). The differences (BL%, FU%) in the use of BMD screening in high-risk patients were as follows: high-risk due to major risk factors, 5% (72%, 77%); high-risk due to minor risk factors, 6% (62%, 68%); high-risk due to major or minor risk factors, 6% (70%, 76%).
The QC intervention substantially improved FP's awareness of major and minor risk factors for OP. The intervention only modestly improved the overall use of BMD assessment in high-risk patients. Future QC interventions should focus on bridging the gap between risk factor identification and BMD assessment.

P492-Tu

The Risk Factors Contributing to Distal Cancellous Bone Loss in Rheumatoid Arthritis

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The nature of osteoporosis in rheumatoid arthritis (RA) is multifactorial—periarticular bone loss is an early radiological sign of RA, generalized osteoporosis has also been described as the frequent consequence of glucocorticosteroid (GK) treatment. In our cross-sectional study, we evaluated 59 RA patients (38 females, 21 males, mean age 48.3 ± 8.7 years, mean duration of RA 12.1 ± 9.5 years) in order to determine the extent to which different factors (GK use, RA duration, age, gender, menopause, BMI, c-reactive protein, and rheumatoid factor) contribute to bone loss in the peripheral skeleton. We also aimed to prove the diagnostic value of phalangeal quantitative ultrasonometry (QUS) in such patients. Patients were subdivided in three groups: 21 patients (13 females, 8 males, mean age 46.3 ± 9.1 years) with high dose steroid treatment (GK dose >7.5 mg/day); 18 patients (12 females, 6 males, mean age 47.8 ± 8.1 years) with

moderate dose steroid treatment (GK dose 2.5–7.5 mg/day); 20 patients (13 females, 7 males, mean age 50.8 ± 7.3 years) with steroid free RA treatment regimen. 20 age-matched persons (12 females, 8 males, mean age 48.7 ± 9.1 years) without RA served as control. Measurements were done by DBM Sonic 1200 ultrasound device at hand fingers (IGEA, Italy). Bone mineral density results measured by ultrasound and expressed as *T*-score and *T*-score are shown in the following table. Within RA groups, multiple regression analysis showed that duration of RA ($P < 0.5$), female gender ($P < 0.02$), and GK dose ($P < 0.02$) were associated with reduced peripheral bone mass. We conclude that: (1) ultrasound bone density measurement at the hand fingers is suitable to asses properties of peripheral trabecular bone in RA patients; (2) peripheral bone density is reduced in RA patients indicating more severe bone loss if compared with age-matched controls; (3) the main predicting factors of bone loss were prolonged RA course, female gender, and higher GK dose.

Table

	GK >7.5 mg (n = 21)	GK 2.5–7.5 mg (n = 18)	GK free (n = 20)	Controls (n = 20)
T-score (SD)	−1.67 ± 1.58**	−1.15 ± 0.89*	−0.75 ± 1.04	−0.38 ± 0.79
Z-score (SD)	−1.94 ± 1.53**	−1.22 ± 1.15*	−0.91 ± 1.12	−0.51 ± 1.01
Normal BMD (%)	8 (38.1%)	9 (50%)	14 (70.0%)	17 (85%)
Osteopenia (%)	8 (38.1%)	7 (38.9%)	4 (20.0%)	2 (10.0%)
Osteoporosis (%)	5 (23.8%)	2 (11.1%)	2 (10.0%)	1 (5.0%)

* $P < 0.05$ vs. control group; **$P < 0.01$ vs. control group.

P493-Su

Fall-Related Risk Factors in Men Compared to Women Referred for Open Access Bone Densitometry

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Both osteoporosis and falls are important risk factors for low trauma fractures. Most studies of fall-related risk factors have focussed on older women. In this study of patients referred for open access bone densitometry, we wanted to see if there were differences in fall-related risk factors in men compared to women.

We examined data from consecutive patients, >65 years over a 12-month period. Patients underwent DXA and tests of fall risk. These were binocular-corrected visual acuity (VA), ability to stand heel toe for 10 s (a test of static balance), heel toe walking for 4 steps (a test of dynamic balance), and ability to do standups ×5 without arm use (a test of lower limb neuromuscular function).

There were 65 men (mean age 73 years) and 251 women (mean age 74 years). A history of a fall in the previous 12 months was reported by 106/251 (42%) of women but only by 15/65 (23%) of men ($P = 0.05$). Despite this lower rate of reported falls in men, there were no differences in fall-related risk factors. Thus mean VA was 0.63 (SD 0.28) in men vs. 0.62 (SD 0.29) in women ($P = 1.0$). The proportion of individuals with abnormal heel toe standing was 26/65 (40%) in men vs. 112/251 (44%) in women ($P = 0.7$), abnormal heel toe walking in 14/65 (22%) men vs. 57/251 (22%) in women ($P = 1.0$), and inability to do standups 6/65 (9%) in men vs. 23/251 (9%) in women ($P = 1.0$).

These preliminary findings show that despite men reporting fewer falls in the last 12 months compared to women, there were no differences in fall-related risk factors. This suggests that the predictive capacity of the evaluated fall-related risk factors is influenced by gender. Future work on fall-related risk factors should evaluate men and women separately.

P494-Mo

Bone Mineral Density of Arabian Women: Relation to Anthropometric and Lifestyle Factors and to Vitamin D Status

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Objectives: To study the effects of physical and environmental factors on bone mineral density (BMD) of United Arab Emirates (UAE) women.

Methods: Three hundred women volunteers (age range, 20–85 years) completed a questionnaire on reproductive, nutritional, and lifestyle factors. Fat-free mass (FFM) was estimated by bioelectric impedance. Muscle strength was measured by handgrip dynamometer. BMD was determined by dual-energy X-ray absorptiometry. Serum 25 hydroxy-vitamin D (25OHD), parathyroid hormone (PTH), osteocalcin (OC), and urine deoxypyrdinoline (Dpd) were measured.

Results: Peak standardized BMD of spine and femur in healthy young UAE women (age range 20–29 years) was significantly lower than the reference range for US Caucasian women (−0.54 SD, $P < 0.0001$ for spine, and −0.27 SD, $P < 0.05$ for femur). There were significant associations between BMD, and age, years post-menopause, number of pregnancies, FFM, body fat percent (BFP), body mass index (BMI), and muscle strength. Multiple regression analysis showed that BFP and number of pregnancies were independent determinants of BMD in premenopausal

women (standardized coefficient Beta +0.36, −0.30; respectively), and age, years post-menopause, and BFP, in postmenopausal women (standardized coefficient Beta −0.28, −0.33, +0.30, respectively). Hypovitaminosis D (25OHD <50 nmol/L) was present in 97% of the subjects. 25OHD levels were lowest in spring and summer. 25OHD levels correlated significantly with dietary vitamin D intake, serum albumin, PTH, urine calcium/creatinine, and urine Dpd, but not with BMD at any site. Multiple regression analysis showed that serum albumin, PTH, and urine Dpd were independent determinants of 25OHD level (standardized coefficient Beta +0.19, −0.26, −0.19, respectively). However, PTH levels were in the normal range for most subjects with Hypovitaminosis D, suggesting that PTH should not be used as a surrogate marker for Hypovitaminosis D in this population.

Conclusions: Peak standardized BMD of UAE women is significantly lower than US Caucasian women. This may be due in part to increased number of pregnancies together with prevalent vitamin D deficiency. The latter is probably related to insufficient vitamin D intake in the setting of limited sunlight exposure especially during the hottest period of the year when most UAE women stay indoors during daylight hours.

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P495-Tu

Risk Factors of Osteoporosis in Urban Asian Indian Women Presenting for a Preventive Health Check-Up

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Aim: In this study, we aim to define the risk factors of osteoporosis in a select group of urban Asian Indian women.

Methods: The study was a case-control, interview-based study. Study population included women who had undergone spinal and femoral bone mineral densitometry (BMD) measurements as part of post-menopausal preventive health check-up in a private health center in New Delhi (Max medical center) during the period 2002–2003. BMD was measured by axial dual energy X-ray absorptiometry (DXA) using a Lunar Prodigy machine. *T* value was computed for definition of osteoporotic and normal groups in this study based on WHO classification. Case group included 121 osteoporotic women and an equal number of age- and sex-matched controls with normal BMD was chosen as control group. Estimation association of risk factors with osteoporosis has been calculated by Odds ratio and Multiple Logistic Regression Analysis has been used for adjustment the most relevant factors: age, weight, height.

Result: The significant risk factors in the present study population with their Odds ratios (in parenthesis) were as follow: osteoporosis in mother or sister (3), history of fracture in relatives (9.6), lower education defined as less than class 12 (2.2), duration of menopause greater than 13 years (2.34), history of fracture (2.1), weight less than 60 kg (4.8), height less than 155 cm (3.4), BMI less than 25(3.3). Pure vegetarianism (2.2) and usual or higher sugar consumption (3.2) were found to be significant risk factors. Regular consumption of milk (0.4), almond (0.4), and fruits (0.1) appeared to be significant protective factors. Menopausal symptoms (0.40) and information about osteoporosis (0.2) also reduced the risk significantly. After age adjustment, all of the above factors remained significant. However, when data were adjusted for height and weight, only some nutrition factors (milk, almond, and fruits as protective factor and sugar use as risk factor) remained significant.

Conclusions: Osteoporosis in this group of rather well to do Indian Asian women also appears to be associated with several known risk factors that are well described in the literature. The overall risk that is likely to be due to pure vegetarianism is fortunately offset by the demonstrated protective role of certain nutritional dietary components of such diets (such as milk, almonds, and fruits). The latter are indigenous to the regional life styles and can be exploited in preventive educational strategies on osteoporosis in this population.

P496-Su

Factors Influencing Bone Mass in University Students

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Background: Acquisition of high peak bone mass is one of the important factors for prevention of osteoporosis. Factors influencing bone mass including anthropometry, biochemical bone markers, life style, and nutritional status were analyzed in university students.

Methods: Subjects were 469 male university students without diseases associated with bone metabolism. Life style and nutritional status were assessed by questionnaire. Broadband ultrasound attenuation (BUA) and speed of sound (SOS) at calcaneus were measured by ultrasonography (Sahara, Hologic, USA), and estimated bone mineral density (BMD) was calculated. Serum total alkaline phosphatase (TALP), N-mid osteocalcin, and type 1 collagen C-terminal telopeptide (ICTP) were measured.

Results: Age was 22.82 ± 2.37 years, height was 174.78 ± 5.00 cm, weight was 68.99 ± 8.84 kg, body mass index (BMI) was 22.56 ± 2.55 kg/m^2. BUA was 75.45 ± 16.73 dB/MHz,

SOS was 15.59 ± 25.21 m/s, and estimated BMD was 0.55 ± 0.10 g/cm^2. TALP was 80.21 ± 19.10 U/L, N-mid osteocalcin 24.14 ± 7.31 ng/mL, and ICTP was 4.54 ± 1.24 ng/mL. BMI had significant positive correlation with BUA ($r = 0.194$) and estimated BMD ($r = 0.100$). N-mid osteocalcin had significant negative correlation with BUA ($r = -0.127$), SOS ($r = -0.122$), and estimated BMD ($r = -0.132$). TALP had significant negative correlation with SOS ($r = -0.101$) and estimated BMD ($r = -0.097$). ICTP had a tendency to correlate negatively with BUA, SOS, and estimated BMD. There were no significant correlations between bone mass and nutritional status such as calcium, protein, coffee, and alcohol intake. There were no significant correlations between bone mass and life style including smoking and exercise.

Conclusion: BMI was a significant factor influencing bone mass of young men. Bone mass was lower in subjects with high bone turnover rate.

P497-Mo

Pilot Case Control Investigation of Risk Factors for Osteoporosis in an Urban Indian Population

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Despite the high prevalence of osteoporosis in Indians, there have been no previous studies examining the risk factors for hip fracture in this population. We carried out a case control investigation comprising 100 case subjects (57 women and 43 men) admitted with a first hip fracture into one of three hospitals across New Delhi. The 100 controls were age- and sex-matched subjects. Information from all subjects was obtained through a questionnaire-based interview. The importance of risk factors for hip fractures was assessed with univariate and multivariate logistic regression.

Univariate analysis identified protective effects for increased activity, exercise, calcium and vitamin supplements, almonds, fish, paneer (cottage cheese), curd (plain yogurt), and milk. However, tea and other caffeinated beverages were significant risk factors. In women, hormone/estrogen therapy appeared to have a marginal protective effect. For all cases, decreased agility, visual impairment, long-term medications, and chronic illnesses increased the risk of hip fracture. The multivariate analysis confirmed a protective effect of increased activity and also showed a decrease in hip fracture risk with increasing body mass index (odds ratio (OR) 0.024, 95% confidence interval (CI) 0.006–0.10 and OR 0.81, 95% CI 0.68–0.97, respectively). Individuals who take calcium supplements have a decreased risk of hip fracture (OR 0.076; CI 0.017–0.340), as do individuals who eat fish (OR 0.094; CI 0.020–0.431), and those who eat paneer (OR 0.152; 0.031– 0.741). Tea drinkers have a higher risk of hip fracture (OR 22.8; 95% CI 3.73–139.43). Difficulty in getting up from a chair also appears to be an important risk factor for hip fractures (OR 14.53; 95% CI 3.86–54.23).

Conclusions: In the urban Indian population, dietary calcium, vitamin D, increased body mass index, and higher activity levels have a significant protective effect on hip fracture. On the other hand, caffeine intake and decreased agility increase the risk of hip fracture. Based on these findings, future studies should be done in order to direct primary preventive programs for hip fracture in India.

P498-Tu

Differences in Markers of Bone Turnover Between Older Chinese and British Adults

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The incidence of hip fracture is low in Shenyang, northern China (1), and older adults in Shenyang have better vitamin K status compared with British counterparts (2). The aim of this study was to examine whether there are any differences in markers of bone turnover between the two populations which might explain the difference in fracture risk. Subjects were older men and women aged 60–83 years from Shenyang, China (58 men, 59 women), and Cambridge, UK (67 men, 67women). Fasting plasma, collected in early morning between 7 and 9 am, was analyzed for total calcium, albumin, parathyroid hormone, 25-hydroxyvitamin D, and osteocalcin. Two-hour fasting urine was analyzed for deoxypyridinoline and creatinine. These assays were performed using same methodology in the same laboratory. All samples were collected in the summer (August and September). Comparison between the two populations was made by analysis of covariance using natural log-transformed data adjusted for sex and age. Total plasma calcium and albumin concentrations were significantly higher in the Chinese group compared with the British group [mean difference (SE)%: +2.0 (0.8)%, $P = 0.02$ and +9.9 (1.0)%, $P < 0.001$, respectively], but once adjusted for plasma albumin concentration using country-specific correction factors the difference in plasma calcium was not significant [+0.5 (0.7)%, $P = 0.4$]. Mean 25-hydroxyvitamin D concentration was significantly lower in the Chinese group [−8.5 (3.7)%, $P = 0.02$], but there was no significant difference in parathyroid hormone concentration between the two populations [−2.3 (4.7)%, $P = 0.6$]. Marked differences in bone formation and resorption markers were found; mean osteocalcin and urinary deoxypyridinoline/creatinine concentrations were significantly lower in Chinese compared with British subjects [−31.4 (5.4)%, $P < 0.001$ and −41.2 (6.3)%, $P < 0.001$, respectively]. These results suggest that compared with British counterparts, older Chinese adults have lower bone turnover. This may be related to their better

vitamin K nutrition status (2) and may contribute to the lower incidence of hip fracture in Shenyang.
This study was supported in part by the Nestle Foundation.
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P499-Su
Serum Leptin and BMD in Obese Postmenopausal Women
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Introduction: Obesity is considered one of the protective factors in epidemiology of postmenopausal osteoporosis. Leptin, a circulating peptide of adipocyte origin, has emerged as a potential candidate responsible for the above effect due to its dual effects on bone tissue, skeletal maturity/signaling pathway. The aim of the study was to assess whether leptin can be an independent predictor of BMD in obese postmenopausal women.
Materials and methods: Seventy-one postmenopausal obese women and ten postmenopausal women with normal body weigh (control group) participated in the study. Women were recruited in Outpatient Osteoporosis Department in Institute of Agricultural Medicine in Lublin (Poland). Bone mineral density was measured in lumbar spine (BMD L2–L4) and femoral neck using DXA method. Serum leptin was determined using RIA method.
Results and conclusions: Mean values of serum leptin (3.7) (BMI ± study group were: 9.61 < (1.6) ± 25), control group, 12.04 (2.3) (BMI ± (1.8) (BMI 30–39.9), 11.94 ± (BMI 25–29.9), 11.90 > 40), and the differences with control group were statistically significant (0.01). Serum leptin in all study group (BMI > 25) correlated negatively with lumbar spine (L2–L4) and femoral neck BMD (*P* values 0.57 and 0.69, respectively). In subgroup with BMI 25–29.9, Serum leptin was negatively correlated with femoral neck BMD (*P* = 0.04), although a slight (non-significant) positive correlation with BMD L2–L4 was also observed. Circulating leptin showed a strong negative correlation with bone mineral density in postmenopausal women with I degree of obesity.

P500-Mo
The Evaluation of Risk Factor Affecting Bone Mineral Density in Menopausal Jordanian Women
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Aim: The objective of this study is the determination of risk factors contributing to low bone mineral density (BMD) in a random group of postmenopausal Jordanian women.
Patients and methods: The design of the study was a randomized prospective one to investigate menopausal Jordanian women. Three hundred eighty-four menopausal women ageing between 39 and 75 years were included, all women were referred for the first time to undergo bone density measurement, those with a history of using drugs that may affect BMD were excluded from the study. Bone mineral density (BMD) was measured using Dual energy X-ray absorptiometry (DEXA). Measurements of the left hip and spine (L1–L4) were recorded, while information concerning other factors thought to influence BMD was collected through face-to-face interviews prior to the test and a questionnaire filled.
Results: Age of patients and number of years since menopause showed the strongest inverse relation of left hip and spine BMD, while the strongest positive relation, in our study, was a regular high calcium intake as well as daily and regular physical activity throughout life. The onset age of menopause did not show significant correlation with the values of BMD.
After adjustment for age and actual age of menopause, we observed a small negative effect of smoking and large consumption of coffee and tea on BMD. The accumulation of repeated pregnancy events without a recovery interval was not associated with lowered bone mineral density while lactation seems to have a positive effect on BMD values.
Conclusion: Osteoporosis is a complex and multifactorial process. While some factors are determined by nature such as age and menopause, others might be controllable by sufficient intake of calcium at an early age, increasing physical activity, and integrating this as a part of a healthy life and not just as an occasional one.
This draws the attention for the need for multifactorial prevention strategies, which most effectively need to be instituted at an early age, before peak bone mass is achieved.

P501-Tu
Relationship Between Mortality and Baseline Bone Mineral Density, Bone Loss, Weight Loss, and Weight Fluctuation in Elderly Men and Women
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Low bone mineral density (BMD) has been suggested as a risk factor for mortality in women. However, BMD is affected by weight and weight change. The present study examines the association between baseline BMD, bone loss, weight loss, and weight fluctuation and all-cause mortality risk in both elderly men and women.

Data from 1059 women and 644 men aged 60+ years as at 1989 of Caucasian background who have participated in the Dubbo Osteoporosis Epidemiology Study were analyzed. All-cause mortality was recorded annually between 1989 and 2004. Incidence of fractures, confirmed by X-ray and personal interview, was also ascertained during the follow-up period. Traumatic and pathological fractures and fractures of the skull, cervical spinal, or digits were excluded from the analysis. Bone mineral density at the femoral neck was measured by dual energy X-ray absorptiometry (GE-LUNAR) at baseline (1989) and approximately every 2 years afterward. Information of concomitant diseases, including cardio-vascular diseases (CVD), all type cancer, and type I/II diabetes mellitus was also recorded.

After adjusting for age and concomitant diseases in a Cox's proportional hazards model, the following factors were independently significant predictors of mortality in men: BMD loss (hazards ratio (HR): 1.6; 95% CI, 1.0–2.4) and weight fluctuation (HR: 1.2; 95% CI, 1.0–1.3). In women, baseline femoral neck BMD (HR: 1.3, 95% CI: 1.1–1.5), BMD loss (HR: 1.6, 95% CI: 1.2–2.2), weight loss (HR: 1.2, 95% CI: 1.1–1.4), and weight fluctuation (HR: 1.1, 95% CI, 1.0–1.2) were independent predictors of mortality risk.

These data suggest that in addition to low BMD, BMD loss and weight change were also significant predictors of all-cause mortality in elderly men and women, independent from age and concomitant diseases.

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P502-Su

In Osteoporotic Men and Women a Low Creatinine Clearance of <65 ml/min Significantly Increases the Risk for Falls and Fractures

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Objectives: 3n community-dwelling elderly men and women, a low creatinine clearance (CrCl) of <65 ml/min was recently described as a new significant and independent risk factor for falls. The aim of this study was to analyze if a low creatinine clearance of <65 ml/min is also a risk factor for falls and fractures in osteoporotic men and women.

Materials and methods: In 5313 German men and women diagnosed with osteoporosis we analyzed in a cross-sectional study, the prevalence of having experienced falls and fractures during the last 12 months according to the renal function, which we assessed with the CrCl, was calculated with the established Cockcroft–Gault formula. According to a CrCl cut-off of 65 ml/min, the prevalence of falls and fractures was assessed in multivariate-controlled logistic regression models. The *P* values are two-sided.

Results: A low CrCl of <65 ml/min was found in this osteoporotic population in 60.9% ($N = 3238$) of participating men and women ($N = 5313$). The low CrCl of <65 ml/min was associated in this population in multivariate controlled analyses, compared to a CrCl of <65 ml/min ($N = 2075$ res. 39.1% of participating men and women), with a significant increased risk of experiencing falls (OR 1.69, 95% CI 1.50–1.91, $P < 0.0001$) and an increased risk for multiple falls (37.1% vs. 22.6%, OR 1.63, 95% CI 1.42–1.87, $P < 0.0001$). A CrCl of <65 ml/min was also, compared to CrCl of <65 ml/min, in osteoporotic men and women associated with a significant increased risk for fractures: total vertebral fractures (607/3238 vs. 268/2075; $P < 0.0001$), fall-associated vertebral fractures (490/1775 vs. 181/773; $P = 0.027$), hip fractures (253/3238 vs. 78/2075; $P < 0.0001$), and radial fractures (285/3238 vs. 104/2075; $P < 0.0001$).

Conclusion: Similar to community-dwelling elderly, a CrCl of <65 ml/min is in osteoporotic men and women associated with a significant increased risk for falls. Furthermore, for the first time, a low CrCl of <65 ml/min in osteoporotic men and women has been found to be associated with a significantly increased risk of vertebral, hip, and radial fractures.

P503-Mo

Biphasic Relationship Between Bone Mineral Density and Weekly Running Distance in Healthy Males

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Background: Regular physical exercise is associated with increased bone mineral density (BMD). However, high endurance male runners (i.e., >90 km/week) may have reduced BMD, possibly in relation to low sex hormone serum levels. In this cross-sectional study, we investigated the relationship between BMD and running distance (RunD) in healthy middle age male runners and assessed the interaction with sex hormones status, calciotropic hormones, and biochemical markers of bone remodeling.

Methods: We studied 74 healthy males: 56 runners (age 36 ± 6 years, body mass index (BMI) 22 ± 2 kg/m^2, RunD 42 ± 21 km/week) and 18 sedentary controls, matched for age and BMI (age 35 ± 6 years, BMI 23 ± 2 kg/m^2, RunD <5 km/week). RunD was evaluated over 8 weeks with an auto-administered daily running record. BMD was assessed with dual X-ray absorptiometry (QDR 4500) at the levels of upper limb (arm-BMD), lower limb (leg-BMD), L2–L4 lumbar vertebrae (L2-4BMD), and whole body (total-BMD). Fasting morning blood samples were collected for LH, FSH, prolactine, estradiol (E), testosterone, sex hormone binding protein (SHBG), parathormone, IGF-I, and osteocalcine. In a stepwise linear regression, we analyzed the relationship

between BMD and serum variables, with age, age^2, body weight, RunD, and RunD^2 forced into the model (squared variables indicate non-linear relationships).
Results: In multivariate stepwise regressions, arm-BMD, leg-BMD, and total-BMD were positively correlated with RunD and body weight, negatively with RunD^2 and osteocalcine, but not with any other serum variables (R^2 = 0.21 to 0.24 for the whole prediction equations). L2–L4 BMD was independent from RunD and negatively correlated with osteocalcine. From the prediction equations, maximal BMD at any site except vertebrae was associated with about 40–60 km/week RunD, and lower BMD than controls with over 80–110 km/week RunD. The Pearson correlation coefficient between E, which is known to play a major role in male bone homeostasis, or SHBG, and RunD was $r = -0.21$, $P = 0.07$, and $r = 0.29$, $P < 0.05$, respectively.
Conclusions: Men who run about 50 km/week present the highest BMD at any site except vertebrae whereas men who run more than about 100 km/week are expected to present a lower BMD than sedentary individuals. A biphasic relationship between BMD and running distance is thus shown, with a possible contribution of low estradiol with very long running distances.

P504-Tu
Osteoporotic Risk in Women with Coronary Arteriosclerosis
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A relationship between arteriosclerosis (AT) and osteoporosis (OP) has been suggested.
Objectives: To evaluate the relationship between AT and OP in women with coronary disease and analyze the possible pathogenic mechanism associated to both diseases.
Subjects and methods: We studied 71 ambulatory women (45–75 years old) who had undergone coronariographic studies, 44 with arteriosclerotic plaques in coronary arteries (CA), and 27 with no evidence of plaques (control), all of which showed no OP antecedents. Data about their medical history, lifestyle, calcium intake, and concomitant pharmacologic treatments were collected. Bone mineral density (BMD) at lumbar spine (LS) and femoral neck (FN), and spine deformations at dorsal and lumbar spine, were measured by DXA and Lateral RX, respectively. Finally, sexual and calciotropic hormones, bone remodeling markers, inflammatory activity factors, and serum homocysteine levels were quantified by RIA and ELISA.
Results: AC and control women were comparable in age, years of menopause, physical activity, smoking, maternal antecedents of bone fractures, calcium intake, percentage of women with arterial hypertension, and treatments with statins and bone active drugs. Control women were significantly more obese than AC women (BMI 32 ± 4 vs. BMI 33 ± 4, $P = 0.045$) and showed higher weight-adjusted BMD at all localizations, being significant at LS ($P = 0.045$). There were no significant differences in levels of serum Ca, calciotropic and sexual hormones, bone remodeling markers, inflammatory activity factors, and serum homocysteine between AC and Control women. Spine deformations were more frequent in AC women (34%) than in controls (17.4%), OR = 2.5 (0.66–9.38) IC 95%. Women with AC and spine deformations also showed more years of menopause (21 ± 7 vs. 14 ± 9, $P = 0.024$) lower BMD at FN (0.655 ± 0.07 vs. 0.793 ± 0.07 $P = 0.004$) and significantly higher serum levels of RANKL (0.13 ± 0.11vs0.05 ± 0.06 pmol/L $P = 0.027$).
Conclusions: Women with AC present lower BMD and a slight increase in the incidence of spine deformations than women controls. The serum level of RANKL was the only biochemical factor studied that showed to be increased in AC women and spine deformations. All together, these results suggest that there is an association between the coronary AT and OP. Although multiple pathogenic mechanisms may be involved, our results show that neither inflammatory activity nor serum homocysteine levels seem to play a clear role in this association.

P505-Su
Selection of Postmenopausal Women for Bone Densitometry: Can We Use the Same Screening Tools at Different Ages?
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The prevalence and nature of risk factors for low BMD may vary with age. Hence, the value of different screening tools used for selecting women for bone densitometry may also vary with age. We assessed and compared the value of different screening strategies in three groups of postmenopausal women selected from the general population: 60–70 years (n = 399), 70–80 years (n = 392), and >80 years (n = 3628). The strategies considered were (1) presence of at least one common referral criteria (personal history of fracture, maternal history of hip fracture, low weight, early menopause), (2) simple measurement of weight, and (3) calculation of a risk score based on age-specific predictive models. The main criteria for comparison were the sensitivity and positive predictive value (PPV) for osteoporosis. Since women with osteopenia plus other risk factors may also justify a treatment, we also calculated the number of selected women who had such characteristics in order to assess the overall number of women who might be proposed a treatment after BMD screening.
The prevalence of osteoporosis was equal, respectively, to 14%, 31%, and 51% in the age groups 60–70, 70–80, and

80+. The percentage of women with at least one of the common referral criteria was equal, respectively, to 31%, 46%, and 49%. For approximately the same percentage of women selected, the sensitivity of weight was significantly higher: 52%, 71%, and 62%, respectively. Whatever the age group, to have a sensitivity around 80%, more than half of the population must be tested. Using more complex scores did not significantly improve the discriminant value of assessment. A high PPV for osteoporosis (around 80%) could only be obtained for older women with very low weight (45 kg or less). If women with osteopenia plus other risk factors are also considered at high risk, the number of women selected for a bone examination who might be proposed a treatment is increased, especially in the younger age group. Using common referral criteria as compared to weight, the number of selected women who have osteoporosis is lower, but the number of selected women who have osteopenia plus at least two other risk factors is higher. Hence, the overall number of women who might be proposed a treatment after BMD testing is approximately the same.

In conclusion, whatever the age group, weight may be the simplest and most discriminant screening tool to identify women at high risk of low BMD.

P506-Mo

Indicators of Poor Health and Physical Frailty Predict Bone Loss at the Hip

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We have previously observed that indicators of frailty such as low serum albumin, low serum thyroid hormone T3, and poor physical strength predicted vertebral fracture but not non-vertebral fracture. The aim of this study was to see whether these same factors predicted the rate of bone loss from the hip and whether the rate of bone loss from the hip was associated with an increased risk of vertebral fracture.

We carried out a 10-year prospective study of a population-based group of 375 women, ages 50 to 85 years; at baseline, we measured bone mineral density (BMD) of the lumbar spine and femoral neck, collected fasting blood samples for biochemical measurements, and obtained medical and life-style data by questionnaire. Incident vertebral fractures were determined by a single radiologist from spinal radiographs at 0, 2, 5, 7, and 10 years and non-vertebral fractures were confirmed by radiologist reports. BMD measurements were also repeated at 2, 5, 7, and 10 years, and the annual percentage rate of bone loss was calculated from a regression line. Only those subjects who attended for 3 or more visits were included in this analysis (n = 255).

Older age (but not height, weight, or body mass index) was associated with greater annual bone loss (Spearman's rho = −0.17, P = 0.007). Indicators of poor health were associated with bone loss, and these included low serum albumin levels (rho = 0.25, $P < 0.001$) and poor self-assessed health on a scale of 1 to 5 (rho = −0.15, P = 0.016). These predicted bone loss independently of age and of each other. Physical weakness and lack of stamina were associated with bone loss, and these included poor grip strength (rho = 0.25, $P < 0.001$) and self-assessed difficulty in standing (rho = −0.21, P = 0.001) or sitting (rho = −0.13, P = 0.037) for continuous periods. These measures were also independent of age and, except for prolonged sitting, from each other. By logistic regression, age-adjusted FN bone loss was also associated with the risk of incident vertebral fracture during the study (P = 0.013, relative risk = 1.70 for each SD of percentage rate of bone loss). The rate of change at the lumbar spine was not associated with incident fractures.

We conclude that factors relating to poor health and physical frailty predict not only vertebral fractures as previously shown, but also the rate of bone loss at the femoral neck. This rate of bone loss is also significantly associated with the incidence of vertebral fracture.

P507-Tu

Relationship Between Anthropometrics Variables with Bone Mineral Content (BMC) in an Urban Population. Preliminary Study

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Introduction: Overweight is associated with higher bone mineral density; however, the relationship with height has been less studied.

Objectives: To evaluate the relationship between bone mineral content (BMC) with body mass index (BMI) and height in women over 45 years old.

Materials and methods: During the year 2004 we studied 341 women between 45 and 84 years old. None of them were under glucocorticoid therapy or oncological treatment. BMC was measured at lumbar spine L2–L4 (LS) and total hip (TH) employing dual-energy X-ray absorptiometry (DEXA; LUNAR DPX). Patients were classified according to age (G1: 45 to 60 years, G2: 61 to 84 years), height (E1: ≤150cm, E2: >150cm), and BMI (<25 vs. ≥25 and <30 vs. ≥30 kg/m^2). A t test for unpaired samples and Spearman's correlation coefficient (r) were used for statistical analysis.

Results: BMC was significantly lower in G2 vs. G1 at LS ($P < 0.0001$) and TH ($P < 0.0001$). Height was significantly shorter in G2 ($P < 0.001$) as well. No difference was found

in BMI and weight between both groups. Patients with BMI ≥25 showed significantly greater BMC at TH ($P < 0.0001$) and at LS ($P = 0.0019$). When they were divided by age, this difference was maintained at both sites in G2 but only at TH in G1. A further division of the population into BMI <30 and ≥30 showed significantly lower BMC at both sites in G1 while this was only observed at TH for G2. In G1, no significant correlation was found at LS between BMC and weight or BMI, but it was significant for TH ($r = 0.34$; $P < 0.001$ for weight and $r = 0.39$; $P < 0.0001$ for BMI). In G2 there was a significant correlation at both sites for the same variables (LS: $r = 0.33$; $P < 0.0001$ for weight and $r = 0.29$; $P < 0.0001$ for BMI; TH: $r = 0.42$; $P < 0.0001$ for weight and $r = 0.40$; $P < 0.0001$ for BMI). Considering height, BMC was significantly lower both at TH ($P = 0.02$) and LS ($P = 0.04$) in E1 vs. E2. There were no significant differences between BMI in E1 vs. E2 ($P > 0.05$).

Conclusion: BMC was significantly higher in overweight patients independently of age. Although there were no differences for BMI when the population was divided by height, BMC was significantly lower within those with shorter stature.

P508-Su

Soft Tissue Composition and Femoral Bone Mineral Density in Hip-Fracture Women

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Body weight is a strong predictor both of bone mineral density (BMD) and of fracture risk, but the respective roles of fat and lean mass were not clearly defined. Our aim was to evaluate the association between soft tissue composition (fat and lean compartments) and femoral BMD in hip-fracture women.

We evaluated a total of 270 Caucasian women admitted consecutively to our Division of Physical Medicine and Rehabilitation because of an original hip fracture. A total of 14 of the 270 women were excluded because their hip fractures were caused either by major trauma or by cancer affecting the bone. All of the 256 remaining women suffered from fractures that were either spontaneous or caused by minimal trauma. 34 women could not be assessed by DXA because of early discharge, refusal, or concomitant severe diseases, and were not included in the study. The final study sample included 222 women. Both BMD (at the unfractured femur) and soft tissue body composition were assessed by dual-energy X-ray absorptiometry (DXA), 22.1 ± 9.1 (mean ± SD) days after fracture occurrence.

At Spearman rank test, we found a significant positive correlation between BMD assessed at total proximal femur and both fat mass (rho = 0.640; $P < 0.001$) and lean mass (rho = 0.493; $P < 0.001$). The difference between the two correlation coefficients was significant (difference 0.147; 95% CI 0.055–0.239; $P < 0.001$). Linear multiple regression was performed, including BMD assessed at total proximal femur as the dependent variable, and the following ones as independent variables: fat body mass, age, body height, lean body mass, hip-fracture type (cervical or trochanteric), number of concomitant diseases, number of drugs in use, and time between fracture occurrence and DXA assessment. Fat body mass was the only independent variable significantly associated with BMD ($R^2 = 0.366$; $P < 0.001$). The results both of bivariate correlation and multiple regression were similar when femoral BMD was assessed at other four sites (femur neck, trochanter, intertrochanteric area, and Ward's triangle).

Data show that fat but not lean body mass was a major determinant of femoral BMD in elderly Caucasian women with hip fracture. The fat–bone relationship we observed may be explained by various mechanisms, including genetic factors that regulate both fat mass and bone properties, and a number of bone-regulating hormones whose secretion is related to fat mass.

P509-Mo

Calcium Homeostasis and Bone Metabolism During Lactation and Post-Weaning

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Aim: To characterize post-partum calcium homeostasis and bone metabolism in relation to lactation status.

Background: Prospective studies have demonstrated a loss of bone mineral density (BMD) during lactation and a gain post-weaning. The hormonal mechanisms responsible for these changes are less well described.

Material and methods: We followed 91 healthy women for 9 months postpartum (pp) with baseline at 21 ± 7 days pp and first and second follow up at 120 ± 14 and 270 ± 14 days pp, respectively. We categorized them according to breastfeeding at each visit. At each visit, we performed dual energy X-ray absorptiometry (DEXA) of the whole body, the lumber spine, the hip, and the forearm together with ultrasonography of the heel (BUA, SOS, and Stiffness). We also draw blood samples for determination of calciotropic hormones (Vitamin D metabolites, PTH, PTHrP) and markers of bone turnover (osteocalcin, bone specific alkaline phosphatase), and collected 2-h urine for NTx/creatinine excretion.

Results: Between baseline and 1st follow-up, BMD decreased at all sites ($P < 0.001$) with a subsequent increase between 1st and 2nd follow-up ($P < 0.001$). Changes in BMD between baseline and 2nd follow-up differed significantly between women who continued breast-feeding and women who stopped. In those who continued BMD at 2nd follow-up had decreased 1.97% at the lumbar spine, 3.57% at hip, and

0.38% at the whole body, whereas BMD had increased 0.61% at the lumbar spine ($P < 0.001$ compared with breastfeeding), had decreased 1.67% at the hip ($P = 0.001$), and had increased 0.83% ($P < 0.001$) at the whole body in those who stopped. There was no difference in forearm BMD changes between the groups. Ultrasonographic variables did not change over time. At 2nd follow up, breastfeeding was associated with higher levels of prolactin (71.7% $P < 0.001$), bone-specific alkaline phosphatase (19.8%, $P < 0.01$), osteocalcin (19.7%; $P < 0.01$), and NTx/creatinine (34.3%, $P < 0.001$), whereas PTHrP, PTH, 25-OHD, and $1,25(OH)_2D$ remained identical between the groups.

Conclusion: Changes in BMD were related to lactation status. Persistent lactation was associated with higher prolactin levels, increased bone turnover, and continuous bone mineral loss, which could not be explained by differences in PTH, PTHrP, or $1,25(OH)_2D$.

P510-Tu

Relationships Between Fat Mass, Llean Mass, and Bone Mineral Density in Healthy Eugonadal Romanian Women

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It has been shown that bone mass and fracture risk are highly positively related to the body mass index (BMI). However, these relationships may also be influenced by body composition. Several studies indicate that in young adults fat-free mass explains up to 20–25% of the variance in lumbar bone mineral density (BMD). On the other hand, there is evidence that in postmenopausal women the adipose tissue content is strongly related to BMD. We aimed to evaluate the relationships between body composition and bone mass in a group of 36 Romanian premenopausal, eugonadal women, with a mean age of 34 ± 6 years. Subjects had a mean BMI within normal limits (24.9 ± 5 kg/mxm). The total body composition (total fat mass, trunk fat mass, fat-free mass), the bone mineral content (BMC), and the BMD were measured by means of dual-energy X-ray absorptiometry (DXA). As expected, BMI was positively related to both BMD and BMC ($P < 0.05$). It was noticed that despite a BMI towards the upper normal limit premenopausal women from our study group had an increased total body fat mass of 38.3% of the total body mass. About 45% of the total body fat mass was distributed on the trunk. A positive relationship was seen between the total body fat mass and BMC ($P < 0.05$). In addition, BMC was positively related to the trunk fat mass content ($P < 0.05$). A tendency towards a positive association between fat-free mass and both BMC and total BMD was observed; however, the correlation was not statistically significant. These results indicate that total and trunk fat mass play significant roles in the maintenance of bone mass in healthy eugonadal Romanian adult women.

P511-Su

Bone Mineral Density and Low-Density Lipoprotein Cholesterol in Saudi Postmenopausal Women

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Objectives: To assess the relationship between bone mineral density (BMD) and plasma lipids in Saudi postmenopausal women.

Subjects and methods: A total of 790 Saudi postmenopausal women living in Jeddah area were studied. The inclusion criteria were: menopausal status (defined as amenorrhea for a minimum of 12 months), age 45–65 years, serum triglycerides (TG) <3.50 mmol/L, absence for at least 6 months before clinical assessment, or treatment with hormone replacement therapy or with drugs affecting bone or lipid metabolism. The serum levels of total cholesterol (c), low-density lipoprotein-c (LDL-c), high density lipoprotein-c (HDL-c), and TG were measured together with bone turnover markers (formation: serum osteocalcin (sOC), bone alkaline phosphatase (sBAP), carboxyterminal propeptide of type 1 collagen (sPICP); and resorption: C-telopeptide fragments of type 1 collagen (sCTX), Urinary (uCTX), cross-linked N-telopeptide of type 1 collagen (uNTX), and deoxypyridionoline cross-links (uDPYR)). BMDs of the spine (L2–L4) and femur were determined using dual-energy X-ray absorptiometry (DXA) technique and women were classified with osteoporosis, osteopenia, or normal BMD according to WHO criteria. The relationships between BMD and lipid variables were examined using univariate analysis by means of Chi-square test and by multivariable analysis using multiple logistic regression. ANOVA was used to examine differences among groups according to LDL-c tertiles.

Results: A significant relationship was found between the prevalence of osteopenia and age, body mass index (BMI), years since menopause (YSM), and LDL-c levels. Women in the upper tertile of LDL-c showed more than double probability of being osteopenic compared with women in the lower tertile of LDL-c (52% vs. 23%, $P < 0.001$), respectively. Bone turnover markers (namely, sOC, sBAP, sCTX, uNTX, and uDPYR) were significantly increased in postmenopausal women in the upper LDL-c tertile as compared with that in the lower LDL-c tertile ($P < 0.05$). Using multivariable analysis, only LDL-c, YSM, and BMI were significantly associated with the prevalence of osteopenia in the postmenopausal women examined.

Conclusions: Increased LDL-c levels in postmenopausal women were associated with greater probability of lower BMD values and osteopenia. These results suggest that increased LDL-c levels should be regarded as an additional risk factor for lower BMD values in postmenopausal women.

P512-Mo

Growth Retardation and Osteopenia in Adolescents and Young Adults Treated with Hemodialysis

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Bone disease and osteoporosis are the major causes of morbidity in end stage renal failure. This study was performed to asses the prevalence and severity of osteopenia and growth retardation in 42 young adults and adolescent dialysis patients, 23 females and 19 males, aged 14–25 years, mean age 19.9 ± 3.85, with duration of hemodialysis from 1 to 12 years, mean 4.9 ± 3.5. Bone mineral density of the lumbar spine L1–L4 was measured using dual energy X-ray absorptiometry by Hologic QDR 4500 system. Height and weight were recorded, and body index calculated. Bone age was assessed by analysis of wrist X-rays films. Laboratory investigations included intact parathyroid hormone, serum total and ionized calcium, serum phosphate, osteocalcin, alkaline phosphatase. Mean bone mineral density corrected for gender and age, *Z* score, of the lumbar spine was −2.01 ± 1.6. 59.5% had osteopenia or osteoporosis. 45.2% had growth retardation. Growth retardation and a low lumbar bone mineral density were closely associated, $P < 0.001$, with age at start of hemodialysis and pubertal status, duration of hemodialysis and weight, $P < 0.001$. Laboratory results did not correlate significantly with bone mineral density of the lumbar spine. Our results indicated that end stage renal disease and hemodialysis are associated with serious skeletal abnormalities in adolescent and young adults. The start of dialysis before puberty is associated with high risk of growth retardation and developing osteopenia.

P513-Tu

Effect of Immobilization and Neural Influence on Tibial Fracture in Rats

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Purpose: To investigate the effect of immobilization and neural influence on fracture healing.
Relevance: Fracture healing is a complex process which involves a series of dynamic events and, ultimately, the normal integrity as well as the biomechanical properties of bone could be restored. Many studies have shown that immobilization as well as nerve innervation could cause changes in bone and which might, in turn, significantly affect the healing of a fracture. Since immobilization and neural injury are complications that are usually associated with fracture, it is essential for us to understand the role of these two factors in order to establish appropriate protocol in treating fractures.
Subjects: Thirty-six matured female Sprague–Dawley rats at 12 weeks of age, around 300 g, have been used in this study.
Methods and materials: All the rats have received a diaphyseal transverse fracture of the right tibia with an intramedullary pin fixation. They were assigned randomly in equal number into 3 groups: control group, tenotomy group, which received a tenotomy of the right patella tendon, and de-innervation group, which received a neurectomy of the right sciatic nerve. The rats were sacrificed for peripheral quantitative computed tomography (pQCT) at day 21 post-fractures.
Analysis: One-way ANOVA was used for all the statistical comparisons between the three groups.
Results: Significant difference was found in both total Bone Mineral Density (BMD) and total Bone Mineral Content (BMC) among three groups ($P < 0.05$). Post hoc analysis (LSD) revealed that control group has a significantly higher total BMD than the neurectomy group ($P = 0.01$). It has also been found that control group has a significant higher total BMC when compared to other two groups (tenotomy, $P < 0.05$; neurectomy, $P < 0.01$).
Conclusions: The results suggest that immobilization as well as nerve innervation could affect fracture healing to different extent.
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P514-Su

Effects of Cyclosporin A on the Remodeling of Alveolar Bone in Rats

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Background: Cyclosporin A (CsA) is used as an immunosuppressant agent. CsA therapy induces side effects such as nephrotoxicity, hepatoxicity, gingival overgrowth, and osteoporosis. Several studies for CsA-induced osteoporosis have been reported from clinical and basic aspects. Osteoporosis is thought to be one of risk factors of periodontal disease, which is induced by periodontopathic bacteria and resulted in alveolar bone loss. In this study, we

investigated the effects of CsA on the remodeling of rat alveolar bone.

Materials and methods: Fifteen-day-old rats were fed powered diet containing or lacking CsA for 8–30 days. On days 8, 16, and 30, plasma calcium level was measured by MXB method and plasma parathyroid hormone (PTH) and osteocalcin (OCN) levels were determined by ELISA. After the extraction of mandible, histomorphometric analyses were performed to examine morphologic changes in alveolar bone. Lingual alveolar bone proper around mesial root of the first molar was analyzed by micro-CT. The distance between alveolar bone crest (ABC) and cement-enamel junction (CEJ) was measured in two-dimensional image from the micro-CT. Decalcified semi-thin sections were stained with tartrate-resistant acid phosphatase (TRAP), and then histomorphometrical measurement was performed.

Results: Plasma calcium level in CsA-treated rats was normally maintained during the experimental period. Concentrations of plasma PTH and OCN in CsA-treated rats were significantly increased compared to the controls on day 8, whereas no difference was observed on days 16 and 30. Micro-CT analysis revealed that volume and thickness of alveolar bone proper in CsA-treated rats were decreased on days 16 and 30. However, the distance between ABC and CEJ was not changed by CsA treatment. Also, there was no change in the number of osteoblasts and osteoclasts on the bone surface during experimental periods.

Conclusion: As the present results showed the decreasing effect of CsA on volume and thickness of alveolar bone, it is possible that CsA administration may be a risk for periodontal disease progression.

P515-Mo

Regulation of Bone Balance-Sequential Micronutrition

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Osteoporosis is becoming a real public health issue. Bioresearch and Partners' objective is to exploit a new nutritional approach: micronutrition which can be used in prevention as well as to help and strengthen classic therapeutics. Micronutrisequences are a combination of microgram-dosed dietary substances organized in sequence. The micronutritional approach developed by Bioresearch and Partners is a new mode of utilizing nutrients which target an action on the global regulation of bone balance, the nutrients have been selected to participate in a nutritional strategy aiming to help the restoration of bone metabolism homeostasis. The aim of the nutritional intervention was the measurement in monotherapy in an open study of bone balance regulation by the dosage of biological markers of bone formation (osteocalcin) and resorption (24-h urine desoxypiridonoline at the creatinine dosage), as well as the increase of bone density observed with bone density test in menopaused women presenting a disturbance of bone balance markers and osteopenia or osteoporosis (BMD). The inclusion in the trial was excluding any treatment. The nutritional intervention lasted 2 years. Were excluded of the trial the patients who were immobilized or taking corticoids, having osteomalacia or unstable diseases: diabetes, thyroid, Crohn disease, kidney, or active hepatic diseases. Nutrisequence (capsules containing micronutrition granules) was taken three times a day during 3 months.

Population studied: 24 women aged 64 in average. All 24 patients are followed on bone markers balance, 10 are complementarily followed in absorptiometry.

Results: Of the 17 cases out of 24 cases which were presenting at the beginning of the trial, a bone balance with an excessive increase of dpyr, 94% re-stabilized their bone balance, 82% totally normalized their balance (in 17.3 months in average).

Results on bone density: Of the 10 cases followed in BMD: 7 patients out of 9 had osteopenia or osteoporosis and showed an increase of the trabecular density by 7%.

Conclusion: The regulation of bone markers (82%) and the increase of bone density (+7%) offer a satisfactory response to the required therapeutic aims in a population presenting an excessively negative bone balance. Micronutrisequence is efficient and perfectly adapted to a systematic preventive action and can be associated with any other drug therapeutics.

P516-Tu

Densitometric and Textural Analysis on Digitized X-rays of Trabecular Bone and its Relationship with Biomechanical Properties

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The biomechanical properties of bone can only be directly determined by destructive tests. For this reason, it is important to develop a noninvasive technique which can predict the biomechanical properties of bone. Fractal analysis on digitized conventional X-rays is a useful technique to analyze trabecular texture patterns. In conjunction with computed assisted densitometry, as a variable related to BMD, it can be a promise technology to indirectly determine the biomechanical properties. The purpose of this study is the evaluation of the correlation between the biomechanical properties of bone samples and their densitometric and textural properties using a new computer-assisted image analysis system.

Methods: 31 bone cylinder samples corresponding to femoral condiles, vertebrae, and hips were employed. Craneo-caudal (CC) standardized radiographs were performed and, subsequently, digitized at 12 bit grayscale, obtaining images of 2048 × 2048 pixels. Each squared region of interest was obtained and analyzed, being characterized by a six-dimen-

sional feature vector: medium optical density level, 2D and 3D fractal dimension (2DFD and 3DFD, respectively), and 3 autocorrelation functions between them. Multidirectional fractal analysis (anisotropic degree (AD) and anisotropic index (AI)) was used to determinate anisotropy. A compressive biomechanical testing was performed to determine Young modulus (YM) and maximum stress (MS).

Results: For all the samples, biomechanical properties (YM and MS) were correlated with 2DFD ($r < 0.473$, $P < 0.01$; $r < 0.432$, $P < 0.05$, respectively). However, there was no correlation between 3DFD and both YM and MS ($r < 0.057$ and $r < -0.045$, respectively). Autocorrelation functions showed the most significant statistical correlation with both YM ($0.596 < r < 0.633$, $P < 0.01$) and MS ($P < 0.05$, $0.362 < r < 0.515$). A direct correlation was found between both YM and MS, and AD ($r < 0.569$, $P < 0.01$; $r < 0.449$, $P < 0.05$, respectively) and inverse correlation ($P < 0.01$) with AI ($r < -0.637$ and $r < -0.463$, respectively).

Conclusions: The radiographic textural analysis based in fractals combined with computer-assisted densitometric image analysis improves the correlation with biomechanical properties showed by textural analysis alone. This analysis should become an accessible, useful, and low cost technique to evaluate the biomechanical properties of trabecular bone.

P517-Su

Patterns of Mineralization in Children with Secondary and Syndrome Associated Osteoporosis

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The Pediatric Bone Health Clinic at the Hospital for Sick Children in Toronto is evaluating an increasing number of children with chronic illness and syndromes for probable osteoporosis. Often, commonly used investigative tools such as bone mineral density (BMD), radiographs, bone turnover markers, and other biochemistry do not allow for diagnostic certainty and add little to our understanding of the underlying pathophysiology. To more accurately describe the bone disease in these children with low BMD and/or fractures, we now undertake transcortical bone biopsies to assess bone histology and turnover by histomorphometry. Using this approach in a small study sample, we have previously reported that approximately 40% of these children have histomorphometric evidence of an adynamic form of osteoporosis. To further understand these findings, we have used the newer technique of backscattering electron microscopy (BSE) to determine the degree of bone mineralization in these biopsies.

We report here the pattern of mineralization in 13 children with various clinical diagnoses in relation to the more standard histomorphometric parameters used to diagnose and follow osteoporosis. Group 1 comprised 9 patients with chronic illness such as: ALL 1, BMT 2, Chronic liver disease 1, Rheumatological 5; 5 of 9 patients had received long-term glucocorticoids. In this group, we found uniformly low bone formation and poor bone mineralization by BSE as well as low to very low BMD (Z score, -5.5 to -2.0). Group 2 comprised 4 patients with syndrome-associated osteoporosis (Stuve–Wiedemann syndrome 1, Ehlers–Danlos Syndrome 1, Larsen syndrome 1, undefined 1). These patients in addition to a low to very low BMD had a very high rate of bone formation with over 30% of the bone surfaces covered by osteoid and with an average osteoid width of 15 μm corresponding to an osteomalacic syndrome. However, they all had a normal degree of bone mineralization as shown by BSE.

In conclusion, histomorphometric and mineralization patterns appear to differ between children with chronic illness and syndrome-associated osteoporosis. These findings also suggest that in children, mixed disorders of osteoporosis and mineralization defects may be more common than previously believed.

P518-Mo

Corticosterone Treatment Produces Rapid and Profound Reductions in Circulating Osteocalcin Levels in Mice

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Corticosterone is the natural circulating bioactive glucocorticoid in mice. The skeletal effects of corticosterone treatment in mice have not been assessed. Dexamethasone treatment has previously been reported to reduce osteocalcin circulating levels in mice and these changes correlated with development of osteopenia (1). The aim of this study was to evaluate the effects of different corticosterone treatment regimes on circulating levels of osteocalcin, as a surrogate for effects on bone formation, and relate these to corticosterone levels.

Corticosterone was provided to 6-week-old male Swiss mice by single subcutaneous injection in sesame oil or by implantation of a commercial pellet (Innovative Research, USA). Doses used ranged from 1 to 50 mg/kg for injections and from 1 to 50 mg for implants. In an additional study, 10 mg commercial pellets was implanted weekly for 3 weeks. Corticosterone levels increased rapidly following an injection of corticosterone, but returned to baseline by 8 h. The commercial implants produced a rapid increase in corticosterone levels. However, even though the pellets were marketed as providing slow release over 21 days, corticosterone levels returned to baseline by day 7.

Osteocalcin was dose dependently suppressed 50% by 8 h following corticosterone injection but returned to control levels by 12 h. Osteocalcin levels were suppressed 75% following implantation of a 10 or 50 mg corticosterone

pellet, remained suppressed at day 7, but rebounded to baseline by day 14. Dosing with repeated implantation of 10 mg pellets at day 7 intervals produced elevated levels of corticosterone that returned to baseline levels prior to reimplantation. Interestingly, osteocalcin levels were stably suppressed 75% using this dosing regimen.

Pharmacologic doses of corticosterone act to profoundly suppress bone formation as assessed by circulating osteocalcin levels. Maintaining sustained suppression of osteocalcin required repeated implantation with slow release pellets at day 7 intervals. Suppression of osteocalcin correlated with circulating levels of corticosterone, but there was evidence of delay between clearance of corticosterone and recovery of osteocalcin levels.

1. McLaughlin et al. Glucocorticoid-induced osteopenia in the mouse as assessed by histomorphometry, microcomputed tomography, and biochemical markers. Bone. 30:924–30, 2002.

P519-Tu

Glucocorticoid-Induced Low Bone Mass in Children and Adolescents: A Longitudinal Study on a Large Sample

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266 young patients (3–20 years) on long-term glucocorticoid (GC) therapy underwent multiple evaluations of bone density over 3 to 14 years (mean 6.9 ± 6.2). Cumulative doses of GCs (prednisone equivalent) were calculated.

The diseases were nephrotic syndrome (NS, 38), cystic fibrosis (64), juvenile rheumatoid arthritis (42), systemic lupus erythematous (SLE, 18), Duchenne muscular dystrophy (28), acute lymphatic leukemia (12), asthma (12), autoimmune hepatitis (12), and transplants (Tx: 25 kidney, 10 liver, 5 lung).

Bone mineral density (BMD) was measured with DXA (Hologic QDR 2000) at lumbar spine and on total body. BMD was expressed as absolute value (mg/cm^2) and as *Z*-score (calculated vs. sex- and age-matched healthy Italian samples). Areal BMD was adjusted for body surface (BMD/BS) to minimize the effect of body size. Vertebral BMD was adjusted for vertebral volume (VV) and BMAD was calculated: BMAD (g/cm^3) = BMD × [4/(3.14 × width)]. Control values were similarly adjusted for BS and VV.

Independently of primary disease and patients' age, GCs had a major effect on trabecular bone (spine vs. total body). The BMD decrease was dose-related: cumulative dose<10 g = −23%; 10–30 g = −40%; >30 g = −68%. There was significant correlation between spine *Z*-score BMD vs. cumulative GCs ($r = -0.84$, $P < 0.001$), also considering specific diseases separately.

Spine bone loss was higher during the first year of GC therapy and continued at a lower rate thereafter (on average: 1st year, 16%; year 2, 5–6%; year >5, 3.5%). Bone loss in the 1st year was different for different diseases (e.g., in Tx: highest for lung, lowest for kidney).

In children followed with DXA for up to 12 years, GCs influenced bone mass in a different way in relation to age at disease onset and disease characteristics. Full recovery (e.g., in NS) with GC suspension before puberty led to normalization of bone mass. On the contrary, as in patients with SLE, starting GCs during puberty induced a bone loss (while BMD was normal at diagnosis).

83 subjects (31%) had at least 1 fragility fracture, 33 more than one. Overall, 161 fractures (39 vertebral) occurred in the group.

This longitudinal study indicates that GCs exert a detrimental effect on bone even in young patients, inducing bone loss and fractures and altering the possibility to reach the expected bone accrual. The relationships between bone density and cumulative dose of steroids clearly underline the absolute need to use the minimum effective dose of GCs.

P520-Su

The Influence of Treatment with Glucocorticoids on Cortical Bone Structure and Vertebral ragility: Comparison Between Mmenstrual and Postmenopausal Women

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Since vertebral body is the predominant site of trabecular bone, the trabecular bone mass and microarchitecture play an important role in vertebral bone strength. Recently, we showed that architectural properties of cortical bone and the index of cortical flexural strength were useful to predict vertebral fractures in postmenopausal women. Glucocorticoid (GC) treatment leads to progressive bone loss and the development of fragility fractures. GC-induced vertebral fragility is generally considered to depend on trabecular bone factors. However, the roles of cortical bone factors in the vertebral fragility by GC treatment remains little known. To evaluate the possibility of the contribution of cortical bone factors to GC-induced vertebral fragility, we analyzed the cortical geometric parameters of radius using peripheral quantitative computed tomography in women with GC treatment (GC+) and normal controls matched to age, gender, body size, and menstrual state (GC−). One hundred six postmenopausal women (mean age GC+; 60 years, GC−; 63) and 84 menstrual women (35, 34) were enrolled in this study. GC+ subjects underwent oral GC treatment for more than 6

months (the current mean dose converted to prednisolone; 11 (postmenopausal), 10 (menstrual) mg/day, the mean treatment duration; 87 and 88 months, respectively). In menstrual women but not in postmenopausal women, total bone area, periosteal circumferences, and strength strain index (SSI), a cortical bone strength index, were significantly lower in GC+ group than in GC− group. Similar results were obtained from menstrual women with Cushing syndrome. We compared cortical parameters between the presence (Fr+) and absence (Fr−) of vertebral fractures in GC+ groups of each menstrual state. In postmenopausal GC+ group, there were no significant differences in any cortical parameters between Fr+ and Fr− groups. In contrast, in menstrual GC+ group, total bone area, periosteal circumferences, cortical area, cortical thickness, and SSI were significantly lower and treatment duration was significantly longer in Fr+ group than in Fr− group. Menstrual Fr+ women showed slender and thin cortical bone, which indicated that their cortical bone was structurally weak. In conclusion, the present findings suggest that cortical bone factors play an important role in vertebral fragility in GC-treated menstrual women and that marked differences exist in mechanisms of GC-induced vertebral fragility between menstrual and postmenopausal women.

P521-Mo

A Multi-Center Secondary Care Based National Audit of Bone Prophylaxis in Glucocorticoid Induced Osteoporosis

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The risk of fragility fracture is increased substantially in those who are prescribed oral glucocorticoids, and recent evidence-based guidelines recommend empirical bone protective therapy in individuals aged over 65 years. This analysis examines the adherence to these guidelines for hospital inpatients in England and Wales.

Glucocorticoid-induced osteoporosis screening in older people (GIOSCOPE) is an audit tool designed to evaluate the prescribing prevalence of bone prophylaxis in patients aged over 65 years receiving oral glucocorticoids. This tool was utilized on inpatients who had medications and case notes reviewed. Information on previous fracture history, DXA measurement, and prescription of calcium and vitamin D was also recorded.

618 inpatients prescribed glucocorticoids from 9 hospitals were available for analysis. Their mean age was 77.2 (SD 7.2) years and 58% were female. 203 (33%) patients were currently prescribed a bisphosphonate. Of the 415 patients not on bisphosphonate therapy, 183 patients had a commitment to glucocorticoids for more than 3 months; 56 of these patients had a potential contra-indication to bisphosphonate therapy. Thus, there were 127 patients who were eligible for a bisphosphonate but were not on treatment. Patients not receiving GIO prophylaxis were significantly more likely ($P = 0.003$) to be male (26% vs. 16%) and slightly older (78.2 vs. 77.1 years, $P = 0.1$). History of previous fracture or DXA did not predict appropriate treatment.

279 (45%) of all patients on glucocorticoids were also prescribed calcium and vitamin D supplement. While supplementation was more prevalent in those on long-term glucocorticoids and those prescribed a bisphosphonate, 21% were not co-prescribed calcium and vitamin D.

Approximately two-thirds of hospital inpatients prescribed glucocorticoids for 3 months or more, but not bisphosphonate therapy, are not receiving any appropriate bone prophylaxis. Men appear to be at higher risk for not receiving prophylaxis. Identification of these high risk patients via secondary care and initiation of prophylaxis is a clinical priority.

P522-Tu

Relationship Between Serum Parathyroid Hormone and Bone Mass among Black and White Adolescent Girls

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Background: Hypovitaminosis D is a prevalent problem in United States particularly during winter. The fact that Blacks have higher bone mineral density than Whites has led some to assign less importance to defining the role of vitamin D insufficiency in Blacks. However, they have much lower levels of vitamin D.

Specific Aim: Assess the relationship between vitamin D indices and bone mass in adolescent black and white girls.

Methods: We recruited 30 Black and 12 White healthy adolescent girls, 12–14 years old, during late winter. After obtaining informed consent, subjects were evaluated by medical history and a physical exam. Pubertal development was assessed by Tanner staging. The height was measured by Harpenden Stadiometer and weight was recorded. Fasting blood sample was drawn for assessment of serum 25-OHD(RIA, DiaSorin) and PTH(Nichols). A food frequency questionnaire was completed to assess dietary intake of calcium and vitamin D. We performed DXA (Hologic QDR 4500, pediatric software version 9.0) and peripheral Quantitative Computed Tomography on radius (pQCT, Stratec 960, Norland).

Results: The subjects ranged from Tanner stage II to V with majority in III/IV with a mean age of 12.8 years. The mean dietary calcium and vitamin D was 580 mg/day and 70 IU/day, respectively. The Blacks had significantly lower

serum 25OHD (27.8 nmol/L vs. 47.1 nmol/L, $P = 0.0001$) and higher PTH (44.7 pg/ml vs. 33.5 pg/ml, $P = 0.0055$) when compared to Whites. The serum 25-OHD and PTH levels were inversely related, $r = -0.31$, $P = 0.04$. The unadjusted Pearson correlation coefficient (r) between bone mass and serum PTH, 25-OHD are reported in Table 1. The significant relationships remain unchanged when adjusted for variables like chronological age, Tanner stage, and BMI.

Conclusion: An inverse relationship exists between serum PTH and bone mass among adolescent Black and White girls. This indicates that lowering serum PTH levels can potentially result in higher bone accretion during adolescence in both Blacks and Whites. Influence of vitamin D3 supplementation on achievement of peak bone mass among adolescents from all races should be studied longitudinally.

Table 1
Pearson correlation coefficients

Bone mass	PTH (r) Blacks, $n = 30$	P value	PTH (r) Whites, $n = 12$	P value
TBBMC	−0.45	0.01	−0.43	ns
BMD spine	−0.40	0.02	−0.24	ns
BMD total hip	−0.51	0.004	−0.57	0.05
Total BMD 1/3 radius, pQCT	−0.38	0.05	−0.34	ns

P523-Su

Early Serum IGF-I Response to Oral Protein Supplements in Elderly Women with a Recent Hip Fracture

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Protein malnutrition plays an important role in the occurrence and the outcome of hip fracture in the elderly. Correction of protein undernutrition with oral protein supplements improves the clinical outcome of elderly with hip fracture and is associated with higher IGF-I levels 6 months after the fracture. However, the early IGF-I response to protein supplements in elderly is not known. We investigated the time course of serum IGF-I response after 7, 14, and 28 days of protein supplement of 20 g daily in 45 women aged 81.3 ± 1.1 years (mean ± SEM) starting 10.0 ± 0.5 days after surgery for hip fracture. Serum IGF-I levels increased significantly as early as by 7 days of protein supplementation (+54 ± 10%, $P < 0.01$) reaching +68 ± 13% ($P < 0.001$) by 28 days. As compared to a control group of 12 elderly women with a recent hip fracture who refused to take any protein supplement, the difference in the increase of serum IGF-I levels, expressed in absolute values, was already maximum and significant after 7 days (33.6 ± 6.9 vs. 6.0 ± 3.6 μg/L, $P = 0.04$). In contrast, IGFBP-3 changes were similar in both groups. This suggests a higher IGF-I bioavailability under protein supplements. Since end-organ responsiveness has been suggested in protein depletion and since IGF is known to increase bone remodeling, we measured serum osteocalcin, which turned out to be similar in both groups. This may suggest that a longer exposure to protein supplement would be required to improve bone sensitivity to IGF-I. In conclusion, our study shows an early and specific increase of serum IGF-I levels under protein supplements with almost 80% of this effect observed already by 1 week of oral protein supplements.

P524-Mo

Glucuronic Acid Increases the Bone Mineral Density in the Diabetic Rats

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Background and aim of study: Glucuronic acid is one of the constituents of hyaluronic acid. Several dietary supplements, such as glucosamine, hyaluronic acid, have been reported to be effective for the improvement of the conditions of the damaged skin.

Skin damage and osteopenia are phenomena known as type1 diabetes complications. We focus on glucuronic acid and examine the potential of this substance to modulate the osteopenia associated with streptozotocin (STZ)-induced diabetic rats.

Methods: Osteopenia models were induced by streptozotocin (STZ) (50 mg/kg intraperitoneally) in 6-week-old male Wistar rats. Glucuronic acid was administered orally (400 mg/kg) for 4 weeks after STZ injection. The blood and femur were harvested. Blood sugar content was compared by Glucose C-Test. Bone mineral density (BMD) of the femur was measured by dual energy X-ray absorptiometry, Dichrom SCAN PCS-600. The freeze-crushed epiphyses of the femur were extracted with 4 M guanidine hydrochloride. Collagen in the extracts was analyzed by Western blotting with anti-type1 collagen antibody. The knee joints sections were stained with hematoxylin and eosin (HE), and with safranin O.

Results: Metaphyseal (adjacent to the knee joints) BMD in the glucuronic acid treatment group was significantly higher than that in the STZ control (Diabetic control) group ($P < 0.02$). Western blotting demonstrated the signal intensity of α1 and α2 chain collagens was elevated by glucuronic acid administration. Histological findings showed STZ treatment reduced the growth plate thickness. Glucuronic acid treatment improved this change of growth plate in STZ rats.

Conclusion: Glucuronic acid inhibited osteopenia in the diabetic rats. This indicates intake of glucuronic acid could stimulate the extracellular matrix metabolism in the bone. These facts suggest the nutritional approach for the complications of diabetes.

P525-Tu

Sodium and Potassium Effects on Bone Mass in Rats

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High sodium intake may reduce calcium retention by increasing urinary calcium loss while alkaline potassium salts have opposite effects. Therefore, theoretically, increased potassium intake may protect bone mass in individuals with high sodium intake. We evaluated effects of both high sodium and high potassium intake on bone mass in growing rats.

Six-week-old female Wistar rats were randomly assigned into four groups according to the drinking solution (compounds were dissolved in deionized water): control (deionized water only), 1.2% sodium chloride, 1.2% potassium citrate, and 1.2% sodium chloride plus 1.2% potassium citrate. All rats were fed on marginal (0.33%) calcium in diet. After 10-week exposure, we evaluated bone mineral density (BMD) and content (BMC) of the whole body and 24-h urinary calcium, potassium, sodium, magnesium, and phosphorus excretion. Subsequently, we killed all animals and removed right femurs to assess bone weights and bone calcium.

Body weight gain did not differ among groups. Sodium-loaded rats drank more and had greater urine volume. High sodium chloride intake increased urinary calcium, sodium, potassium, phosphorus, and magnesium excretion. Potassium supplementation increased urinary potassium excretion and decreased urinary excretion of calcium, phosphorus, and magnesium. Neither sodium nor potassium intake affected total body BMD and BMC or femur weights. However, both femoral calcium content and concentration were lower in sodium-loaded rats and potassium supplementation diminished these sodium effects. Potassium supplementation itself had no effect on bone calcium compared to controls.

In conclusion, under our experimental conditions, we found that increased potassium intake protects bone mass in sodium-loaded growing rats. Therefore, increased potassium intake may have beneficial effects on bones during growth and development in children consuming higher sodium in diet.

P526-Su

Effect of *Carthamus Tinctorius* l. Extracts on Bone Resorption in Ovariectomized Rats

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Carthamus tinctorius L. (Safflower) seeds are used in traditional Eastern folk medicine to promote bone healing and prevent osteoporosis. We have previously shown that *Carthamus* extract inhibits bone resorption in vitro. The aim of this study was to investigate the effect of *Carthamus* seed extracts on prevention of bone loss in the ovariectomized rat model. In a pilot study, 3-month-old female Wistar rats were either sham-operated (SH; $n = 10$) or ovariectomized (OVX; $n = 30$). The OVX rats ($n = 10$) received either a control diet or were supplemented with 0.25% or 1% *Carthamus* seed extract. Urinary deoxypiridinoline (DPD) excretion, a marker of bone resorption, was measured periodically for 3 weeks. OVX induced an increase in urinary DPD which was reversed by 1% *Carthamus* seed extract at day 21. In a follow-up study, 3-month-old female Wistar rats were randomized to 7 groups ($n = 15$/group) as follows: SH, OVX control diets, OVX fed control diet supplemented with 0.1% or 0.5% of a Carthamus seed extract or 1% Carthamus oil-cake extract or 0.125% soy isoflavones for 90 days. Bone mineral density (BMD) was measured in vivo at the vertebral and femur sites using the Piximus densitometer at day 90 following OVX. Vertebral BMD was reduced by 10% ($P < 0.05$) in the OVX group compared to SH. The groups fed 0.5% Carthamus extract, 1% oil cake, and 0.125% isoflavones all showed an equivalent vertebral BMD which was intermediate between SH and OVX. No effect was observed with 0.1% of the *Carthamus* extract. Less significant changes were observed in total femur BMD than in vertebral BMD among the groups. In conclusion, *Carthamus* extract shows potential to prevent OVX-induced bone loss but further studies are required to establish the dose-response and active compounds.

P527-Mo

Fat Soluble Vitamins and Bone Mineral Density in Postmenopausal Women: Results of a Cross-sectional Study

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Calcium and vitamin D play a key role in pathogenesis, prevention, and treatment of osteoporosis. Fat soluble vitamins A and K are also certainly involved in bone health. Nevertheless, the influence of vitamin E on bone metabolism is less clearly established. Thus, an insufficient dietary intake of this vitamin may increase substantially the risk of hip fracture, whereas a more adequate intake seems to be protective, at least in current smokers. Additionally, high-dose vitamin E supplementation can stimulate bone formation and improve bone quality in old mice. Notwithstanding these data, the role of vitamin E on bone metabolism and postmenopausal bone loss is still controversial. Therefore, in this study, we assessed the relationships between vitamins E and A and bone mineral density (BMD) in 232 community dwelling healthy ambulatory postmenopausal women in Córdoba (Spain). BMD was measured by dual-energy X-ray absorptiometry (Hologic QDR1000; Hologic, Waltham, MA, USA) at lumbar spine (LS) and femoral neck (FN). Retinol (vitamin A) and alpha-tocopherol (vitamin E) were measured simultaneously by a domestic automated, high-performance liquid chromatography method (HPLC) previously reported [Quesada et al., J Steroid Biochem Mol Biol (2004): 89–90:473–7].

The average alpha-tocopherol concentration in serum was lower in the osteopenic–osteoporotic (T-score < -1) group of postmenopausal women (12.6 ± 1 vs. 10.9 ± 0.5 μg/mL; $P < 0.03$). Yet, the retinol concentration in serum was higher in osteopenic–osteoporotic postmenopausal women (T-score < -1). There was a correlation between alpha-tocopherol concentration in serum and bone mineral density in lumbar spine ($r = 0.176$; $P < 0.03$), and there was a significant negative linear correlation between retinol and BMD at FN ($r = -0.162$) but not at LS.

Our data clearly indicate that fat soluble vitamins E and A (besides vitamin D and K) are attractive candidates for bone health in postmenopausal women, through adequate intake and optimal serum levels. The mechanisms underlying this action and its relevance to the osteoporosis pathogenesis deserve further investigation in order to allow its correct use in supplementation and fortification of food, juices, and dairy products.

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P528-Tu

Vitamin Deficiency and Bone Loss in Inflammatory Bowel Disease

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Patients with inflammatory bowel disease (IBD) were evaluated for their bone mineral density (BMD) at lumbar spine, total hip, and radius. Their plasma levels of 25-dihidroxy vitamin D; 25(OH)D, vitamin K (phyloquinone, PK; menaquinone 4, MK-4; menaquinone-7, MK-7), intact PTH, and indices of vitamin K deficiency (protein induced by vitamin K; PIVKA and undercarboxylated osteocalcic; ucOC) were measured. Their food intake was also evaluated. The subjects consisted of 26 patients with Crohn's disease (CD) and 25 patients with ulcerative colitis (UC). BMD, as expressed as age-adjusted Z value, was -1 to -2 SD, which was significantly lower in CD than in UC. Plasma concentration of 25(OH)D, PK, MK-4, and MK-7 was far below the reference value in Japanese population. Again they were lower in CD than in UC. Intact PTH level was significantly higher in CD than in UC. Plasma levels of 25(OH)D or PK correlated with BMDs. However, their food intake of vitamin D and K was far above the current requirement in Japan and did not correlate with corresponding plasma levels. In contrast, their fat intake correlated with plasma concentration of vitamin D and K. Thus, severe malabsorption due to the inflammation in their intestine and restricted fat intake was strongly suspected. There was no significant difference in BMD between patients who were under glucocorticoid treatment and those who were not. Our results show that bone loss is an important clinical problem in IBD and that nutritional factors are quite likely to be involved in its pathogenesis.

P529-Su

Protein Intake: Effects on Phalangeal Bone Ultrasound in Healthy Women

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The effect of protein intake and other nutrients on bone mass is unclear and debated in the literature. Increasing dietary protein results in an increase in urinary calcium. However, there are no definitive nutrition intervention studies that show a detrimental effect of a high protein diet on the skeleton and the hypothesis remains unproven. In order to contribute to clarifying these discrepancies, we evaluated bone status using an ultrasound device that measures the amplitude-dependent speed of sound (m/s) transmitted through the phalanges in 940 healthy Caucasian women with high protein intake (ages 16 to 85 years) and body mass index between 18 and 32 kg/m^2. Nutrient intake was assessed using a 7-day record questionnaire. The study subjects were taking no medication and had no disease known to affect mineral metabolism that could interfere with calcium metabolism. Women were stratified according to their protein, calcium, and vitamin-D intake, and calcium/phosphorus and calcium/protein ratios. The mean calcium

intake was 1143 ± 520 mg/day. 5.42% of the women had a protein intake <0.8 g/kg/day, 49.36% between 0.8–1.5 g/kg/day and 45.22% >1.5 g/kg/day. The amplitude-dependent speed of sound was significantly and negatively correlated with age, weight, body mass index, and protein intake (P = 0.0009 to <0.0001), and positively correlated with height, and calcium/phosphorus and calcium/protein ratios (P = 0.04 to $P < 0.0001$). Amplitude-dependent speed of sound differed with protein intake < or >125g/day, and was greater in the group with <125 g/day ($P < 0.0001$). We conclude that protein intake needs to be adjusted to <125 g/day to minimize bone loss in women with high protein intake and to reduce the risk of fracture.

P530-Mo

Isocaloric Low-protein Diet has a Major Negative Impact on Bone Mass and Microarchitecture of the Mandibular Aalveolar Bone in the Rat

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Isocaloric protein undernutrition has deleterious effects on bone, muscle mass, and the somatotrop and gonadotrop axis. These alterations resemble some clinical manifestations of anorexia nervosa, even though the latter is characterized by low energy intake as well. To address the issue of mandibular bone quantitative and qualitative characteristics under protein under-nutrition, we investigated the micro-architecture and density of the alveolar bone of the mandible in a rat model. Isocaloric low-protein diet is known to cause estrogen deficiency after approximately 6 weeks. In order to differentiate between the effects of undernutrition and estrogen deprivation, we also studied ovariectomized animals. Forty-four 6-month-old female Sprague–Dawley rats underwent transabdominal ovariectomy (OVX) (n = 22) or sham operation (SHAM) (n = 22), and pair-fed isocaloric diets containing either 15% or 2.5% casein (SHAM 15% [n = 11], SHAM 2.5% [n = 11], OVX 15% [n = 11], and OVX 2.5% [n = 11]) for 16 weeks. At the end of the experiment, the animals were sacrificed and their left hemi-mandible was excised. Bone mineral density (BMD) and bone microstructure parameters of the alveolar process were measured using dual-energy X-ray absorptiometry (DXA) and micro-computed tomography (micro-CT). The alveolar process height was also measured. Protein undernutrition led to significant reduction of BMD of the molar alveolar process ($P < 0.05$), decrease of bone volume fraction (volumetric density) ($P < 0.01$), and of both trabecular ($P < 0.05$) and cortical thickness ($P < 0.001$). The height of the alveolar process was not influenced. Ovariectomy reduced significantly only the trabecular number ($P < 0.05$). Based on the findings of the present study, it seems that protein undernutrition has a strong negative impact on bone mass and microarchitecture of the mandibular alveolar bone, possibly in an estrogen-independent manner.

P531-Tu

Evaluation of Sex Steroids, Bone Turnover Markers, and Bone Mineral Density in Saudi Men with Osteoporosis

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Objectives: To evaluate the relationship between sex steroids, intact-PTH, bone turnover markers, and bone mineral density (BMD) in Saudi men with osteoporosis as compared with healthy age-matched controls.
Subjects and methods: A prospective study was conducted on randomly selected Saudi men (age > 50 years) with osteoporosis (n = 116), and compared with age-matched healthy controls (n = 131), living in the Jeddah area. Serum levels of sex steroid hormones total T, bio-T, total E2, bio-E2, free T, Androstenedione (A), and SHBG were determined, together with bone formation: sOC, sBAP, and sPICP, and resorption: sCTX, uCTX, uNTX, and uDPYR markers. Serum intact-PTH and minerals (Ca, PO_4, and Mg) were also measured. BMD of the spine (L2–L4) and femur were determined by DXA and men were classified with osteoporosis according to WHO criteria. ANOVA was used to examine the differences among various groups. Correlations were carried out using multiple linear regression analysis.
Results: Men with osteoporosis showed significantly lower BMD values than that obtained for age-matched healthy controls ($P < 0.000$). Sex steroid hormones were decreased in men with osteoporosis as compared with controls with the most significant changes obtained for bio-T ($P < 0.005$), A ($P < 0.000$), total E2 ($P < 0.000$), and bio-E2 ($P < 0.000$). Bone formation and resorption markers exhibited significant increases in men with osteoporosis as compared with controls. Men with the lower bio-E2 quartile values showed significantly high levels of biochemical bone markers as compared with the corresponding men of the highest quartile of bio-E2. BMD values of the spine (L2–L4) and neck femur were significantly lower in men of the lowest bio-E2 quartile as compared with those obtained in the corresponding highest quartile of bio-E2. Multiple regression analysis showed that serum bio-E2 contributed significantly to

explain the variations in biochemical bone markers studied. Similarly, age and BMI explained some of the variations in biochemical bone markers studied.

Conclusions: Significant changes were observed in bone turnover markers and BMD in patients with osteoporosis and related to changes in bio-E2. Multiple regression analysis showed that serum bio-E2 contributed significantly to explain the variations in biochemical bone markers studied.

P532-Su

In Men with Vertebral Fracture Cancellous Bone is More Affected than Cortical Bone but is not Associated with Increased Bone Breakdown

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Men with idiopathic vertebral osteoporosis (IVO) are shorter, lighter, have low spine and femoral BMD, and less lean body mass (LBM). IVO is of uncertain cause. Vertebral bone is predominantly cancellous and can be lost in states of increased turnover. Increased bone breakdown has been reported, but this is probably an anomaly because creatinine-corrected urine-based markers are artificially elevated due to lower creatinine excretion. Hormone results remain controversial but SHBG is generally raised. We have investigated (1) bone breakdown in IVO by measuring a serum marker \betaCTX, thus removing the need of creatinine correction for urine volume and the influence of LBM; (2) IGF-I, IGFBP-3, and also SHBG to investigate whether IGF is associated with LBM in these patients; and (3) bone mineral content (BMC) in cancellous (spinal) and cortical (lower limb) bone compartments is affected equally in these patients. We measured whole body BMD (wb-BMD), BMC and lean mass (wb-BMC, LBM), and BMC in the lumbar spine (LS-BMC) and in the lower left limb (LEG) by Hologic QDR4500 and serum \betaCTX, IGF-I, IGFBP-3 by immunoassay in 47 patients with low-trauma vertebral fracture (60 ± 10, 40–79 years) and 151 healthy men (58 ± 10, 40–78 years). Groups were compared by ANOVA. Our male patients with IVO are shorter, lighter, and have lower wb-BMD than controls. Wb-BMC and LBM are lower. Serum \betaCTX is not significantly different and does not correlate with BMC. IVO is not associated with either IGF-I or IGFBP-3, but IGFBP-3 correlates weakly with LBM ($r^2 = 0.29$, $P = 0.001$). SHBG is elevated and shows weak inverse correlations with both IGF-components in both groups ($P < 0.05$). BMC in both cancellous (LS) and cortical (LEG) bone compartments is also lower with a small but significant reduction in the spine/leg ratio (0.10 ± 0.01 vs. 0.11 ± 0.01 $P < 0.01$). Serum-based markers show no evidence of enhanced bone collagen breakdown in IVO at their time of referral. Whole body BMC and BMD are lower in men with IVO, who tend to have a greater relative reduction in cancellous bone.

Table

	IVO	Controls
Height (m)	1.71 ± 0.08*	1.75 ± 0.06
Weight (kg)	74.0 ± 11**	81.8 ± 11
wb-BMD (g cm^2)	1.061 ± 0.11**	1.191 ± 0.10
wb-LBM (kg)	54.6 ± 6.9**	59.9 ± 6.8
wb-BMC (kg)	2.2 ± 0.4**	2.7 ± 0.4
LS-BMC (g)	45.3 ± 10.2**	60.1 ± 12.5
LEG-BMC (g)	453.1 ± 72**	546.7 ± 83
SHBG (nmol/l)	55.5 ± 35*	42.0 ± 20

*$P < 0.01$; **$P < 0.001$.

P533-Mo

Large Population-based Geographic Variations in DXA BMD in Men and Women Across Europe. Results From the Network for Male Osteoporosis (NEMO) Study

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Patterns of BMD change with age and between-population variations have been mostly studied in women, but little is known about men. As part of the Network for Male Osteoporosis (NEMO) study, we analyzed BMD data from population-based sample of 5139 men and 6601 women aged 19–95 years (mean = 60 years, SD = 14) from 39 centers across Europe to assess between center variations in mean BMD levels; effects of age, weight, height; and between-gender contrasts. BMD was measured at the femoral neck, trochanter, and/or L2–L4 spine using DXA densitometers manufactured by Hologic ($n = 16$), Lunar ($n = 19$), Norland ($n = 4$), and Sopha ($n = 1$). Densitometers were cross-calibrated with the European Spine Phantom. Analysis was by linear regression and polynomial terms in age, weight, and height were tested to assess curvature of associations. Differences in effects by center were evaluated by testing for interactions with center.

There were highly significant between-center differences in mean BMD levels in both genders for the 3 regions ($P < 0.0001$), except female spine. The difference between the highest and lowest BMD center in men ranged from 1.3 population SDs at the femoral neck to 1.6 SDs at the spine. The differences were larger in women ranging from 1.9 to 2.8 SDs, respectively. In men, femoral neck and trochanter BMD ($n = 4724$) were best described by a 3-degree (femoral neck) and 4-degree (trochanter) polynomial in age ($P = 0.001$), 2-degree polynomial in weight ($P < 0.0001$), and a negative linear effect of height ($P < 0.037$). None of these effects differed significantly by center ($P > 0.05$) and they explained 28% and 21% of the total variance in femoral neck and trochanter BMD. Spine BMD ($n = 3773$) was best

described by a 2-degree polynomial in age and weight ($P < 0.001$), with the linear term for age significantly differing by center ($P = 0.0004$) and 19% of the total variance was explained. In women, femoral neck and trochanter BMD ($n = 6195$) were best described by 4-degree polynomial in age, 2-degree polynomial in weight, and a negative linear effect for height (trochanter). The effect of age and weight significantly differed by center ($P < 0.009$) and explained 42% and 36% of the variance in femoral neck and trochanter BMD, respectively. Spine BMD in women ($n = 4936$) was described by 3-degree polynomial in age and linear effects for weight and height, explaining 17% of the variance. In conclusion, there is considerable geographic variation across Europe in BMD and effects of age and weight varied by center in women.

P534-Tu

Comparison of DEXA, pQCT, and Quantitative USG in Assessment of Interval Bone Mineral Density Changes in Adolescent Dancers and Non-dancers

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Objective: To compare the interval bone mineral density (BMD) changes of the axial and appendicular skeleton in a group of collegiate dance students undergoing intensive training and a group of non-exercising adolescent females over a 20-month interval using standard dual energy X-ray absorptiometry (DEXA) of the lumbar spine and hip, quantitative peripheral CT scans (pQCT) of the distal radius and tibia, and quantitative USG (QUS) of the os calcis.
Methods: Twenty-six full time collegiate dance students were recruited from a tertiary Performing Arts Institute. All were healthy. All subjects had basic anthropometric measurements, a full hormonal profile, pelvic ultrasound, bio-impedance estimation of body fat, and DEXA, pQCT, and QUS to determine bone density. The measurements were then repeated 20 months later. The same interval measurements were made in a group of 14 non-exercising adolescent females of comparable age. The interval changes measurable by each of these methods were compared.
Results: The mean age of the dancers (18.63 years) and controls (18.6 years) was similar. The dancers had lower BMI (18.2 kg/cm^2 vs. 19.2, $P = 0.037$) and body fat percentage (19.1% vs. 23.6%, $P = 0.001$) compared to controls. There was no significant difference in the basic anthropometric or initial BMD measurements as measured by DEXA, pQCT, or QUS between the two groups. Comparing the interval changes at the 20-month reassessment, all three modes of BMD assessment showed positive increments in both dancers and controls. DEXA showed that the dancers had significantly larger interval increments in lumbar spine BMD ($P = 0.006$) as well hip BMD (neck of femur, $P = 0.004$; Ward's triangle, $P = 0.025$) compared to controls. QUS similarly showed a larger interval increment in the dancers (soundness 18.1 vs. 6.99, $P = 0.033$), but pQCT was unable to demonstrate any difference in increments between the two groups.
Conclusion: While both dancers and controls showed positive BMD increments over the observed 20-month period, dancers undergoing regular intensive weight-bearing exercises have higher BMD increments compared to non-exercising females. Such differential increments were evident on DEXA of the axial skeleton, as well as quantitative USG measurement of the os calcis, but could not be discerned from pQCT measurements of the appendicular skeleton. Further evaluation is needed to distinguish whether such observations could be due to site-specific BMD changes in response to exercise.

P535-Su

Development of a Cost-effective Small-scale Loading Machine for in Vivo, ex Vivo, and in Vitro Osteoporosis Research

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Mechanical testing machines are commonplace in mechanical research and thus are routinely found in biomechanical testing facilities. Those not having access to such equipment or the experience to fabricate a single purpose system must justify the expense of the commercial machine with limited use. To remedy this, we set out to build a loading machine that was ideal for small scale testing such as in vitro cell stimulation, ex vivo organ culture stimulation, and in vivo limb loading, as well as routine biomechanical testing such as material and structural property determination. Here we describe the development of the cost-effective (under $10,000.00) system and illustrate the flexibility of the system in a variety of research studies related to osteoporosis. The loading system allows motor-driven, bi-directional vertical movement. Planar, manual movement is provided by a milling machine table with a 100 mm $\times$ 150 mm travel and a resolution of 0.0254 mm. The table is affixed to an aluminum base plate that allows for vertical mounting of a linear slide capable of 100 mm of travel. The slide is driven by a servo motor and controller with proximity and home switches. Data acquisition is enabled by a scanner that interfaces with load cells (0.0625–22.5N), the linear displacement sensor, and strain gages. Accuracy of both the load cell and displacement sensor was 0.9999. Once assembled and calibrated, the loading machine was utilized in a variety of small-scale studies including structural and material property determination of transgenic mouse long limbs; in vitro stimulation of osteoblastic cells

via three-point bending of substrate with morphological analysis using AFM, quantification of gap junctional intercellular communication, prostaglandin, osteoprotegerin, and RANKL protein; and ex vivo rat and in vivo mouse long bone stimulation studies. The performance of our system in these studies verifies that we were successful in our attempt to develop a portable, cost-effective, user-friendly, flexible small-scale loading machine that is ideal for osteoporosis research. Since biomedical research is a multidisciplinary area in which scientists from biology, engineering, and medicine bring their respective expertise together to further the advancement of medical science and discovery, one goal of research should be to make the technology of the respective professions accessible to all researchers.

P536-Mo

Association of Amount of Physical Activity with Cortical Bone Size and Trabecular Volumetric Bone Mineral Density in Young Adult Men—The Good Study

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Background: Physical activity (PA) is believed to have positive effects on the skeleton and possibly help in preventing the occurrence of osteoporosis. Neither the lowest effective amount of PA needed to induce an osteogenic response nor its effect on bone density and size of the different bone compartments, i.e., trabecular and cortical bone, has yet been clarified.
Methods: In the present population-based study, we investigated the amount of all types of PA in relation to areal bone mineral density (aBMD), trabecular and cortical volumetric BMD (vBMD), and cortical bone size, in 1068 men, 18 to 19 years old, included in the Gothenburg Osteoporosis and Obesity Determinants (GOOD) study. aBMD was measured by dual X-ray absorptiometry, while cortical and trabecular vBMD and bone size were measured by peripheral quantitative computerized tomography.
Results: The amount of PA was associated with aBMD of the total body, radius, femoral neck, and lumbar spine, as well as with the cortical bone size (increased thickness and periosteal circumference) and trabecular vBMD, but not with cortical vBMD, or length of the long bones. The lowest effective amount of PA was 4 h or more per week. aBMD, cortical bone size, and trabecular vBMD were higher in subjects who started their training before age 13 than in subjects who started their training later in life.
Conclusions: Our data indicate that 4 h or more per week of PA is required to increase bone mass in young men and that exercise prior to and during the pubertal growth is of importance. If these findings in young men can be transferred to the elderly population, this knowledge could help in outlining guidelines to the general public in order to optimize bone health.

P537-Tu

Regional Changes in Body Composition 3 Months to 30 Years After Traumatic Spinal Cord Injury (SCI): Results of a Cross-sectional Study in 100 Paraplegic Men

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Background: It has been shown earlier that paraplegia induces severe and sustained infralesional cortical and trabecular bone loss (Osteoporosis Int 2004; 15:180–9). However, little is known on the long-term changes in body composition (lean mass, fat mass, and bone mass) at the supra- and infralesional levels.
Aim: To document the long-term supralesional (arms) and infralesional (legs) changes in body composition with time since SCI in paraplegic men.
Patients and methods: One hundred paraplegic men (age 18–60 years) with complete motor posttraumatic medullary lesion T1-L3 and total motor and total or partial sensory loss (Frankel stage A or B) since 3 months to 30 years were included in this cross-sectional study. Body composition was assessed on whole body scans performed by DXA (Hologic QDR 4500A™). Total mass (TM), lean mass (LM), fat mass (FM), and bone mass (BM) of arms and legs were determined and expressed as grams. In addition, the ratios of LM/TM, FM/TM, and BM/TM were calculated in percent. The influence of time since SCI on these parameters was then tested using logarithmic regression analysis.
Results: Means ± SD of the relative distribution of tissues in arms and legs are shown in the table. With time since SCI, TM, LM, FM, and BM of the arms increased (r^2 = 0.3, $P < 0.0001$; $r^2 = 0.2$, $P < 0.0001$; $r^2 = 0.1$, $P < 0.01$; and $r^2 = 0.1$, $P < 0.01$, respectively). A non-significant trend towards a decrease in LM/TM was accompanied by a non-significant trend towards an increase in FM/TM with time since SCI (both $P < 0.1$), while BM/TM was significantly reduced ($r^2 = 0.15$, $P < 0.0001$). In the legs, TM and LM did not change significantly with time since SCI, while FM increased ($r^2 = 0.1$, $P < 0.05$) and BM dramatically decreased ($r^2 = 0.4$, $P < 0.0001$). Furthermore, with time since SCI, LM/TM, and BM/TM decreased (r^2 = 0.1, $P < 0.05$, and $r^2 = 0.5$, $P < 0.0001$, respectively), whereas FM/TM increased ($r^2 = 0.1$, $P < 0.01$).
Conclusion: In paraplegic men, 3 months up to 30 years post SCI, total, lean, fat, and bone mass increase with time since SCI in the arms, possibly as a result of intensive physical training and use of the arms. In the legs, total mass

remains unchanged, bone mass is lost extensively, while lean mass is partly substituted by fat mass.

Table

	Supralesional (arms)	Infralesional (legs)
LM/TM (%)	78.9 ± 6.5	64.6 ± 10.2
FM/TM (%)	16.5 ± 6.8	32.3 ± 10.6
BM/TM (%)	4.6 ± 0.6	3.1 ± 0.8

P538-Su

Long Periods of Disuse Rescue the Osteogenic Response to Mechanical Loading that is Suppressed by Aging

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It is recognized that blunting of the osteogenic responses to load bearing in the aged underpins the decline in bone's fracture resistance. Many studies showing that the mechanical threshold for load-induced bone formation does indeed increase with age. Using a new tibial loading model, we recently found that sciatic denervation (SN) in mice increases the new cortical bone formation in response to loading. This raises the intriguing possibility that the blunted capacity for load-induced osteogenesis in aged bones may also be rescued by such treatment. In this study, we have therefore examined aged bone's response to defined load-engendered mechanical strains with and without a prolonged period of disuse, imposed by SN. Accordingly, female C57Bl/J6 mice were assigned into 2 groups. Right limbs of 18-month-old mice in one group (n = 5) were loaded (13 N) to engender peak strains of 2000 mE on the lateral midshaft of the tibia; left limbs were used as controls. A second group (n = 5) was submitted to right limb SN 100 days before they were 18 months old, kept for 100 days, and then loaded after calibration (7 N) to generate identical peak strains (2000 mE) on the tibiae; contra lateral tibiae used as controls. All animals were loaded on alternate days for 3 weeks, received double fluorochrome labels, and killed 3 days later. Confocal images of transverse sections from 3 defined diaphyseal sites of the tibiae were analyzed. Periosteal enclosed bone area (PEB), endosteal area (EA), and new bone formation (NBF) were measured. We found that aged tibiae failed to produce any increases in NBF in response to peak strains of 2000 mE; EA and PEB areas in these loaded tibiae were also similar to their contra lateral controls. In contrast, we found that disuse (SN) produced marked decreases in PEB area (P = 0.008) and increases in EA. Loading to identical peak strain levels of SN tibiae surprisingly produced significant new bone formation (P = 0.001) and reversed the loss in PEB area. Current studies are aimed at addressing whether this effect is also evident in trabecular bone. Our findings support the notion that aged bones in a normal functional situation exhibit a reduced response to loading. This capacity to respond can, however, be rescued by the imposition of periods of disuse. Establishing the basis of this disuse-related re-sensitization to lower loads, but similar peak strain magnitudes, would allow the decline in fracture resistance associated with ageing to be reversed.

P539-Mo

Influence of Spasticity on Body Composition of Paraplegic Men After Traumatic Spinal Cord Injury (SCI): Results of a Cross-sectional Study in 100 Paraplegic Men

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Background: After SCI, demineralization at infralesional sites occurs. It has been hypothesized by some authors that spasticity might be a protective factor of immobilization osteoporosis. However, little is known about the effects of spasticity on lean, fat, and bone mass in paraplegics.

Patients and methods: 100 paraplegic men with complete motor posttraumatic medullary lesion T1-L3 and total motor and total or partial sensory loss (Frankel A or B) since 3 months to 30 years were included in this study. Patients were allocated to one of two groups depending on the presence or absence of spasticity. Body composition was assessed using DXA (Hologic QDR 4500A™). Total mass (TM), lean mass (LM), fat mass (FM), and bone mass (BM) of legs were determined. Additionally, ratios of BM/TM, LM/TM, and FM/TM were calculated. These parameters were then correlated with time since SCI for the two groups separately using logarithmic regression analysis.

Results: 97 patients had a complete dataset for TM, LM, FM, and BM, and were included in the analysis. 45 patients were spastic and 52 were not (table). Mean TM and BM of the legs were not significantly different between spastic and non-spastic paraplegics. However, spastic paraplegics had a higher LM and a lower FM of the legs compared with non-spastic patients. Over time since SCI, TM of the legs did not change significantly in either group. Whereas in the non-spastic group, LM/TM significantly decreased, and FM/TM significantly increased, these ratios did not change significantly in the spastic group. However, both groups showed a similar significant logarithmic decrease of BM with time of paraplegia.

Conclusion: In paraplegic men up to 30 years post-SCI, the presence of spasticity was not associated with higher bone mass of the legs. Although LM of the legs was better preserved over time since injury in spastic patients, infrale-

sional bone was lost independently of muscle tone in the entire population of paraplegics.

Table

Means ± SD	Spastic (n = 45)	Non-spastic (n = 52)	P
Age (years)	37.3 ± 9.5	38.1 ± 9.8	0.7
Height (cm)	177.7 ± 7.2	176.8 ± 8.3	0.6
Weight (kg)	69.9 ± 8.6	72.3 ± 12.3	0.3
Time since SCI (months)	139.3 ± 99.6	113.9 ± 92.3	0.2
TM (g)	30,314 ± 4037	30,203 ± 5726	0.9
LM (g)	21,050 ± 3046	18,047 ± 4248	<0.001
FM (g)	8318 ± 2313	11,240 ± 4876	<0.001
BM (g)	946 ± 284	916 ± 244	0.6

P540-Tu

Relationships Between Body Weight and Vertebral Bone Architecture in Primates that Exhibit a 48-fold Range in Body Weight

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Species of larger body weight must modify their behavior, adapt their skeleton to support the load, or risk fracture, an event that may severely impact the animal's Darwinian fitness. To better understand the structural origins of vertebral fragility and gain insight into the mechanisms that govern adaptation of a bone to load, we investigated the relationship between lumbar vertebral body bone structure and body weight (BW) in 30 lumbar vertebrae from ten closely related primate species. Species ranged in BW from 42 g (*Microcebus rufus*) to 2050 g (*Eulemur fulvus*). Intact last lumbar vertebrae were scanned with micro-CT and all parameters quantified in 3D. Regressions between BW and architecture were compared to predictions we developed for the geometric scaling of trabecular bone architecture in lumbar vertebrae, assuming axial compression as the dominant loading regime (Fajardo, 2004). The bone volume fraction and degree of anisotropy were independent of BW. Regression slopes between the remaining cancellous bone features and BW were consistent with predictions for geometric similarity. Trabecular thickness increased (slope = 0.33, $P < 0.0005$, $r^2 = 0.88$) whereas trabecular number (slope = −0.28, $P < 0.013$, $r^2 = 0.66$) decreased with increasing BW. The thickness and volume of the anterior vertebral cortex increased isometrically with BW ($r^2 = 0.68$, $P = 0.003$ and $r^2 = 0.71$, $P = 0.02$). The cross-sectional area of the vertebral body positively correlated with BW, with the slope approximating a value nearly greater than isometry (slope = 0.90, $P = 0.005$, $r^2 = 0.65$). In summary, this cross-species analysis of closely related primates shows that cancellous bone structural variables are either unchanging with BW or maintaining geometric similarity. These results imply either that lumbar vertebral cancellous bone in larger species is under-built for its BW or that in smaller species it is over-built. Clearly, the vertebral body functions as a composite structure with a combination of cancellous and cortical bone providing structural competence. Our findings imply that the vertebral body cancellous bone does not adapt to the greater load incurred by increasing body weight and therefore suggest that it serves primarily to distribute the load to the cortical shell.

P541-Su

Effect of Long-term Administration of Antiepileptic Drugs on Bone Health Parameters in Outpatient Population: High Prevalence of Osteopenia and Vitamin D Deficiency

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Antiepileptic drug (AED) use is identified as being associated with increased risk of osteopenia, osteoporosis (Op), and vitamin D deficiency (VDD).

Objective: To assess the prevalence of osteopenia, Op, and VDD in patients (pts) receiving long-term AED.

Materials and methods: We prospectively studied 40 patients receiving AED on outpatient basis from more than 1 year. Bone mineral density (BMD) was measured by DXA Hologic QDR 4500 while serum 25 vitamin D (VD) was measured by RIA. One patient was excluded from study. None of these patients were on calcium/VD supplementation in the last 1 year.

Results: Of 39 patients, mean age was 26.5 + 10.9 years (19 females, 20 males). Mean duration of AED was 35.08 + 21 months. 18 patients were on carbamazepine, 14 on phenytoin or phenytoin and phenobarbitone combination, while 7 were on sodium valproate. VDD (25 VD level <15 ng/ml) was found in 82% patients while severe VDD (VD <5 ng/ml) was present in 41% patients. Clinical evaluation showed 42.3% patients had body ache, 48.8% had back pain, 52.9% had easy fatigability, and 8.3% had proximal muscle weakness. Mean BMD at spine 0.855 + 0.17 g/cm^2 and at hip 0.815 + 0.12, which was significantly lower than normal controls ($P < 0.05$). Op was present in 31.5% at spine and 7.1% at hip, while osteopenia was seen in 36.8% at spine and 57.1% at hip. 31.5% at spine and 35.7% at hip had normal BMD. Mean T score at spine was −1.9, hip −1.3, and forearm was −2.5, while Z scores were −1.5 at spine, −1.1 at hip, and −1.7at forearm. No significant difference in BMD and VDD was observed among different AED groups.

Conclusion: There is a high prevalence of osteopenia and VDD in patients receiving long-term AED. There was no significant difference observed among different AED. This may be because of the small number of patients included in study. All those patients who are on long-term AED should be screened for Op and VDD. It would

be more appropriate if these measures are taken at the start of AED.

P543-Tu

Reduced Osteocyte Density at the Iliac Crest in Fragility Hip Fracture Patients

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Increased bone fragility may be associated with an accumulation of microdamage due to impaired damage repair mechanisms. Microdamage is repaired by stimulating a targeted bone remodeling response and has been shown to induce osteocyte apoptosis, which may provide an important local signal to remodel a damaged area of bone. Osteocyte deficiency may lead to impaired function of the osteocytic network to detect microdamage for repair. This study examined osteocyte density in trabecular bone from the iliac crest of patients with a fragility hip fracture (Fx) compared to age-matched controls (C). Iliac crest biopsies were obtained from patients undergoing hip arthroplasty surgery for a fractured neck of femur (5 females, 4 males, aged 79 ± 10 years [mean ± SD]) and from non-fracture controls at autopsy (3 females, 3 males, aged 72 ± 11 years). Undecalcified bone tissue blocks were processed into resin. Three sections of 5-μm thickness, cut at 200-μm intervals, were decalcified in 10% EDTA and stained with H and E. The following parameters were measured: number of osteocytes per bone mineral area (N.Ot/B.Ar [#/mm^2]), total lacunae per bone mineral area (N.Lc/B.Ar [#/mm^2]), percentage of empty lacunae (%.empty.Lc), bone volume fraction (BV/TV), specific surface of bone (BS/BV), and trabecular thickness (Tb.Th). The fracture group had significantly reduced N.Ot/B.Ar (Fx: 132.8 ± 62.9, C: 301.6 ± 51.1, $P < 0.0001$) and N.Lc/B.Ar (Fx: 213.0 ± 48.9, C: 374.7 ± 48.5, $P < 0.0001$). Conversely, %.empty.Lc was significantly higher for the fracture cases (Fx: 40.7 ± 18.9, C: 19.8 ± 7.0, $P < 0.03$). The trabecular bone architectural parameters, BV/TV, BS/BV, and Tb.Th, were not different between the fracture and control groups. The reduction in osteocyte density and total lacunar density at the iliac crest in hip fracture patients is consistent with iliac crest data reported for vertebral fracture patients [1]. Greater variance in femoral head osteocyte viability has been reported for hip fracture patients; 1 in 4 patients had less than 25% osteocyte viability [2]. Our data suggest that the reduced number of osteocytes and increased percentage of empty lacunae in the iliac crest of hip fracture patients may be due to a significant loss of osteocyte viability. Furthermore, osteocyte deficiency, with no bone architectural change, suggests that bone from hip fracture patients may be mechanically compromised due to a defective repair-sensing osteocytic network.

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P544-Su

Oxidative Stress Induces Bone Loss in Ovariectomized Mice Through Induction of Bone Marrow Restricted Activation of Dendritic Cells and T Cell Activation

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In physiologic and stimulated conditions, osteoclast (OC) formation occurs in the bone marrow (BM) but not in the spleen or lymph nodes despite the presence in all lymphoid organs of the necessary precursors and cytokines, but the mechanism of this phenomenon is unknown. We have previously reported that ovx stimulates OC formation and induces bone loss by increasing T cell activation and T cell TNF production through upregulation of Ag presentation by macrophages. In this study, we investigated the hypothesis that a greater stimulation of Ag presentation and thus of T cell TNF production may specifically occur in the BM. Professional Ag presenting cells include B cells, macrophages, and dendritic cells (DC). We found that ovx does not increase Ag presentation by B cells. However, ovx increased by ~4-fold antigen presentation by BM DC, while it had no effect on Ag presentation by splenic DC. Furthermore, ovx was found to increase DC MHCII expression by 70% and the percentage of CD4+ T cells expressing the early activation marker CD25 by 2-fold in the BM but not in the spleen, demonstrating that ovx specifically stimulates DC-mediated, MHCII-restricted, Ag presentation and T cell activation in the BM. In vitro co-culture experiments showed that BM DC from ovx mice increased T cell production of TNF by 2-fold, while those from both sham mice and splenic DC from sham and ovx mice did not. Thus, a selective activation of DC explains how ovx specifically upregulates TNF production in the BM. An increase in reactive oxygen species (ROS) in the BM has been reported to stimulate TNF production leading to OC formation and bone loss following ovx. Since redox state is a powerful regulator of immune cells function, we asked whether BM-specific DC activation is induced by ovx through an oxidative stress-dependent mechanism. In vivo treatment with the anti-oxidant N-Acetyl cysteine (NAC) abrogated the ovx-induced increase in Ag presentation by BM DC and the increased expression of MHCII. Furthermore, NAC significantly decreased ovx-induced bone loss. In summary, the data show that ovx is followed by a BM-specific DC activation that leads to increased Ag presentation, T cell activation, and T cell TNF production. The data demonstrate that DC represents a novel estrogen target and that

ROS modulation of DC function may play a pivotal role in the mechanism of ovx-induced bone loss.

P545-Mo

Gender Differences in Serum Leptin and Ghrelin: Relationship to Body Composition and Bone Mineral Measures—A Cross-sectional Opposite Sex Twin Study

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Background: In recent studies, much attention has been paid to the plasma peptide leptin (and to a lesser extent ghrelin) and its relationship to bone and body composition measures. The results of these studies are contradictory.
Aims: The aims of the present study were to use the opposite sex twin model to determine if there were gender differences in the relationship between serum levels of leptin and ghrelin and body composition/bone mineral measures and how any such differences were influenced by age.
Methods: Seventy-nine pairs of opposite sex twins participated in the study. We measured plasma levels of leptin and ghrelin using standard kits. Body composition and bone mineral measures were obtained by dual energy X-ray absorptiometry. To examine the effect of age, the twins were divided into two age groups: under 50 years old (38 pairs) and over 50 years old (41 pairs).
Results: There were significant gender differences in leptin levels in all age groups (15.6 ± 19.5 in men vs. 32.1 ± 28.1 in women, $P < 0.001$). Ghrelin levels differed between males and females only in the older age group (1109 ± 404 in men vs. 1210 ± 424 in women, $P < 0.001$). Leptin was positively associated with fat mass ($r = 0.46$, $P < 0.01$ for men and $r = 0.42$, $P < 0.01$ for women under 50 age and $r = 0.73$, $P < 001$ and $r = 0.57$, $P < 0.001$ for men and women over 50, respectively) and its distribution measures in both genders of all age groups. We did not find associations between serum leptin levels and lean mass or any of the bone measures in ether gender at any age group. In the older age group, ghrelin was negatively associated with fat mass measures in both genders and tot fat mass/total lean mass ratio in women ($r = -0.44$, $P < 0.01$). Hip BMD was the only bone measure significantly associated with ghrelin levels in females under 50 age group ($r = -0.39$, $P < 0.01$). Ghrelin did not show significant associations with any of the body composition parameters in ether gender under age 50.
Conclusion: There are gender differences in leptin and ghrelin levels in different age groups. The relationships between leptin and ghrelin levels and body composition measures are similar in both genders of studied age groups. Leptin was not significantly associated with any of the bone measures in ether gender at any age. Ghrelin was negatively associated with hip BMD in women under 50.

P546-Tu

Bone Mineral Density and Functional Electrical Stimulation in Acute Spinal Cord Injury

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Aim of the study: A pilot study was carried out to examine the effects of isometric electrical stimulation on bone mineral density (BMD) in the acute phase in patients who had suffered a trauma-induced spinal cord injury paraplegia.
Methods: 6 men and 1 woman, aged between 17 and 50, were recruited into the study, 5 subjects within 4 weeks of their spinal cord injury and 2 subjects within 8 weeks of their injury. Four subjects were treated with isometric electrical stimulation to both legs for 1 h, 5 times per week (STIM); and three subjects acted as controls (CTRL). BMD measurements of the lumbar spine (L2–L4), femoral neck, and trochanter were made at 8, 12, and 16 weeks post-injury. Measurements of serum parathyroid hormone (PTH) and osteocalcin (OC), and urine deoxypyridinoline (DPYR), were made at the same time points.
Results: BMD fell during the observation period in the spine, femoral neck, and trochanter, and the magnitude of the fall at each site was similar in both the STIM and CTRL groups. When the values for the whole group were combined, the BMD in the spine, femoral neck, and trochanter decreased by 2%, 8%, and 10%, respectively, during the observation period. PTH (measured in 5 subjects) showed low or undetectable values when measured at 8 weeks. The values remained low in 4 of the 5 subjects at 12 weeks. Interestingly, there was an increase in OC values in 4 out of 5 subjects between 8 and 12 weeks. DPYR was elevated at 8 weeks (in 5 subjects that were measured), indicating that there is evidence of increased bone resorption at this early acute post-spinal cord injury phase.
Conclusion: Our observations have shown that isometric electrical stimulation did not confer any benefit on BMD when applied during the acute phase of the spinal cord injury (i.e., from 4 weeks post-injury).

P547-Su

Primary Amenorrhea and Bone Turnover

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A 21-year-old adult female (height: 173 cm, weight: 58 kg) with normal secondary characteristics was presented with intense joint and muscle aches in the upper and lower limbs bilaterally, and low bone mineral density in the lumbar spine.

Her family history and her physical examination are unremarkable. Her medical history starts at the age of 15 years, because of primary amenorrhea combined with generalized weakness, undue fatigue, and anxiety. At that time, biochemical results showed elevated gonadotrophins (FSH, and LH), low estradiol levels, normal thyroid function, and prolactin levels. Adrenal function was normal. Ultrasonography revealed normal uterus. The cytogenetic examination showed normal female karyotype (46,XX) without numerical and structural chromosomal abnormalities. The diagnosis of hypergonadotrophic amenorrhea with normal karyotype was proposed at this point, and the patient received oral contraceptives.

The first determination of bone mineral density (at age of 20 years) in the region of lumbar spine revealed osteopenia (DPXL: L2–L4: 1.013 g/cm^2, Z score: −1.9, age matched: 82%). The treatment was the same during this 5-year time interval, with the addition of calcium and vitamin D supplements only for the last year.

At present, the clinical condition changes with intense and constant pains in all joints of upper and lower limbs and tension headaches. The new laboratory findings unfolded increased bone turnover with elevated levels of bone resorption markers in urine (Pyrillinks-D, CTx, NTx). Subsequent densitometric evaluation of bone mass showed a slight increase of 2% in areal BMD of spine (DPXL: L2–L4: 1.024 g/cm^2, Z score: −1.7, age matched: 84%), and osteopenia of the same magnitude in both hips (DPXL: neck right: 0.833 g/cm^2, Z score: −1.3 neck left: 0.817 g/cm^2, Z score: −1.5).

Finally, an additional cytogenetic examination with the application of fluorescence in situ hybridization (FISH) pointed out the existence of a specific Y-linked genomic region namely the SRY gene (Sex determining region of the Y chromosome) in 5% of mesophasic nuclei.

This case highlights the complexity of the pathophysiology of the hormonal and bone metabolism and physicians should be aware of the particular cause of bone turnover imbalance.

P548-Mo

Calcitonin Influences Bone Blood Flow in Rats

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CALCITONIN INFLUENCE BONE BLOOD FLOW (BBF).

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Cessation of estrogen production in a biological system causes enhanced bone remodelation and simultaneously enhanced BBF. The aim of this animal experiment was to answer the question, if administration of calcitonin influence enhances BBF after estrogen depletion.

Materials and methods: Female rats were 4 weeks old (VUFB Konarovice). Oophorectomy was performed by standard approach from the dorsum of animal. Calcitonin (Miacalcic, Novartis, Švýcarsko) 10 IU in 0.2 ml of isotonic saline solution was administered subcutaneously 3 days in a week for a time of 4 weeks.

BBF was determined by use of radioactive, Sr-labeled microspheres (1).

The original value of BBF (ml/min/g bone tissue) can be influenced by minute blood volume. From this reason, we use rather radioactive-Sr-labeled microspheres capture as marker of BBF.

Experiment arrangement: 60 female rats were allotted in 4 groups, 15 animals in each group: (I) Control group (sham operation); (II) Oophorectomized group; (III) Sham operation + calcitonin; (IV) Oophorectomized + calcitonin.

Results: Mean ± SD: Group: I II III IV. BBF in tibia-microsph. capture (%): 0.18 ± 0.01, 0.27 ± 0.02a, 0.23 ± 0.02, 0.22 ± 0.02. BBF in femur-microsph. capture (%): 0.36 ± 0.02, 0.54 ± 0.05a, 0.42 ± 0.04, 0.40 ± 0.04. Osteocalcin (ng/ml): 17.7 ± 1.2, 32.7 ± 1.8a, 15.1 ± 0.8b, 21.9 ± 0.8b,c. a, statistically significant in relation to group I; b, statistically significant in relation to group II; c, statistically significant in relation to group III; all: $P < 0.05$.

Conclusion: Calcitonin, given according the scheme mentioned above, restrict BBF, which is significantly enhanced after oophorectomy.

Reference:

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P549-Tu

Cyclase Activating Parathormone (CAP) and CAP/CIP Ratio are Increased in Elderly Women with Low Bone Mineral Density (BMD) of the Radius

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Introduction: Aging causes an increase of parathormone (PTH) secretion and, subsequently, increased bone resorption, which is a main cause of senile osteoporosis. In last years, two fractions of PTH were discovered, cyclase activating and cyclase inhibiting peptides (CAP and CIP).

It is not known, however, whether CAP and CIP play a role in the trabecular bone resorption and pathogenesis of senile osteoporosis.

Methods: To answer this question, we compared 29 elderly women (>70 years of age) with *T* score of ultradistal radius lower than −2.5 (mean age 74.8 ± 3.4 years, BMI 26.0 ± 3.9 kg/m^2, creatinine clearens 59.9 ± 12.9 ml/min/1.73m^2, serum Ca 2.33 ± 0.25 mmol/l) and 31 women with *T* score higher than 2.5, appropriately matched with regard to age, BMI, and kidney function (mean age 73.6 ± 3.4 years, BMI 26.7 ± 3.8 kg/m^2, creatinine clearens 59.3 ± 20.6 ml/min/1.73m^2, serum Ca 2.4 ± 0.13 mmol/l). Mean BMD of the ultradistal radius was 0.271 ± 0.053 g/cm^2 in the first group and 0.313 ± 0.049 g/cm^2 in the second one ($P < 0.0001$), and median *T* score −3.48 and −1.4, respectively. Patients with secondary osteoporosis and smokers were not included.

In every patient, serum concentrations of intact PTH, CAP, and CIP were assessed, and CAP/CIP ratio was calculated. BMD of the ultradistal radius was assessed using dual energy X-ray absorptiometry (DXA, Lunar). The Mann–Whitney test was used to compare the groups.

Results: Patients with low BMD did not differ from those with higher BMD with regard to serum iPTH (32.1 ± 16.7 vs. 25.8 ± 19.4 pg/ml, $P = 0.066$) and CIP (10.8 ± 6.8 vs. 9.9 ± 7.6 pg/ml) concentrations; however, serum CAP concentration (21.3 ± 10.7 vs. 15.9 ± 12.4 pg/ml, $P < 0.05$) and CAP/CIP ratio (2.19 ± 0.83 vs. 1.73 ± 0.90 pg/ml, $P < 0.05$) were significantly higher in the low BMD group.

Conclusion: Serum CAP concentration and CAP/CIP ratio are higher in elderly women with lower BMD of trabecular bone in ultradistal part of radius.

P550-Su

Evaluation of Bone Mineral Density in Hypogonadic Women

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Osteodensitometry in hypogonadic women with diabetes mellitus (DM) and/or obesity may reveal controversial and interesting data.

Many studies have examined the relationship between the presence of DM and bone density. In general, type II DM (DMII) contrary to type I DM (DMI) has been associated with increased bone mineral density (BMD).

Obesity represents a complex disease with important complications. Among the endocrine complications of obesity hypogonadism affects especially women and may lead to premature development of osteoporosis.

The aim of this study was to assess the correlation hypogonadism–osteoporosis in obese women with DM.

We have also tried to establish if there are possible differences related to the body mass index (BMI) and respectively to body fat distribution.

We have analyzed 71 women, aged 25 to 45 years, with obesity and primary hypogonadism. Depending on the presence of DM, we distributed them in 3 groups: group I, 15 women that associated DMI; group II, 23 women with DMII; and the control group, 33 women without DM.

We included in our study only patients diagnosed with DM for about 5 years and presenting obesity for about 10 years.

We have calculated the BMI (body mass index) and the WHR (waist-to-hip ratio) and we have measured the BMD at the level of lumbar spine and the femoral neck (DXA). All patients have decreased concentrations of estradiol and progesterone and increased concentrations of gonadotropins: FSH and LH.

Most of the patients with class I obesity in all three groups have had osteoporosis and osteopenia (53.9% and 23% in group I, 75% and 25% in group II, and 54.5% and 18.2% in group III). Also in most of the women that associated peripheral obesity and DMI or DMII, we have found decreased BMD, especially osteopenia.

In conclusion, in the obese, hypogonadic women, the presence of DMI may lead to a decrease of BMD, whereas DMII permitted the maintenance of a normal BMD. The abdominal obesity and class II and III obesity had proven to be protective factors against osteoporosis, probably due to a higher extent of androgen aromatization in estrogens in the fat tissue.

P551-Mo

Prevalence and Determinants of Osteoporosis in Adults with Anorexia Nervosa

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Background: Patients with anorexia nervosa are at major risk of osteoporosis and fracture. This could be related to multiple nutritional and endocrine abnormalities, including amenorrhea, and suppressed insulin-like growth factor-I (IGF-I). The relative contribution of these factors and the skeletal site specificity are not well established.

Subjects and methods: In a prospective cohort analysis, we investigated 96 adult patients (88 women and 8 men, mean age 23.8 ± 8.1) with anorexia nervosa. BMD of the anterior–posterior lumbar spine and femoral neck was measured by DXA. The following parameters were determined: anthropometric variables, amenorrhea duration, estrogen use, fracture history, as well as IGF-I and biochemical markers of bone remodeling. Standard multivariate regression model was constructed for each skeletal site by using age, total duration of amenorrhea and of anorexia nervosa, BMI, and IGF-I as covariates.

Results: The prevalence of osteopenia (−1 SD ≥ *T*-score > −2.5 SD) and osteoporosis ($T \leq -2.5$ SD) at one or more skeletal site was 53% and 20%, respectively. The corre-

sponding BMD *Z*-scores were −1.23 ± 1.3 SD at lumbar spine and −1.0 ± 1.1 SD at femoral neck. Six percent had experienced a low trauma fracture. Duration of amenorrhea was a significant predictor of *Z*-score BMD at lumbar spine ($r = -0.01$, $P < 0.0001$) and femoral neck ($r = -0.006$, $P < 0.03$). Serum IGF-I level was a significant independent predictor of femoral neck *Z*-score ($r = 0.006$, $P < 0.000\,1$), but the correlation with spine BMD was only borderline ($P = 0.052$). In contrast to post-menopausal osteoporosis, bone formation (as indicated by osteocalcin) was not elevated: 21 ± 13 μg/l (N: 8.8–39.4). Osteocalcin was positively correlated to IGF-I ($r = 0.06$, $P < 0.002$). No factor seems to influence the level of bone resorption.

Conclusion: In a large cohort of adult patients with anorexia nervosa, spine and hip BMDs were related to hypogonadism, whereas IGF-I was the most significant predictor of bone loss at the femoral neck level.

P552-Tu

Evaluation of Sex Steroids, Bone Turnover Markers, Bone Mineral Density in Saudi Postmenopausal Women with Osteoporosis

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Objectives: To evaluate the relationship between sex steroids, intact-PTH, bone turnover markers, and bone mineral density (BMD) in Saudi postmenopausal women with osteoporosis as compared with healthy postmenopausal controls.

Subjects and methods: A prospective study was conducted on randomly selected Saudi postmenopausal women with osteoporosis ($n = 172$) or with normal BMD ($n = 111$) who were living in Jeddah area. The serum levels of sex steroid hormones including total-T, bio-T, A, total-E2, bio-E2, DHEA, and DHEAs were measured. Also serum SHBG levels were measured and bone turnover markers (formation: sOC, sBAP, sPICP; and resorption: sCTX, uCTX, uNTX, uDPYR) together with serum intact-PTH, Ca, PO4, and Mg.BMD of the spine (L2–L4) and femur (various sites) were determined using DXA technique and women were classified with osteoporosis according to the WHO criteria. ANOVA was used to examine differences among the groups according to BMD status. Correlations were carried out using multiple linear regression analysis.

Results: Women with osteoporosis exhibited markedly lower BMD values at the various skeletal sites examined as compared with that of controls. Women with osteoporosis exhibited significant decreases in the following hormones: total-T, free T, bio-T, A, total-E2, bio-E2, DHEAs, and DHEA with increased SHBG as compared with that of corresponding controls. In age- and BMI-adjusted postmenopausal women, the BMD values of the spine (L2–L4) correlated positively with total-T, free-T, bio-T, FAI, total-E2, bio-E2, DHEAs, DHEA, and SHBG. Significant negative correlations were obtained between BMI and sBAP, sPICP, sCTX, and uCTX. Significant negative correlations were obtained between sOC and sBAP and BMD values at various skeletal sites of the femur. Significant negative correlations were obtained between all bone resorption markers and BMD values of the spine (L2–L4). After adjustment for age, BMI, and other confounders, bio-E2 and bio-T were found to be significant predictors of BMD at the spine (L2–L4) and bio-E2 and DHEAs to be predictors of BMD values of the femur at all skeletal sites examined.

Conclusions: Significant decreases in sex steroids and adrenals in postmenopausal women with osteoporosis were evident and accompanied by marked changes in BMD and bone turnover markers. In postmenopausal women, bio-E2, bio-T, and DHEAs were found to be independent predictors of BMD values: a differential effect of androgens on BMD at the spine (L2–L4) and femur.

P553-Su

Relation of Homocysteine, Folate, Vitamin B12, and Methylenetetrahydrofolate Reductase C667T Polymorphism to Bone Mineral Density in Saudi Postmenopausal Women

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Objectives: To assess the relationship between plasma total homocysteine (t-Hcy), folate, and vitamin B12 together with methylenetetrahydrofolate reductase (MTHFR) C667Y polymorphism with bone mineral density (BMD) in Saudi postmenopausal women.

Subjects and methods: A total of 278 Saudi postmenopausal women (5–60 years) who were randomly selected and living in Jeddah area were studied. Restriction fragment length polymorphism was used for genotyping of MTHFR polymorphism. Plasma levels of tHcy, folate, and vitamin B12 were measured together with BMD of the spine (L2–L4) and femur (determined by DXA technique) and other clinical characteristics among MTHFR genotypes (CC, CT, and TT) were also examined. Pearson's correlations were used to assess the correlation between BMD values with variables including MTHFR genotypes, log plasma tHcy, vitamin B12, age, years since menopause, BMI, and PTH. Women were classified into three groups according to CC, CCT, and TT

genotypes. Chi-square statistics were applied. ANOVA was used for comparison of variables among MTHFR genotypes.
Results: BMD of the spine (L2–L4) ($r = -0.23$, $P < 0.01$) and that of neck femur ($r = 0.25$, $P < 0.001$) exhibited significant negative correlations with log plasma tHcy and positive correlations with plasma folate [$r = 0.15$, $P < 0.02$ for spine (L2–L4); $r = 0.18$, $P < 0.01$ for neck femur], with no evident correlations between MTHFR polymorphism and BMD values studied. Adjustment for folate and vitamin B12 removed the relationship. Age (6.3%) and plasma folate (17.5%) contributed significantly to the prediction of variation in plasma tHcy. Plasma folate contributed significantly to the variation in BMD of spine (L2–L4) (2.2%) and neck femur (3.6%), respectively.
Conclusions: Hyperhomocysteinemia related to folate deficiency but without significant contribution by MTHFR polymorphism, independently, was associated with decreased BMD values which may contribute to the pathogenesis of osteoporosis in Saudi postmenopausal women.

P554-Mo
Free Testosterone is a Positive while Free Estradiol is a Negative Predictor of Bone Area in Elderly Swedish Men—MR OS Sweden
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Osteoporosis-related fractures constitute a major public health concern in women as well as in men. The fracture risk is dependent on several factors, including bone mineral density (BMD) and bone size. Previous studies have indicated that serum levels of estradiol but not of testosterone are associated with BMD in elderly men. The predictive role of sex steroids for bone size in elderly men is unclear. The aim of the present study was to investigate if free estradiol or free testosterone levels are associated with BMD and/or bone area in elderly Swedish men. In the Swedish part of the MrOs study (in total 3000 Swedish subjects), serum sex steroid levels have so far been measured for 977 men (age 75, SD 3) and bone parameters were assessed using DXA. Serum levels of testosterone, estradiol, and SHBG were measured, and free levels of testosterone and estradiol were derived from mass action equations. Height, weight, physical activity, smoking habits, and calcium intake are factors known to influence BMD. These factors were therefore included together with free estradiol and free testosterone levels in regression models. Free estradiol but not free testosterone was a strong independent positive predictor of BMD at all location measured (free estradiol total body beta = 0.136 $P < 0.001$; spine beta = 0.135, $P < 0.001$; trochanter beta = 0.137 $P < 0.01$; total hip beta = 0.151, $P < 0.01$). Interestingly, free testosterone was an independent positive predictor while free estradiol was an independent negative predictor of bone area in the total hip (free testosterone beta = 0.127, $P < 0.01$; free estradiol beta = −0.128, $P < 0.01$) and in the trochanter (free testosterone beta = 0.121, $P < 0.01$; free estradiol beta = −0.132, $P < 0.01$).
In conclusion, free estradiol but not free testosterone is a strong positive predictor of BMD and free testosterone is a positive while free estradiol is a negative predictor of bone area in elderly Swedish men. As bone strength is determined both by BMD and bone size, these findings might indicate that both estradiol and testosterone levels are determinants of bone strength in elderly men.

P555-Tu
Relationship Between Osteopenia and Lumbar Intervertebral Disc Degeneration in Ovariectomized Rats
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Ovariectomy (OVX) can cause bone loss in rats, but little is known about how it also induces lumbar intervertebral disc degeneration (LVD). This study investigated how estrogen deficiency affected intervertebral discs in OVX rats. Thirty 3-month-old female Sprague–Dawley rats were divided randomly into three equal groups. The baseline control group (BL) was killed at the beginning of the experiment. An ovariectomy was performed in 10 rats (OVX group) and another group of 10 rats was subjected to a sham surgery (Sham group). The OVX rats were untreated after the surgery to allow for the development of moderate osteopenia. Bone mineral density (BMD) measurement and bone histomorphometric analysis were applied to the segments of lumbar spines in all rats killed 6 months after postsurgery. The pathological changes of intervertebral discs were observed and the degree of LVD was scored by a histological scoring system. The BMD of the spines (L3–L5) in the OVX group decreased significantly compared with the Sham group. The bone volume indices in the OVX group were significantly lower, but the bone turnover rate parameters were significantly higher than those in the Sham group ($P < 0.01$). The histological scores for LVD in the OVX group were significantly higher than those in the Sham group ($P < 0.01$). There existed a significant negative correlation between the BMD and Grade 2 of the discs in the

OVX rats ($P = 0.042$). In conclusion, LVD occurs in the OVX rats and the degeneration of cartilage end plates may be a pathogenic factor in disc degeneration.

P556-Su

Biochemical Markers of Bone Turnover in Dogs

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Measurement of bone markers for assessment of bone metabolism in clinical veterinary practice is not fully established. The aim of this study was investigation of standard bone markers in dogs treated in outpatient clinic and their relationship to clinical data. Blood samples were obtained during routine diagnostic procedures in 89 dogs and the following bone markers measured in sera by commercial kits: bone alkaline phosphatase, osteocalcin, and carboxy-terminal telopeptide of type I collagen. Clinical data included age, body weight, sex, neutered or no-neutered status, and diagnosis. The results showed a statistically significant and positive correlation between the measured bone markers osteocalcin and telopeptide ($r = 0.408$; $P = 0.0005$), osteocalcin and bone alkaline phosphatase ($r = 0.291$; $P = 0.006$), and telopeptide and bone alkaline phosphatase ($r = 0.249$; $P = 0.02$). Correlation between osteocalcin and age was statistically significant and negative ($r = -0.315$; $P = 0.003$), and positive between telopeptide and body weight ($r = 0.325$; $P = 0.002$). No difference for bone markers existed between sexes or diagnosis groups, i.e., between control animals, bone-related diseases, and other conditions. The observed association between bone formation and resorption markers confirmed coupling of bone cell actions. Osteocalcin decrease with age revealed changes of skeletal growth, and the positive relationship of telopeptide with body weight indicated the impact of muscle mass on bone turnover. Utilization of bone marker measurement in veterinary medicine could be considered for evaluation of bone turnover and study of bone metabolism. Determination of bone markers for diagnostic purpose in dogs requires further investigation.

P557-Mo

Characterization and Assessment of Chronic Pain in a Rat Fracture Model

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Chronic bone pain in osteoporosis is a commonly encountered clinical problem. Previously, we have shown that a closed femur fracture model in the rat can be used to model the enduring pain that follows bone fracture. In order to further characterize the hyperalgesia observed in this model, we have investigated commonly used pain medications for their effects on mechanical hyperalgesia and spinal GFAP staining. Male Sprague–Dawley rats received either a sham surgery or a right femur closed fracture with intramedullary pin (CF + PIN) or a pin insertion only (PIN). Three weeks after surgery, the rats were tested for mechanical hyperalgesia using the von Frey technique. In addition, mechanical hyperalgesia was evaluated in 3 groups of animals that were administered escalating doses of calcitonin, gabapentin, or ketorolac. At the conclusion of the experimental period, the lumbar spinal cord was harvested and stained for GFAP. Compared to intact and PIN control, mechanical hyperalgesia was increased in the CF + PIN animals ($P < 0.01$), and inhibited dose-dependently by gabapentin but not by calcitonin or ketorolac ($P < 0.01$). Spinal GFAP sensitization was inhibited with both gabapentin and ketorolac. Interestingly, while the inhibition of mechanical hyperalgesia and spinal GFAP staining by gabapentin appeared to be highly correlated, ketorolac, which is known to delay fracture repair, inhibited spinal GFAP sensitization without alleviating mechanical hyperalgesia. In conclusion, the inhibition of persistent pain by gabapentin but not by calcitonin or ketorolac suggests that the fracture pain in this model is of neuropathic origin. Furthermore, these findings demonstrate the potential of this model to investigate and characterize the mechanisms associated with the development of chronic bone pain following fracture.

P558-Tu

An Ovary-intact Mouse Model to Study Bone Loss Induced by Estrogen Deficiency

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Surgical removal of the ovaries (OVX) is currently the standard animal model used to elucidate the mechanisms underlying bone loss induced by estrogen deficiency. However, this model does not adequately reflect the physiological status of ovary-intact menopausal women. Previous studies have determined that repeated dosing of mice with the occupational chemical, 4-vinylcyclohexene diepoxide (VCD), selectively destroys primordial and primary ovarian follicles by acceleration of the natural process of atresia resulting in premature ovarian failure (Borman et al., 1999). VCD-treated mice have increased plasma LH and FSH; decreased plasma progesterone and 17β-estradiol; decreased ovarian and uterine weights, with no alterations in body, adrenal, kidney, or spleen weights (Mayer et al., 2004). The goal of this study was to evaluate skeletal changes in an ovary-intact model with premature

ovarian failure. Three-month-old female B6C3F1 mice were dosed with VCD (160 mg/kg, i.p., $n = 8$) or vehicle ($n = 8$) for 15 days. An additional group ($n = 4$) was treated identically with VCD, then received subcutaneous estrogen pellets (0.5 mg, 90 d release) 38 days after the onset of dosing (VCD + E). Mice were killed 120 days after the onset of dosing (7 months of age). DXA (PIXImus, GE Lunar) and micro-CT (12 μm voxel size, Scanco Medical AG) were used to assess BMD and microarchitecture, respectively, of the excised L5 vertebra and femur. Compared to VEH, VCD-treated mice had significantly lower femoral BMD (−8.8%, $P = 0.001$), lower vertebral trabecular BV/TV (19.3 ± 0.4 vs. 14.6 ± 1.1%, $P < 0.005$) and number (2.9 ± 0.1 vs. 2.5 ± 0.1 mm^{-1}, $P < 0.05$), and increased vertebral trabecular separation (358 ± 13 vs. 417 ± 23 μm, $P = 0.03$). Similar patterns were observed at the distal femur, though they did not reach significance. Consistent with previous reports of anabolic effects of estrogen in mice, VCD + E mice had increased femoral BMD (+40%, $P < 0.0001$ vs. VCD and VEH) and markedly improved trabecular bone parameters at the vertebrae and distal femur compared to both VCD and VEH ($P = 0.04$ to 0.0001). In addition, compared to VCD, VCD + E had increased cortical bone area and cortical thickness at the femoral midshaft ($P < 0.05$). These results indicate that the VCD-treated mouse, which reflects the hormonal status of peri- and post-menopausal women, may be a useful model to study mechanisms underlying menopausal bone loss (Funded by NIH AG021948 to PBH).

P559-Su

Activation of PPAR-gamma by Rosiglitazone Induces Bone Loss Due to Changes in the Phenotype and Function of Bone Mesenchymal Cells

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Rosiglitazone (R) is an oral anti-diabetic agent that is FDA-approved for the treatment of type 2 diabetes. Mechanistically, R sensitizes cells to insulin via the specific activation of the adipocyte-specific transcription factor PPAR-\gamma. In vitro, activation of PPAR-\gamma2 by R inhibits osteoblast and activates adipocyte differentiation of marrow precursors. In vivo, treatment with R induces marked bone loss whereas PPAR-\gamma insufficiency leads to increased bone formation. To elucidate the mechanism by which R induces bone loss, the component of bone remodeling targeted by this drug was examined. Adult C57BL/6 mice (6 months) were fed a R-supplemented diet (20 μg/g/day) for 7 weeks. Bone mineral density (BMD) and bone mineral content (BMC) were measured at the beginning and end of the experiment by DEXA. Bone microarchitecture and histomorphometric parameters were determined in the tibiae, and bone strength was determined by vertebral compression. R treatment decreased BMD (9.64%) and BMC (7.25%) compared to control mice. Micro-CT analysis revealed significant changes in trabecular bone volume, trabecular number and thickness, and increased spacing. R induced micro-architectural changes by decreasing osteoblast number and activity with a corresponding increase in osteoclast number and activity. In addition, bone quality was markedly diminished as R treatment decreased vertebral bone strength by 26% compared with control mice. To better understand the effect of R on bone progenitor phenotype, R was tested in both U-33/\gamma2 marrow-derived cell line, and primary murine bone marrow cultures. U-33/\gamma2 cells represent murine pre-osteoblasts, which ectopically express PPAR-\gamma2. Treatment with R significantly suppressed the ability of U-33/\gamma2 cells to mineralize extracellular matrix, express osteoblast-specific markers, and converted them to fat-laden adipocytes. In co-cultures of U-33/\gamma2 cells and non-adherant bone marrow cells, and in the presence of $1,25(OH)_2D_3$, R enhanced osteoclast recruitment and maturation. Moreover, R increased the RANKL/OPG ratio in both U-33/\gamma2 cells and primary bone marrow cultures. In conclusion, R induces changes in the phenotype of bone marrow mesenchymal cells, which result in suppression of the osteoblast phenotype and enhancement of the support for osteoclast formation. Thus, R effects on the mesenchymal component of bone remodeling may account for the decreased bone formation and increased bone resorption, and bone loss in R-treated animals.

P560-Mo

Isocaloric Low Protein Intake Decreases Titanium Implant Osseointegration

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Low protein intake is highly prevalent among elderly orthopedic patients and could retard fracture healing. We previously demonstrated that reduced protein intake was deleterious on bone strength. Whether protein intake could influence titanium implant osseointegration is unknown. We measured the resistance to pull-out of titanium rods implanted into proximal tibia of rats receiving an isocaloric low protein diet. Eleven-month-old female rats were fed isocaloric diets containing 2.5% (low protein) or 15% (normal protein) casein from 2 weeks before implantation of 1-mm diameter cylindrical titanium rod in the proximal metaphysis of each tibia. Six and 8 weeks after implantation, the tibias were removed for microtomographic histomorphometry to quantify bone implant contact (BIC) and bone trabecular microarchitecture around the implant. Resistance to implant pull-out was tested by recording the maximal

force necessary to completely loose the implant, i.e., the pull-out strength. All results are expressed as means ± SEM. Significance of difference was evaluated with a two-sided unpaired Student's *t* test (*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$). Pull-out strength was significantly lower in rats fed an isocaloric low protein diet 6 and 8 weeks after implantation (−43%*** and −42%***, respectively) compared with rats fed a normal protein diet. BIC was significantly lower in the low protein group 8 weeks after implantation. BIC and pull-out strength were correlated ($r^2 = 0.57$, $P < 0.0001$). BV/TV was 19.9 ± 2.2* vs. 31.8 ± 3.3% at 6 weeks and 20.1 ± 1.9* vs. 29.8 ± 3.2% at 8 weeks after implantation in the low protein and normal protein intake groups, respectively. Trabecular thickness was 96.2 ± 3.7** vs. 113.0 ± 3.6 mm at 6 weeks and 101.4 ± 2.7** vs. 116.2 ± 3.3 at 8 weeks in the corresponding groups. In a structure model index (SMI) analysis, there was a significant shift to a more rod-like pattern in the low protein diet groups. This was associated with lower plasma IGF-I levels. We conclude that isocaloric low protein intake impairs titanium implant osseointegration, with a decreased strength needed to completely loose the implant and altered bone microarchitecture in the vicinity of the implant (diminished bone relative volume, thinner trabeculae, and a trabecular network shifted to a more rod-like pattern).

Table

	6 weeks normal protein	6 weeks low protein	8 weeks normal protein	8 weeks low protein
Pull-out strength (N)	49.1 ± 3.5	28 ± 3.6***	52.8 ± 4.3	30.8 ± 3.3***
BIC (%)	62.5 ± 4.1	52.7 ± 4.5	63 ± 4.4	49.3 ± 3.5*

P561-Tu

Chemical Modification of Smooth Titanium Implant Surface by Coating with Propylene-tetra-phosphonic Acid Increases their Osseointegration

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Enhancing bone anchorage of titanium implants could potentially lower recovery time of dental implants and increase long-term survival of orthopedic prosthetic material. We investigated the influence on titanium osseointegration of two newly synthesized monomolecular coatings, polypropylene-tetra-phosphonic acid (PTP) and ethylene-triphoshonic acid (ETP) coating, and compared the results to a well-documented rough surface used in dental medicine, the sand-blasted, large-grit, acid-etched (SLA) surface treatment. Fifteen-month-old female rats ($n = 22$) were implanted with a cylindrical pure titanium rod (1-mm diameter) in each proximal tibial metaphysis. Four types of implant were used, a machined (control), a PTP, or ETP-coated machined, and a SLA-treated surface. Rats received in one tibia a treated implant (PTP $n = 7$, ETP $n = 8$, or SLA $n = 7$) and in the other a control one. Treated and control implants were inserted alternatively in right and left tibias. Six weeks after implantation, rats were sacrificed, the tibias collected, and implant pull-out strength was recorded, evaluating thus the degree of osseointegration in fresh specimen. Significance of difference was evaluated with a paired two-sided *t* test after log transformation. With PTP-coated implants, there was a significant increase in pull-out strength of 38% compared with control implants in the contralateral leg ($P < 0.01$). With ETP-coated implants, the 9.6% increase was not significant. The mean pull-out strength of the SLA implants was 103% higher than in control ($P < 0.001$). Thus osseointegration was greater with PTP-coated titanium implants than with ETP. The strongest pull-out force was found with SLA. However, PTP coating, consisting of a monomolecular layer at the titanium surface, might be associated with less direct mechanical retaining effect during pull-out test than SLA, which creates a textured surface (micro-roughness); these tests show that a chemical modification of the implant surface can significantly increase the bone response. Addition of PTP coating after surface roughness treatment should therefore be evaluated. In conclusion, chemical modification of a smooth titanium implant surface by coating with propylene-tetra-phosphonic acid increased their osseointegration. Since PTP and ETP are tetra- and triphosphonic acids, respectively, the difference in their effects on osseointegration and a possible relation to the number of phosphonic groups in a dose-response manner will be investigated.

P562-Su

Accelerated Bone Ageing and Altered Estradiol Levels in DNA Repair Deficient Trichothiodystrophy Mice

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Trichothiodystrophy (TTD) is a rare, autosomal recessive disorder, in which patients present with an array of symptoms, including photosensitivity, ichthyosis, brittle hair and nails, impaired intelligence, decreased fertility, short stature, an aged appearance, and a reduced life span. In addition, skeletal abnormalities have been described.

Three complementation groups exist: XPB, XPD, and TTDA. All three genes code for subunits of the dual functional DNA repair/basal transcription factor TFIIH that is a protein complex involved in the Nucleotide Excision Repair (NER) pathway. We have mimicked a causative point mutation identified in the XPD gene of a TTD patient (TTD1Bel). Previous work has shown that the phenotype of TTD mice very much resembles the symptoms of patients, including the presence of premature ageing features.

In this study, we analyzed long bones of ageing female and male wild-type and TTD mice. TTD females show an accelerated decrease in bone volume and cortical thickness and lack a compensatory increase in perimeter as seen in wild-type females. This lack of periosteal apposition was confirmed by double labeling and histomorphometry. As a consequence, the polar moment of inertia was lower in TTD females indicating reduced bone strength. Mechanical testing showed that from about 52 weeks of age, these differences indeed result in decreased bone strength. From 39 weeks of age, wild-type females showed a significant increase in estradiol levels as well as an increase in weight. In contrast, estradiol levels and weight did not change or even decreased in TTD females. In addition, micro-CT analyses show that age-related changes occur at a later age in male than in female wild-type mice. These temporal differences in ageing between females and males result in failure to detect changes within the life span of male TTD mice.

In conclusion, TTD females display accelerated bone ageing and lack periosteal apposition with ageing. The observed lower serum estradiol levels and weight may explain the bone phenotype via altered mechanical perception and bone formation as well as via direct effects on bone resorption. In addition, an increase in apoptosis due to DNA repair deficiency and damage accumulation in bone cells might influence mechano-perception and result in altered bone formation across the force lines. Interestingly, the retarded bone changes with ageing in male vs. female wild-type mice mimic the human situation.

P563-Mo

Age-related Changes in Vertebral Size and Microarchitecture in Male Mice

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Mouse models are commonly used in the study of skeletal biology. Yet, few data are available on the age-related changes in trabecular bone morphology. The aim of the current study was to gain insight into vertebral fragility and mechanisms underlying skeletal adaptation to mechanical load by determining the age-related changes in vertebral size and microarchitecture in male mice. To do this, we maintained male C57Bl/6J mice under standard conditions and sacrificed them at 4, 6, 8, 12, 24, 52, and 83 weeks of age ($n = 6–9$ per group). Bones were harvested at each time point, and morphology of the 5th lumbar vertebrae evaluated by μCT (μCT40, Scanco Medical AG). The entire vertebra was scanned at 12-μm isotropic voxel size, image data were thresholded using an iterative algorithm (Ridler and Calvard, 1978; Trussell, 1979), and morphometric parameters determined using 3D methods. Body weight increased 3-fold over life, from 16.3 to 45.7 g. Vertebral trabecular (Tb.) BV/TV increased between 4 and 6 weeks (23.8 ± 0.5% to 27.4 ± 0.6%, $P = 0.02$), was stable until 24 weeks, and declined thereafter, with a net 27% decline in BV/TV over life ($P < 0.0001$). Tb number was maximal at 4 to 8 weeks (6.1 ± 0.1 mm^{-1}) and declined thereafter (3.9 ± 0.2 mm^{-1} at 83 weeks), with a 35% decline over life ($P < 0.0001$). In contrast, Tb thickness increased steadily, from 43 to 51 μm (+19%, $P < 0.0001$). Cross-sectional area of the vertebral body and thickness of the anterior cortex increased continually during life, from 13.6 ± 0.3 to 18.2 ± 0.3 mm^2 ($P < 0.0001$) and from 69.6 ± 3.9 μm to 88.3 ± 2.8 μm ($P = 0.0003$), respectively. In summary, we found that (1) peak vertebral Tb BV/TV is achieved by 6–8 weeks of age in male mice; (2) there are synergistic, reciprocal relationships among Tb BV/TV, vertebral cross-sectional area, and thickness of the anterior cortex, and (3) declines in Tb number are accompanied by increases in Tb thickness, a phenomenon previously reported in tibia (Halloran et al., 2002). Interestingly, comparison of these age-related structural patterns within a species resembles the patterns we have also seen between primate species as a function of increasing body size. Altogether, these results indicate that age-related changes in vertebral microarchitecture occur early and continue throughout life, providing a useful model to study the interactions among sex-steroids, modeling and remodeling, and vertebral fragility.

P564-Tu

Central Control of Bone Remodeling by the Vestibular System

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Bone mass and architecture are recognized to be under the control of mechanical strains and hormonal influences. However, other factors are now recognized, and the central and peripheral nervous systems may play important roles. We recently demonstrated that labyrinthectomy is associated with bone loss in weight bearing bones (J Vestib Res. 2004) without any decrease in muscular activity (JBMR, 2004 19 S1; Exp Neurol. 2004). We hypothesized that the

sympathetic nervous system and the vascular system are mediating this bone loss. The effect of labyrinthectomy was searched in a series of Wistar rats and the influence of the beta-blocker propranolol was investigated as a countermeasure. We have compared controls rats (CONT, $n = 8$), rats with bilateral labyrinthectomy alone (LABY, $n = 8$), and rat receiving propranolol (0.5 g/l in drinking water) (LABY + P, $n = 8$). Animals were housed for 30 days postsurgery. DXA analysis was done on the distal femur metaphysis (DFM). Micro-CT analysis was done on the tibia and 3D reconstruction of the models permitted the measurement of trabecular bone volume and microarchitectural descriptors. In control animals, DXA showed a BMD gain of 13.9 ± 0.9% between D0 and D30. A significant decrease of bone gain was observed in LABY compared to CONT between D0 and D30 (+1.9 ± 0.8% vs. 13.9 ± 0.9%, $P < 0.0001$) and a trend to rescue bone gain in LABY + P when compared to LABY (6.8 ± 1.2% vs. 1.9 ± 0.8%, $P = 0.05$). Correlations were searched between DXA and 3D analysis by linear regression analysis. Because both methods showed a trend to a significant bone loss, data were re-computed using principal component analysis. Thus, the sympathetic nervous system could be a mediator of bone loss, controlled by the vestibular system, in the labyrinthectomized rat.

P565-Su

Development of an Experimental Model to Study of Osteoporosis

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An animal model with mice (*Rattus novergicus*) is being used because it allows access to information on the quality and bone structure that otherwise would not be obtained in patients of clinical selection, being therefore fundamental agents to accomplish an effective and safe measurement of bone quality. The proposal of this model is to cause osteoporosis through castration, glucocorticoid administration, or the association of both, in males and females, and to evaluate the moment the disease starts. For the accomplishment of the experiment, 192 adult mice were used (96 males and 96 females). They were divided in eight groups with 24 animals in each: group I, male control; group II, castrated males; group III, males with glucocorticoid therapy; group IV, castrated males with glucocorticoids therapy; group V, female control; group VI, castrated females; group VII, females with glucocorticoid therapy; group VIII, castrated females with glucocorticoid therapy. The collections took place 14, 28, 42, and 56 days after beginning of the administration glucocorticoid in all the groups. It was performed by plasmatic analysis of calcium, phosphorus, and total proteins; quantitative analysis of calcium, phosphorus, and magnesium of humerus; flexing biomechanic test in tibia and compression biomechanic test in lumbar vertebra (L5). Comparing the obtained results, all the experimental groups presented normal plasmatic concentrations of calcium: phosphorus (1:1 to 2:1) and bone concentrations of calcium: phosphorus (2:1) inferior to the normal rate, except for the control groups. Regarding the biomechanic tests with males, group IV showed smaller resistance to bone fracture, followed by group III and group II when compared to the control group. In the groups of females, it was verified that group VI had minimum bone loss, indicated mainly by the unbalance of the concentration of calcium: phosphorus in the bone and by the reduction of the rigidity observed in the biomechanic tests. On the other hand, groups VII and VIII presented inadequate bone concentration of calcium: phosphorus and bone fragility, which was observed in the flexing and compression tests, considering that the group VII presented minor rigidity average and the group VIII smaller resistance to fracture vertebra L5 and in tibia compared to the control group.

P566-Mo

Mechanisms for Bone Growth Arrest and Osteoporosis in Young Rats Secondary to Acute 5-fluorouracil Chemotherapy

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Due to the improved survival rates and with the intensified use of chemotherapy for childhood cancers, it has become apparent that some children or adult survivors show poor bone growth and develop osteoporosis. However, how chemotherapy affects bone growth remains largely unknown. The growth plate cartilage and its adjacent bone-forming region metaphysis are responsible for bone growth in children via endochondral ossification, which relies on the regulated proliferation, maturation, and apoptosis of cartilage cells (chondrocytes) and bone cells (osteoblasts) and their extracellular matrix production and modeling. As a step to investigate possible mechanisms underlying chemotherapy-induced bone growth defects, we examined effects of a single-injected dose of anti-metabolite 5-fluorouracil (5-FU) on cell proliferation, apoptosis, bone formation, and expression of growth factors and matrix molecules in the growth plate cartilage and metaphysis in young rats. Two weeks after 5-FU treatment, total body and tibial lengths were significantly reduced. Consistently, heights of both proliferative and hypertrophic zones and the total growth plate thickness decreased gradually to the lowest levels by days 4–5 before returning to normal by day 10. In the adjacent bone forming region (metaphysis), percent areas of trabecular bone in the 1st and 2nd spongiosa were reduced by day 10. On the cellular level, 5-FU treatment suppressed proliferation of growth plate

chondrocytes and metaphyseal osteoblasts/pre-osteoblasts by day 2 and gradually returned to normal by day 10. Apoptosis was induced rapidly among osteoblasts/pre-osteoblasts (day 2) but delayed until day 5 among chondrocytes. Apoptosis persisted to day 10 in both regions. Expression of mitogenic/survival factors IGF-I and TGF-beta1 was initially suppressed after 5-FU injection and then significantly upregulated during days 4–10. Expressions of matrix molecules in the growth plate (col-2 and col-10) and in the metaphysis (col-1 and osteocalcin) were dramatically upregulated during the repair phase (days 4–10). These data suggest that 5-FU chemotherapy affects bone growth directly by altering expression of growth factors and matrix molecules, inhibiting proliferation, inducing apoptosis, and reducing bone formation at growth plate cartilage and metaphyseal bone.

P567-Tu

Lysine Dose-response on Microarchitecture of Trabecular Bone and Gene Expression of Calcium Transporters in Growing Rats

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There is evidence to suggest that lysine plays a role in generalized and skeletal growth. Supplementation of amino acids, including lysine, has been found to improve bone mineral status in ovariectomized rats on low protein diets. It is unknown if a high lysine intake under protein-replete conditions optimizes peak bone mass, hence reducing the risk of osteoporosis later in life.

In an earlier study, we examined lysine dose-response on bone growth, bone mineral content (BMC), Ca absorption, bone turnover markers, and IGF-1. Briefly, 3-week-old rats were randomized by weight to one of five formulated diets containing 20%, 60%, 80%, 120%, and 160% of lysine requirement (based on National Research Council recommendations) and were equivalent in all other aspects (n = 10/group). Results showed no lysine dose-response in bone size, bone turnover, BMC, and Ca absorption. However, rats fed 20% lysine had significantly lower BMC and IGF-1 levels but higher Ca absorption compared with the other groups.

To further investigate if there was a lysine dose-response in the bone microarchitecture, muCT analysis (muCT 40) was conducted on the femurs for relative bone volume (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), connectivity density, and structure model index (SMI). Furthermore, the gene expression of intestinal and kidney Ca transporters (i.e., ATPase Ca transporting plasma membrane 1, Calbindin3, Ca sensing receptor, Vitamin D receptor) was measured to study the mechanism of lysine on Ca absorption. Duodenal and kidney samples were harvested at sacrifice and RNA extracted for analysis using real-time PCR (ABI Prism 7000 Sequence Detection System) and the comparative CT method for calculating relative gene expression, normalized to eta-actin. muCT results showed that compared to the other groups, rats fed 20% lysine had significantly lower BV/TV (−55–71%, $P < 0.05$) and Tb.Th (−15–20%, $P < 0.05$), and significantly higher Tb.Sp (+13–22%, $P < 0.05$) and SMI (indicating a more rod-like form of trabecular bone) [+16–26%, $P < 0.05$]. No significant differences were found among the other groups.

Preliminary results of gene expression data showed that calbindin3 expression in the kidney was significantly higher in these rats compared to those fed 80% and 160% lysine (+14%, $P < 0.05$).

These results suggest that lysine deficiency compromises skeletal growth and trabecular development and may result in upregulation of some Ca transporter genes.

P568-Su

Effects of Alpha-ketoglutarate (AKG) Administration to Pregnant Sows on Femur Properties in Offspring at Slaughter

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Proper function of central and peripheral nervous system in humans and animals is crucial for proper development and function of skeletal system and whole body. Alpha-ketoglutarate (AKG) that increases amino acid synthesis and is a precursor of the major neuromediator in the central nervous system–glutamate–plays a very important role in the regulation of skeletal system developmental processes. The aim of the study was to evaluate the hypothesis whether administration of AKG in pregnant sows positively influences peak bone mass (PBM) in offspring that is reached at the time of skeletal maturity. This study was performed on pigs born to sows that were administrated AKG at the daily dose of 0.4 g/kg b.w. in the experimental group throughout the two last weeks of pregnancy. Both AKG and placebo were administered orally during the morning meal. At the age of 6 months, the pigs were slaughtered and the right femur was isolated for further analyses. Using quantitative computed tomography (QCT) technique and Somatom Emotion (Siemens) apparatus, the

trabecular and cortical bone mineral density of femur was measured. The mechanical properties were determined in INSTRON 4302 using the three-point bending test. Moreover, bone geometry in terms of cross-sectional area, second moment of inertia, mean relative wall thickness, and cortical index was determined. AKG administration to pregnant sows increased significantly the trabecular bone mineral density ($P < 0.0001$), the maximum elastic strength, and the ultimate strength (both $P < 0.0001$) in 6-month-old porkers. Furthermore, AKG-treated animals were characterized by higher values of the investigated geometrical parameters of the femur (all $P < 0.001$). Obtained results showed positive influence of AKG administration in pregnant sows on skeletal system development and peak bone mass acquisition in offspring when analyzed at slaughter.

P569-Mo

Effects of 3-hydroksy-3-methylbutyrate (HMB) Administration to Pregnant Saws on Femur Properties in Offspring at Slaughter

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Metabolic activity of the essential amino acid leucine includes regulation of protein metabolism in skeletal muscles and whole body. It is believed that the regulation of protein metabolism is induced by leucine and its metabolites like β-hydroksy-β-methylbutyrate (HMB). The aim of the study was to evaluate the hypothesis whether administration of HMB in pregnant sows positively influences peak bone mass (PBM) in offspring that is reached at the time of skeletal maturity. This study was performed on pigs born to sows that were administrated $Ca(HMB)_2$ at the daily dose of 0.05 g/kg b.w. in the experimental group throughout the two last weeks of pregnancy. Both HMB in the experimental sows and vehiculum in the controls ($CaCO_3$ at the dosage of 0.05 g/kg b.w. per day) were administered orally during the morning meal as a water solution. At the age of 6 months, the pigs were slaughtered and the right femur was isolated for further analyses. Using quantitative computed tomography (QCT) technique and Somatom Emotion (Siemens) apparatus, the trabecular and cortical bone mineral density of femur was measured. The mechanical properties of investigated bones were determined in INSTRON 4302 using the three-point bending test. Moreover, bone geometry in terms of cross-sectional area, second moment of inertia, mean relative wall thickness, and cortical index was determined. HMB administration to pregnant sows increased significantly the trabecular and cortical bone mineral density (both $P < 0.0001$), the maximum elastic strength, and the ultimate strength (both $P < 0.0001$) in 6-month-old porkers. Moreover, HMB-treated animals were characterized by higher values of the investigated geometrical parameters of the femur (all $P < 0.0001$). Obtained results showed positive influence of HMB administration in pregnant sows on skeletal system development and peak bone mass acquisition in offspring when analyzed at slaughter.

P570-Tu

Smoking is Associated with Low Peak Bone Mass in Men

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Smoking reduces bone mineral density (BMD) in elderly men and post-menopausal women and increases the risk of hip fractures. Whether smoking decreases peak bone mass (PBM) or increases bone loss in young men is unknown. The Odense Androgen Study is a population-based, observational study on endocrine status, body composition, muscle function, and bone metabolism in men comprising 783 participants aged 20–30 years. Smoking status and medical history were obtained by use of a questionnaire, and the participants received medical examination. BMD of the lumbar spine and hip and total bone mineral content (BMC) were measured using a Hologic 4500-a scanner. Serum estradiol, testosterone, 25-OH-vitamin-D, and biochemical bone markers were measured. A total of 97 participants were excluded due to testicular/chronic disease, excessive alcohol consumption (>6 units/day), abuse of anabolic steroids, serum LH below detection level, or myxedema. Thus, the study comprised 681 participants. Thirty-two percent were current smokers (mean age 25.9 ± 2.9 vs. 25.2 ± 2.9 years, $P < 0.01$). Smokers had lower whole-body BMC (2887 ± 380 vs. 2956 ± 393 g, $P < 0.05$) and total hip BMD (1.06 ± 0.14 vs. 1.09 ± 0.14 g/cm^2, $P < 0.05$) compared to non-smokers. Additionally, smokers had lower body height (181.1 ± 6.6 vs. 183.6 ± 6.7 cm, $P < 0.01$), serum 25-OH-vitamin-D (60.5 ± 27.4 vs. 67.7 ± 27.7 pmol/l, $P < 0.001$), and serum IGF-1 (3.30 ± 0.27 vs. 3.21 ± 0.26 ng/ml, $P < 0.001$). In multiple regression analysis, no significant impact of smoking status on bone mineral measurements was detected when controlling for height, markers of bone turnover, serum IGF-1, and 25-OH-vitamin-D. Both IGF-1 and vitamin D correlated negatively ($P < 0.05$) with femoral BMD when testing for age and smoking status, too. When controlling for BMI, smoking reduced both femoral BMD ($P < 0.02$) (Fig 2) and total BMC ($P < 0.05$), but not BMD in lumbar spine. Additionally, smoking decreased femoral BMD ($P > 0.05$) when controlling for weight. In smokers, no correlations

between pack-years or age at smoking debut and bone mineral measurements were found. Finally, serum estradiol, testosterone, and SHBG did not differ significantly between smokers and non-smokers. We conclude that smoking is associated with approximately 0.2 SD lower PBM in young men. The effect of smoking on bone may be mediated by low serum 25-OH-vitamin-D or IGF-1, and the magnitude is similar to that observed in elderly men and women.

P571-Su

Vitamin D Supplementation Aids Bone Mineral Accretion in Adolescent Girls

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Background: Adequate vitamin D intake protects the elderly against osteoporosis, but relevant evidence that vitamin D would beneficial for the growing skeleton is few. The aim of this 1-year study was to determine in a randomized double-blinded trial the effect of 5 μg and 10 μg vitamin D supplementation on bone accretion of the lumbar spine and femur, bone turnover, and calciotropic hormones in adolescent girls.

Methods: A total of 228 girls, mean age (SD) 11.4 (0.4) years, participated. Their mean intakes of calcium and vitamin D at baseline were 1200 (520) mg/day and 4.8 (2.7) μg/day. Pubertal status and physical activity of the subjects were determined three times during the study. Their BMC was measured by DXA (Hologic QDR 4500). Fasting serum samples were analyzed for serum 25-hydroxyvitamin D (S-25-OHD) with HPLC, intact parathyroid hormone (S-iPTH) with OCTEIA assay, and osteocalcin (S-OC) with ELISA method. Urinary pyridinoline (U-Pyr) and deoxypyridinoline (U-Dpyr) with HPLC were measured in second void urinary samples.

Results: A dose-response effect of the vitamin D supplementation was seen in the femur, in which bone mineral accretion increased by 14.3% with 5 μg and by 17.2% with 10 μg as compared to the placebo group (ANCOVA; $P = 0.012$). However, lumbar spine BMC increased significantly only in the midpubertal girls ($P = 0.028$). The mean concentration of S-25-OHD increased ($P < 0.001$) in the 5 μg group by 5.7 (15.7) nmol/L and in the 10 μg group by 12.4 (13.7) nmol/L, while it decreased by 6.7 (11.3) nmol/L in the control group. Supplementation decreased U-Dpyr, which reflects bone resorption ($P = 0.046$), but had no effect on S-iPTH or S-OC.

Conclusions: Our study indicates that 10–15 μg/day of vitamin D intake reinforced bone mineral accumulation, since 14–17% more mineral was accrued in the femur. In midpubertal girls, vitamin D increased mineral accretion in the spine by 19–26%. We definitely believe that the current vitamin D recommendation for adolescent girls at least in the northern latitudes is too low to ensure sufficient vitamin D status during winter. Intake of vitamin D at rates of 10–15 μg/day aids in maintaining S-25-OHD stability and prevents the increase of S-iPTH concentration during winter. Vitamin D induced BMC gain by decreasing bone resorption. Optimizing bone mineral gain in adolescence is crucial for the future prevention of osteoporosis.

P572-Mo

Association of Genetic Polymorphisms with Bone Mineral Density and Fracture Risk in Osteoporotic Women

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Osteoporosis is a multifactorial disorder, influenced by both environmental factors and a complex genetic background. In recent years, a growing number of possible candidate genes have been identified. We evaluated the role of several polymorphisms for bone mineral density (BMD) and fracture risk in a cohort of osteoporotic patients from our endocrine practice.

Twelve polymorphisms in nine candidate genes were studied, including vitamin D receptor (VDR; *Bsm*I in Intron 8), collagen type1a1 (COL1A1; G1546T, Sp1), estrogen receptor a (ESR1; *Xba*I in Intron 1), osteoprotegerin (OPG; A163G, G209A, T245G in the promoter), interleukin-6 (Il-6, G-174C), transforming growth factor β (TGFB; C-509T, Leu10Pro), lactase (T-13910C), bone morphogenetic protein 2 (BMP2, Ser37Ala), and methylene tetrahydrofolate reductase (MTHFR; Ala222Val).

In 101 women with osteoporosis aged 65 ± 11 years, BMD was measured at the lumbar spine by DXA. 58 patients (age 68 ± 10 years, mean *T*-score −2.5) with one or more osteoporotic fractures were compared with 43 patients (age 61 ± 12 years, mean *T*-score −2.15) without reported fractures. Genotyping was performed after PCR amplification of genomic DNA by direct sequencing or by melting curve analysis using sequence-specific hybridization probes (Light Cycler). Distribution of the respective genotypes was found to be in accordance with published data for European populations.

None of the analyzed polymorphisms was found to be more or less frequent in the group of patients with osteoporotic fractures, and there was no significant

association of one of the polymorphisms with BMD in the patients with or without fractures, although BMD in fracture patients was significantly lower (0.799 g/cm^2 vs. 0.897 g/cm^2, $P = 0.007$). Furthermore, there was no enrichment of combinations of certain genetic risk factors in the fracture group compared to the non-fracture group. In conclusion, at present, our data do not support the idea that a patient's individual risk for loss of BMD or osteoporotic fractures in the clinical practice can be predicted based on molecular genetic testing. Environmental factors, such as lifestyle and concomitant diseases in elder patients, may be much more relevant for development of osteoporosis.

P573-Tu

CYP19-aromatase Haplotypes and Bone Mass

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Aromatase activity is important for estrogen synthesis and bone homeostasis in postmenopausal women. In fact, therapy with aromatase inhibitors induces loss of bone mass and is associated with an increased fracture risk. We explored the relationship of several single nucleotide polymorphisms (SNPs) situated in the region of the CYP19-aromatase gene with bone mass. Genotyping was performed by PCR with Taqman probes and bone mineral density (BMD) was measured by DXA (Hologic) in a group of 286 postmenopausal women.

In women aged over 60, alleles of three CYP19 SNPs situated between the 3′untranslated region (UTR) and the I.2 promoter were associated with significant differences in BMD. Thus, women with A alleles in the 3′UTR (locus rs10046) had higher BMD than those with G alleles. Hip *T*-scores were: AA, -1.3 ± 0.1; AG, -1.3 ± 0.2; GG, -1.9 ± 0.1 ($P = 0.002$). Lumbar spine *T*-scores were: AA, -1.9 ± 0.2; AG, -2.2 ± 0.1; GG -3.0 ± 0.2 ($P = 0.001$). Two other SNPs (rs3784307 and rs1062033) were also associated with significant differences in hip and spine BMD. Those three loci were in strong disequilibrium linkage, and in fact 95% of the women studied had one of three common haplotypes. Hip BMD in women with two copies of the AAG haplotype was 0.886 g/cm^2, whereas it was only 0.738 in women with no copy of that haplotype ($P < 0.001$). Unlike CYP19 SNPs, two other SNPs located in neighbor genes were not related to BMD.

In conclusion, these results suggest that a number of frequent allelic variants of the aromatase gene are important determinants of bone mass in postmenopausal women.

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P574-Su

The Role of Collagen i Alpha1 sp1 Polymorphism and Ultrasound Transmission Velocity in Colles' Fracture Prediction Depends on Body Weight

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To compare the ability of the BMD at distal forearm, COLIA1 polymorphism, and ultrasound stiffness to identify individuals with increased risk of wrist fracture, we studied 183 postmenopausal Czech women with a wrist fracture and 178 postmenopausal controls aged 45–70 years. The genotypes 'Ss' and 'ss' were significantly overrepresented among fracture cases. The BMD measurements at the femoral neck, total femur, and distal forearm as well as ultrasound stiffness of the heel, BUA, and SOS were significantly lower in the fracture cohort. BMD of the distal forearm was the main determinant of susceptibility to the wrist fracture. Weight, the COLIA1 genotype, and ultrasound SOS further strengthened predictive value of BMD. However, we found an interaction between weight and both the COLIA1 Sp1 polymorphism and ultrasound parameters. Presence of the 's' allele as well as low SOS acted as significant predictors of wrist fracture only in heavier women but not in women with body weight lower than 62 kg. In heavier women, both the COLIA1 Sp1 polymorphism and ultrasound parameters acted as independent markers that contributed to BMD to enhance fracture prediction. However, the COLIA1 enabled a higher specificity (specificity 72.4%, sensitivity 44.2%) while SOS enabled a higher sensitivity (sensitivity 73.9%, specificity, 45.7%). We conclude that BMD at total forearm, the COLIA1 polymorphism, and ultrasound SOS are independent predictors of wrist fracture in postmenopausal women. The effect of the COLIA1 Sp1 polymorphism and SOS on wrist fracture risk is more pronounced in patients with a higher body weight.

P575-Mo

LRP5 Polymorphisms are Associated with Osteoporosis in Males

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Background: Male osteoporosis accounts for at least 1/3 of hip fractures, 1/5 of vertebral fractures, and significant excess mortality. Bone mineral density (BMD) variation in men and women is highly heritable, and there is evidence of site- and gender-specific genetic effects. Mutations and polymorphisms of LRP5 have been shown to influence BMD and possibly height, with suggestive evidence that the

genetic effects are greater in men. We sought to investigate the role of genetic variation in LRP5 in male osteoporosis. **Methods:** This study examined the influence of LRP5 gene polymorphisms upon bone mass parameters and height using family-based and case-control approaches. Our family cohort comprised 56 osteoporotic male probands and their relatives (n = 210). A second cohort composed of 78 osteoporotic men with a history of fragility fractures (mean age 57 years, range 26 to 88 years), and 65 healthy male controls (mean age 50.4 years, range 21 to 75 years) was recruited. Association between LRP5 gene polymorphisms and femoral neck (FN) and lumbar spine (LS) BMD Z-scores corrected for height, as well as height corrected for gender, was examined in both cohorts. Within-family association studies were performed by QTDT and linkage analysis by maximum likelihood variance components methods using Genehunter 2.1. Case-control comparisons of genotype and haplotype frequencies between men with divergent BMD were performed by contingency-table analysis.
Results: QTDT analysis of families of osteoporotic male probands identified association between the coding C135242T polymorphism in exon 8 and FN BMD Z-score (P = 0.026). When this analysis was restricted to males only, association was still observed (P = 0.048). Within families, trend towards association between height and the C165215T (A1330V) LRP5 polymorphism was also noted (P = 0.062). No linkage was detected between LRP5 polymorphisms and BMD or height. LRP5 polymorphisms and haplotypes were expressed at similar frequencies in men with and without fragility fractures.
Conclusions: LRP5 gene polymorphisms were associated with femoral neck BMD and height in families of osteoporotic men, and support a role for genetic variation in LRP5 in male osteoporosis.

P576-Tu

A Polymorphism in the Gene Coding for Estrogen Receptor Beta is Associated with Bone Mineral Density in Elderly Swedish Men—MR OS Sweden

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Estrogen is necessary for the male skeleton. Case reports of men who are unable to convert testosterone into estrogen due to loss-of-function mutations in the gene coding for the aromatase enzyme have shown that these men are osteopenic, a condition which is normalized after substitution with estrogen. Bone mineral density (BMD) is positively correlated with serum levels of estrogens in men, and the rate of bone loss in elderly men is negatively correlated with estrogen levels. BMD is partly determined by genetic factors. Due to the known importance of estrogen to skeletal integrity, estrogen-related genes are candidate genes for BMD. In the gene coding for estrogen receptor beta (ERbeta), several polymorphisms have lately been identified. The aim of the present study was to investigate associations between the ERbeta C>T rs1256031 polymorphism and BMD in elderly Swedish men. In the Swedish part of the MrOs study (in total, 3000 Swedish subjects), 464 men (age 73.5 SD 2.2) have so far been genotyped. Bone parameters were assessed using DXA. Life style factors were investigated using questionnaires. Genotype frequencies were as follows: CC 22.1%, CT 49.2%, TT 28.8%. Height, weight, physical activity, smoking habits, and calcium intake are factors known to influence BMD. These factors were therefore included together with ERbeta genotype in a regression model. Using this model, the rs1256031 polymorphism was an independent predictor of BMD in the lumbar spine (beta = 0.114, $P < 0.01$), the trochanter (beta = 0.129, $P < 0.01$), femur neck (beta = 0.110, $P < 0.05$), and total hip (beta = 0.098, $P < 0.05$). In all locations, the CC genotype had the highest, and the TT genotype had the lowest BMD (BMD lumbar spine: CC 1.06 ± 0.2, CT 1.04 ± 0.2, CC 1.01 ± 0.2 g/cm^2). ERbeta genotype was an independent predictor of the bone area in the spine (beta = 0.096, $P < 0.05$) but not in the total hip, the femur neck, or the trochanter. Serum levels of free testosterone and free estradiol were not associated with ERbeta genotype. In conclusion, the ERbeta rs1256031 polymorphism is an independent predictor of BMD in this cohort of elderly Swedish men.

P577-Su

LRP5 Gene Polymorphisms Influence Bone Mineralization and Growth in Children

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Background: Peak bone mass acquired during childhood is the primary determinant of adult bone mineral density (BMD) and osteoporosis risk. Genetic influences are known to determine peak bone mass acquisition. Activating and inactivating LRP5 gene mutations elicit extreme bone phenotypes, while its genetic variants are associated with normal variation of BMD, and in some studies, with adult height. The bone phenotype of animal models of

dysregulated LRP5 function is evident during bone accrual.

Hypothesis: We hypothesized that LRP5 gene polymorphisms influence bone mass acquisition during childhood.

Methods: Association between LRP5 gene polymorphisms and bone size and mineralization was examined in 819 unrelated British Caucasian children (n = 429 boys) aged 9 years. Height, weight, pubertal status (Tanner stage) and total body and spinal bone area, bone mineral content (BMC), BMD, area-adjusted BMC, and area-adjusted BMD were assessed. 140, 79, 12, and 2 girls were described as Tanner stages I–IV respectively. DXA–gene associations were assessed in boys and girls and in girls alone. Association between continuous variables and genotypes was assessed by ANOVA with data corrected for covariates as described.

Results: A coding C141759T polymorphism in exon 10 of the LRP5 gene was associated with spinal BMAD (P = 0.01), spinal BMC (P = 0.01), and area-adjusted spinal BMC (P = 0.01) in both boys and girls adjusted for age, gender, height, and weight. When these results were adjusted for pubertal status (n = 219 girls, 429 boys), the association with area-adjusted spinal BMC and spinal BMAD was strengthened (P = 0.003 and P = 0.002). Carriage of the minor T allele was associated with greater spinal BMD and area-adjusted spinal BMC scores, with a codominant effect apparent. The coding G138351A polymorphism in exon 9 was associated with total body BMC (P = 0.02) and total body area (P = 0.03) in girls following adjustment for age and pubertal status. Haplotyping studies confirm localization of bone mass traits to the LRP5 gene, but no haplotypic associations were stronger than single-point analyses.

Conclusions: LRP5 gene polymorphisms are associated with area-adjusted spinal BMD and BMC and total body BMC and area in children. These results indicate that the influence of LRP5 upon bone mass is relevant to both boys and girls and already evident in pre- and early puberty.

P578-Mo

A Repeat Polymorphism in the CLCN7 Gene Influences Bone Density in Patients with Autosomal Dominant Osteopetrosis (ADO) Type II and in Post-menopausal Women

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We hypothesized that polymorphisms in the CLCN7 gene could be associated with the variability of bone density in autosomal dominant osteopetrosis (ADO II) and in the general population. Therefore, we investigated the frequency of the synonymous SNP rs3751884 in exon 1, the non-synonymous SNP rs12926089 in exon 15 and a polymorphic variable number tandem repeat (VNTR) in intron 8 in a large family with a mild variant of ADO II caused by a mutation in CLCN7. The penetrance of the disorder was exceptionally low (9 affected and 9 non-affected mutation carriers). No association between the phenotype of the carriers and the two SNPs was observed. In all affected carriers, however, we detected only 3 repeat units in intron 8 of the non-mutated allele, whereas the frequency of this allele was significantly lower in other family members (P = 0.02). We then analyzed if the length of the intron 8 VNTR could be associated with a higher bone density in 394 post-menopausal women. After adjustment for age, height, weight, years since menopause and hormone replacement therapy, women with 3 repeat units on both alleles (3/3) had a slightly higher BMD at the femoral neck (0.78 + 0.14 vs. 0.74 + 0.12 mg/cm^2 (P < 0.01) than individuals with higher repeat numbers. In addition, we found a significant association of the 3/3 genotype with lower deoxypyridinoline levels (P = 0.04). In conclusion, a VNTR polymorphism in intron 8 of CLCN7 seems to have a small impact on BMD in ADO II and in the normal population.

P579-Tu

Osteoporosis Pseudoglioma Syndrome: A Novel LRP5 Mutation

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Osteoporosis pseudoglioma syndrome (OPPG) is a rare autosomal recessive disorder caused by inactivating mutations in LRP5 (Low density lipoprotein receptor-related protein 5) localized in 11q13.4. This pediatric syndrome includes early-onset blindness with severe osteoporosis and sometimes other clinical features (1). To date, more than 12 mutations have been described in OPPG. Here, we report a case of OPPG in a 19-year-old boy with a new LRP5 mutation. This patient is from a consanguineous family of Tunisian origin, has already had 8 fractures in childhood (tibias) and surgery for inter-auricular communication. Clinical examination showed a short stature (1.32 m for 42 kg), hypotonia, lower limbs deformities and blindness with microphtalmia. The DXA (Hologic QDR1000, Waltham, MA) results gave a lumbar BMD of 0.409 g/cm^2 and a lumbar Z score of −4.3. Calcemia, calciuria, phosphatemia, phosphaturia, intact PTH and 25OHVitD were normal. Mutation analysis of the LRP5 gene revealed a homozygous 5-base pair insertion (1048_1049insGGACA) in exon 5. This would cause a frame shift

at amino acid 334 (R334fsX51) and leads to the appearance of a stop codon 51 amino acid downstream. Furthermore, the protein would lack 1231 amino acids, and the co-receptor would not be functional. The treatment of this severe osteoporosis includes regular perfusion of pamidronate with no new fracture. This new case of OPPG emphasizes the major role of LRP5 in bone accrual and eye development (2).

(1) Levasseur R, Lacombe D, de Vernejoul MC. LRP5: the gene mutated in osteoporosis–pseudoglioma syndrome and the high bone mass phenotype. Joint Bone Spine 2005 (in press).

(2) Gong Y, Slee RB, Fukai N, Rawadi G, Roman-Roman S, Reginato AM, et al. LDL receptor-related protein 5 (LRP5) affects bone accrual and eye development. Cell. 2001; 107 (4): 513–23.

P580-Su

Identification of Polymorphisms in the MKP1 Gene and their Association with Bone Mineral Density in Men with Asthma

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Mitogen-activated protein kinases (MAPK) play an important role in osteoblast function and regulate osteoblast growth stimulated by PTH and growth factors. Regulation of MAPK activity is mediated, in part, by dephosphorylation of tyrosine and threonine residues that become phosphorylated during the process of MAPK activation. These dephosphorylation reactions are carried out by a family of enzymes termed dual specificity phosphatases (DUSP). The MKP1 gene encodes a DUSP family member, which is responsible for dephosphorylation of ERK, which plays an essential role in osteoblast growth. Moreover, MKP1 has been shown to mediate the inhibitory effects of glucocorticoids on osteoblast growth by dephosphorylating ERK. These observations raise the possibility that MKP1 could be a candidate gene for regulation of bone mass or the response to glucocorticoids. Here, we conducted mutation screening of the MKP1 gene and investigated possible associations between MKP1 polymorphisms and bone density in a population-based cohort of 260 asthmatic subjects. We identified seven SNP on mutation screening, including two promoter SNP, two intronic SNP and three exonic SNP. Two exonic SNP predicted amino acid substitutions, an alanine–threonine change at codon 56 in exon 1 and a tyrosine–histidine change at codon 187 in exon 3, but these were both relatively uncommon (allele frequency ~3.5%). The promoter SNP were in complete linkage disequilibrium (LD) with each other, and there was also strong LD between the other SNP in MKP1. Analysis of 5 informative SNP in relation to BMD values in the asthmatic subjects revealed a significant association between the promoter polymorphism at position −600, relative to the start codon and lumbar spine BMD in men, such that homozygotes for the T allele had BMD values approximately 0.2 g/cm^2 higher than the other genotype groups, after correcting for age, body weight and corticosteroid use (1.40 ± 0.02 g/cm^2 (n = 5) vs. 1.20 ± 0.07 cm^2 (n = 107); P < 0.02). In conclusion, we have found evidence for an allelic association between a SNP in the promoter of the MKP1 gene and LS-BMD in male asthmatics, raising the possibility that MKP1 may contribute to the genetic regulation of bone mass. Caution must be exercised in interpreting these findings in view of the small number of TT homozygotes however, and further studies will be required to confirm and extend these observations.

P581-Mo

Analysis of Murine Chromosomes 11 and 13 Loci that Control Peak Bone Mass

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Previously, we identified two significant quantitative trait loci (QTLs) specifying the peak bone mass on Chrs 11 and 13 by interval mapping in two mouse strains, SAMP2 and SAMP6. The latter strain exhibits a significantly lower peak bone mass than the former strain. The Chrs 11 and 13 loci were designated as Pbd (Peak bone density) 1 and 2, respectively. We constructed four congenic strains to confirm the effects of both loci on murine peak bone mass. P6.P2-Pbd1b and P6.P2-Pbd2b, which have P2-derived intervals transferred onto P6-derived backgrounds, have higher peak bone mass than the background strain, SAMP6. P2.P6-Pbd1a and P2.P6-Pbd2a, which have P6-derived intervals transferred onto P2-derived backgrounds, have lower peak bone mass than the background strain, SAMP2. These results showed that both Pbd1 and Pbd2 loci regulate peak bone mass of SAM strains. The phenotypes of these congenic strains were confirmed by three measurement methods, each utilizing different measurement principles; MD, DXA and pQCT. By cross-mating P6.P2-Pbd1b and P6.P2-Pbd2b strains with their background strain, SAMP6, and identifying those progeny that have recombination within the SAMP2-derived intervals, we have made new congenic sublines (subcongenic strains) that harbor smaller

segments of the donor-derived intervals to contract the QTL regions on Chrs 11 and 13. In this study, we report high-resolution congenic maps on peak bone mass by constructing nine subcongenic strains on Chr 11 QTL (Pbd1) and five subcongenic strains on Chr 13 QTL (Pbd2). Finally, we delineated a 10.9 Mbp on murine Chr 11 and a 5.8 Mbp on murine Chr 13, corresponding to human Chrs 17q and 7p, respectively. Furthermore, genomic DNA sequences of 127 genes around the murine Chr 13 (Pbd2) locus were determined from SAMP6 and SAMP2 strains. In this region,74 known or putative genes were assigned, and 15 cSNPs between the two strains were found.

P582-Tu

The RANKL Inhibitor Osteoprotegerin Causes Rapid and Significant Increases in Bone Density in Rats Co-treated with Parathyroid Hormone

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Combination therapy is a promising approach for osteoporosis, but a recent clinical trial demonstrated that the bisphosphonate alendronate (ALN) significantly blunted the anabolic effects of parathyroid hormone (PTH) in postmenopausal women (Black et al., NEJM 2003). This result suggests that bone resorption may be essential for PTH anabolism. However, in aged ovariectomized rats treated with PTH, the RANKL inhibitor osteoprotegerin (OPG-Fc) had additive effects on bone mineral density (BMD) and bone strength, despite the profound antiresorptive effect of OPG-Fc (Kostenuik et al., Endo 2001). ALN was also recently shown to block the anabolic effects of PTH in elderly men (Finkelstein et al., NEJM 2003). We used OPG-Fc to determine if bone resorption is essential for PTH anabolism in adult male rats. Rats were treated with PBS, or OPG-Fc (10 mg/kg, once/week), or PTH-(1–34) (80 mg/kg, three times/week), or OPG-Fc + PTH for 4 weeks. OPG-Fc or PTH each significantly increased BMD compared to PBS-treated rats. Rats treated with OPG-Fc + PTH had more rapid and significantly greater gains in BMD compared to rats treated with either monotherapy ($P < 0.05$). These data suggest that bone resorption per se is not a requirement for PTH anabolism in male rats. We hypothesized that OPG enhances rather than blunts PTH anabolism because of the favorable remodeling balance induced by RANKL inhibition. We compared the relative balance of bone formation and resorption markers in male rats after a single subcutaneous injection of OPG-Fc (5 mg/kg) or ALN (1 mg/kg). Serum was collected regularly for 4 weeks and assayed for TRAP-5b (a resorption marker) and osteocalcin (a formation marker). The relative balance of formation and resorption was analyzed by subtracting *Z* scores for TRAP-5b from *Z* scores for osteocalcin. The resulting Uncoupling Index revealed significantly higher scores for OPG-Fc versus ALN from day 1 through day 14 ($P < 0.05$), after which the differences became non-significant. This comparison suggests that RANKL inhibition results in greater preservation of bone formation relative to the suppression of bone resorption. In conclusion, these data confirm that the dramatic suppression of bone resorption associated with RANKL inhibition does not blunt, but rather enhances, the ability of PTH to increase BMD. Biomarker data suggest that this phenomenon may be related to a favorable remodeling balance induced by RANKL inhibition.

P583-Su

Pharmacokinetics (PK), Pharmacodynamics (PD), and Safety of AMG 162, a Fully Human Monoclonal Antibody to RANKL, Following a Single Subcutaneous Dose to Healthy Men Aged 50 Years and Older

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AMG 162 is a fully human monoclonal antibody that binds with high affinity and specificity to receptor activator of NF Kappa B ligand (RANKL). This blinded, controlled, single-dose study evaluated the PK, PD, and safety of AMG 162 in healthy men aged >50 years. Forty subjects were randomized 4:1 to receive single subcutaneous (SC) doses of AMG 162 (0.1, 0.3, 1, or 3 mg/kg) or placebo, with subjects stratified by age (50–64 years; >65 years; $n = 20$/stratum). Samples were collected for determinations of serum AMG 162 concentrations and urinary N-Telopeptide/creatinine ratios (uNTx/Cr) throughout study. PD were evaluated as percentage change from baseline of the bone resorption marker uNTx/Cr. After SC dosing, AMG 162 absorption was rapid and prolonged (median T_{max}: 7–10 days). Following peak levels, AMG 162 concentrations declined in a biphasic manner. While levels were above ~1000 ng/mL, serum concentrations declined linearly. Below 1000 ng/mL, AMG 162 clearance increased, and serum levels declined at an increasing rate. The nonlinear disposition of AMG 162 resulted in apparent clearances that decreased 63%, mean exposures that increased ~80-fold, and mean residence times that increased from 21.0 to 49.2 days across a 30-fold change in dose. The PK of AMG 162 resulted in detectable levels 38 weeks after a single SC dose of 3 mg/kg. AMG 162 significantly suppressed uNTx/Cr vs placebo ($P < 0.05$) at all doses. Significant decreases from baseline in uNTx/Cr were observed within 24 h (all doses) and reached average maximum suppressions of greater than 70% (all doses) thereafter. The significant suppression in uNTx/Cr levels continued up through day 113 (0.3–3 mg/kg), after which levels returned towards baseline. The PK and PD of AMG 162 appeared to be well correlated, as uNTx/Cr suppres-

sion was maintained as long as AMG 162 serum levels were above ~300 ng/mL. No differences due to age in either the PK or PD were detected. Only two, non-treatment-related, serious adverse events were reported in 1 subject (lung cancer; pneumonia). No AMG 162 antibody response was reported. Thus, single SC doses of AMG 162 in healthy older men demonstrated nonlinear PK; rapid, profound, and sustained effects on the bone resorption marker uNTx/Cr; and were well tolerated at all dose levels investigated. These results suggest that AMG 162 may be effective in older men to treat conditions related to increased bone loss, such as osteoporosis or bone metastatic diseases.

P584-Mo

Strontium Ranelate Promotes Chemotaxis, Stimulates Proliferation and Induces Genes Involved in the Differentiation of Rat Primary Osteoblasts

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Strontium ranelate is a recently proposed therapy for postmenopausal osteoporosis that acts through an innovative mode of action, both stimulating bone formation and decreasing bone resorption, resulting in the rebalancing of bone turnover in favor of bone formation. While these actions have been demonstrated in vivo and in vitro in various models,[1] the mechanism(s) underlying them remain under investigation. We hypothesize that an extracellular calcium-sensing receptor (CaR), cloned from parathyroid gland, could act as the target for strontium. Evidences in favor of our hypothesis are expression of the CaR in rat primary calvarial osteoblast and the ability of strontium ranelate to activate the CaR in heterologous cell system.[2] The present study investigated the possible involvement of CaR in mediating strontium ranelate effects on rat primary osteoblasts (OB). Strontium stimulated the proliferation of OB by 2–3-fold at 5–15 mM, an effect that is significantly attenuated when the cells are infected with dominant negative CaR by rAAV technology. This result suggests that strontium action is CaR-mediated. Furthermore, 3.5 and 7.5 mM strontium enhanced the chemotaxis of OB by 2- and 3-fold, respectively, as measured using a Boyden's chamber assay. Strontium induced the mRNA for the OB early response gene, c-*fos*, by ~6.0-fold or more, as assessed by QPCR. Similarly, strontium upregulated early OB differentiation marker, matrix Gla protein (MGP), by ~9.0-fold. These genes are also upregulated by elevated calcium. Therefore, strontium emulates the actions of calcium on key parameters of OB function that contribute to expanding the pool of pre-OB (proliferation), recruiting OB and pre-OB to sites of recent bone resorption (chemotaxis), and differentiation to mature, bone forming OB (activation of early response gene and increased expression of MGP). Furthermore, the inhibitory effects of the dominant negative CaR on the mitogenic action of strontium supports a mediatory role for the CaR in vitro and, presumably, in vivo in patients being treated with strontium ranelate for postmenopausal osteoporosis.

[1] Marie, 2001.
[2] Quinn, 2004.

P585-Tu

Strontium Ranelate Decreases In Vitro Human Osteoclastic Differentiation

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Strontium ranelate is a new anti-osteoporotic agent that has demonstrated its efficacy in reducing both vertebral and hip fractures in osteoporotic postmenopausal women. Strontium ranelate acts through an original mechanism of action by increasing bone formation and decreasing bone resorption. This results in a rebalance of bone turnover in favor of bone formation. Several studies have already demonstrated the inhibitory effect of strontium ranelate on osteoclastic bone resorption. The aim of the present study was to investigate the effect of strontium ranelate on in vitro osteoclastic differentiation. For this purpose, two cellular models were used: a human model using PBMC (Peripheral Blood Monocytic Cells) in the presence of RANKL and M-CSF and, secondly, a murine model of RAW 264.7 cells in the presence of RANKL. Strontium ranelate was tested in conditions close to the in vivo situation, i.e. by reproducing the same proportion of strontium ions associated to ranelic acid present in the plasma of patients. Strontium and ranelic acid were tested simultaneously from 0.1 to 24 mM and from 0.001 to 0.24 mM, respectively. Osteoclastic differentiation was assessed by measuring TRAP activity, by counting the TRAP-positive multinuclear cells or osteoclasts-like (OCLs) and by the assessment of the bone resorption activity. Our results indicate that strontium ranelate inhibits the osteoclastic differentiation in both models. TRAP activity, OCLs counting and bone resorption activity decrease in a dose-dependent manner. Significant differences occur at concentration levels from 2 mM of strontium in the human model: OCLs number was evaluated to 70% of the control, TRAP activity to 77% of the control and bone resorption decreases to 48% by bone area assessment ($P < 0.05$). Hydroxylysylpyridinoline measurements confirm the decrease of bone resorption and is significantly decreased at 6 mM (46% of control). In the murine model, significant results are obtained at higher concentrations (i.e. 6 mM of strontium), TRAP activity decreases to 67% and OCLs number represents 78% of the control ($P < 0.05$). Taken together, these data show that

strontium ranelate decreases production of bone resorbing cells. Therefore, osteoclastic precursors are important cellular targets of strontium ranelate when given as a treatment for osteoporosis.

P586-Su
Protein Kinase C are Involved in Strontium-ranelate-induced Osteoclast Apoptosis
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Strontium ranelate is a new osteoporotic medicine that significantly reduces vertebral and hip fracture risks in postmenopausal women. Strontium ranelate acts through an innovative mode of action, both stimulating bone formation and decreasing bone resorption, resulting in a rebalance of bone turnover in favor of bone formation. The intracellular signaling pathways are under investigation. We and others have recently demonstrated that extracellular strontium (Sr) is a full agonist of the extracellular calcium-sensing receptor (CaR)[1] and that Sr inhibits bone resorption by increasing OCs apoptosis[2]. We investigated intracellular signaling pathways which could be modulated by the CaR[3] on both calcium (Ca)- and Sr-induced OCs apoptosis. Whereas phospholipase C was involved in the Sr- and Ca-induced OCs apoptosis, specific inhibitors of the IP3-dependent signaling reduced by 50% the Ca-induced effects and failed to modulate the Sr-induced OCs apoptosis. These results suggested that strontium ranelate, at concentrations ranging from 1 to 24 mM, acts through different intracellular signaling pathways which might be diacylglycerol and protein kinase C (PKC)-dependent. In the present study, purified mature OCs were treated with nonselective PKC inhibitors (calphostin C, staurosporine), PKC activators (phorbol esters: PMA, PDBu) or with an inactive phorbol ester as a negative control. OCs apoptosis displayed a dose-dependent increase reaching 80% and 100% with, respectively, PMA (5 μM) and PDBu (1 mM), while inactive phorbol failed to induce OCs apoptosis. Cells were then cultured with Sr (24 mM) in presence or absence of PKC inhibitors. Calphostin C (1 nM) or staurosporin (10 nM) significantly reduced OCs apoptosis compared to cultures treated with Sr alone. Interestingly, such PKC inhibitors have no effects on the Ca-induced OCs apoptosis, confirming that intracellular pathways involved in Sr or Ca effects are differents. All together, our data indicate that strontium ranelate acts on OCs apoptosis through PKCs, while Ca effects are IP3-dependent. Such results agree with literature data[4] and confirm the major role of the PKC pathway in the regulation of OCs function during the bone resorption process. Among the 11 PKC isozymes, PKC-alpha, -delta and -epsilon are homogeneously expressed by osteoclastic lineage[5], and their role in strontium-ranelate-induced OCs apoptosis remains to be precised. 1 Quinn, 2004. 2 Mentaverri, 2004. 3 Brown, 2003. 4 Moonga, 1996. 5 Teti,1995.

P587-Mo
Sevelamer Prevents Bone Loss in Ovariectomized Rats
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In patients with chronic kidney failure (CKD), hyperphosphatemia is associated with renal osteodystrophy, vascular and soft-tissue calcification, and mortality. In a randomized clinical trial, we recently reported that sevelamer hydrochloride (Renagel®), a non-calcium phosphate binder, is capable of reducing progressive coronary artery and aortic calcification and significantly increasing trabecular bone mineral density (BMD) in CKD (stage 5) patients as compared to calcium containing phosphate binders (acetate and carbonate salts) (JBMR, in press). In the present study, we examined whether Renagel could also prevent bone loss that follows ovariectomy in rats (an animal model of osteoporosis). Six-month-old rats (n = 96) were ovariectomized (OVX) and divided into following groups: (1) Sham; (2) Sham + sevelamer 3%; (3) OVX; (4) OVX + sevelamer 1%; (5) OVX + sevelamer 3%. Sevelamer was mixed with food immediately following OVX, and therapy was continued for 25 weeks. The whole body, hind limbs and lumbar spine bone mineral density (BMD) were monitored by DEXA in vivo throughout the study. Ex vivo measurements of femur, tibia and lumbar spine were performed using DEXA, pQCT and μCT. At 4 and 8 weeks following administration, sevelamer (at both doses) significantly increased BMD at all measured sites. Although sevelamer did not maintain the increased BMD at later time intervals (above 20 weeks) as compared to control animals, sevelamer (3%) progressively increased BMD of sham animals towards 20 weeks following administration, and it was maintained until the termination at week 25 and was confirmed by ex vivo measurement of BMD. These results suggest that sevelamer has a positive effect on bone density and likely bone mass in both normal and osteoporotic animals.

P588-Tu
Effect of Atorvastatin to RANKL/OPG System in Patients with Acute Myocardial Infarction
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Background: The prominent effect of statin therapy is the inhibition of hydroxymethyl glutaryl-Coenzyme A (HMG-

CoA) reductase. This enzyme decreases the mevalonate level and prevents the synthesis of isoprenoids. This inhibition alters osteoclast activity. Osteoprotegerin acts as a decoy receptor of the receptor activator of nuclear factor kB (NF-kB) ligand (RANKL) which is a key regulator of osteoclastogenesis.

Objectives: To evaluate the effects of atorvastatin to RANKL/OPG system in patients with acute myocardial infarction.

Methods: 22 patients (17 men and 5 women, age 61 ± 8 years) referred to Hospital Rio Hortega with the diagnostic of acute myocardial infarction. Blood was drawn in the morning after a 12-h fast. The following parameters were determined: osteocalcin, paratohormone, 25-vitamin D, RANKL and osteoprotegerin. Deoxypiridinoline was determined in urine sample. The same parameters were determined after 12 months. The patients were treated with atorvastatin (20, 40 or 80 mg). All patients gave their written informed consent. Results are expressed as mean ± SD. The differences were tested by the Mann–Whitney nonparametric *U* test. The relationship between RANKL and osteocalcin was studied by Spearman correlation.

Results: The results are showed in Table 1.

A final Spearman correlation test showed a significant positive correlation between RANKL and osteocalcin.

Conclusion: Atorvastatin decreases RANKL and osteocalcin levels in patients with acute myocardial infarction. The atorvastatin can increase the bone mineral density inhibiting the resorption through RANKL/OPG system.

Table

	Initial	Final
Osteocalcin ng/ml	4 ± 2	2.6 ± 1.2 $P = 0.043$
Deoxypiridinoline	5.3 ± 1.7	5.1 ± 1.6
RANKL pmol/l	0.17 ± 0.13	0.10 ± 0.14 $P = 0.02$
OPG pmol/l	12 ± 5	12 ± 4
RANKL/OPG	0.02 ± 0.02	0.01 ± 0.02 $P = 0.04$
25-vitamin D nmol/	41 ± 18	55 ± 22
PTHi pg/ml	45 ± 30	46 ± 30

P589-Su
Oseine–hidroxiapatite Versus Calcium Ccarbonate Effects in Bone Metabolism in Primary Senile Osteoporosis

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Aim: To evaluate the effect of two types of calcium and vitamin D treatment through bone metabolism markers in senile osteoporosis.

Materials and methods: Open, randomized and prospective study. We included women with primary densitometric osteoporosis without fractures. Women were randomized in two groups. Group 1 (G1) received oseine–hidroxiapatite complex (4 pills, 830 mg per pill) with 800 units of vitamin D3 daily. Group 2 (G2) received calcium carbonate (1.000 mg) and 800 units of vitamin D3 daily. The following variables were assessed, at the first year of the study and each year of follow-up: serum calcium, phosphorus, intact parathormone (PTH-i), vitamin D3, total alkaline phosphatases (TAP), bone GLA protein (GLA) and tartrate-resistant acid phosphatase (TRAP), urinary calcium excretion corrected with urine creatinine excretion and bone mineral density in lumbar spine and in femoral neck with DEXA equipe (Hologic QDR 1000).

Results: 115 women were included in the study (G1 = 55, G2 = 60). The two groups were comparable in all variables (body mass index, age, age of menopause, laboratory data and densitometric data). One hundred and eight women completed the first year of treatment (G1 = 52, G2 = 56), ninety-two completed 2 years (G1 = 44, G2 = 48) and fifty-four women completed 3 years (G1 = 25, G2 = 29). Serum GLA levels rise during the 3 years of treatment in G1 (1 year = 0.4 ± 3.11, 2 years = 0.84 ± 3.12, 3 years = 1.86 ± 2.77). In the other group (G2), the bone Gla protein levels were reduced the first and second year and rise the third year (1 year = −0.26 ± 1.85, 2 years = −0.39 ± 1.4, 3 years = 0.31 ± 2.25). The inter-group differences were statistically significant the second year of treatment (G1 = 3.71 ± 3.2 G2 = 2.54 ± 1.37, $P = 0.038$) and the third year (G1 = 4.63 ± 2.8 G2 = 3.32 ± 2.29, $P = 0.05$). There are no differences in the other studied variables.

Conclusions: The oseine–hidroxiapatite complex use is related with a rise of bone formation evaluated between serum levels of bone GLA protein. This bone formation stimulation is greater with oseine–hidroxiapatite complex use than calcium carbonate use. This beneficious effect is present, at least, during 3 years.

P590-Mo
Mononfluorophosphate and Hormone Replacement Therapy: A Randomized Controlled Trial

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High doses of fluoride increase trabecular bone density, but clinical trials have not shown any reduction in vertebral fracture rate. This could be the result of excessive fluoride dose or failure to inhibit bone resorption. We have tested the hypothesis that the antifracture efficacy of fluoride could be enhanced by using a lower dose regimen on the background of established antiresorptive therapy.

80 postmenopausal women with osteoporosis (previous vertebral fracture and/or lumbar spine *T* score < −2.5) who were established for 1 year on hormone replacement therapy (conjugated estrogens ± medroxyprogesterone) were randomized to receive either 20 mg fluoride in the form of

monofluorophosphate (F, $n = 39$) or placebo (P, $n = 41$) daily for 4 years in a double blind RCT. All subjects received calcium supplementation.

Bone density (BMD) was assessed annually by DEXA, and vertebral fractures were assessed by radiography. Bone turnover markers were measured in 20 randomly selected subjects from each group. Bone biopsies were taken from 16 subjects at the end of 4 years' treatment.

Over 4 years, there was no significant loss of BMD from any site in the P group. In the F group, large progressive increases occurred in lumbar spine BMD (16% in anterior and 45% in lateral projections, respectively, both $P < 0.0001$), with the greatest gain seen in the first 2 years. Increments of BMD ranging from 2–5% were also seen at other sites (total body, femoral neck and legs). The formation markers osteocalcin and PINP both showed significant increases in the F group ($P < 0.0005$, $P = 0.01$, respectively), but there was no change in the P group. The resorption marker βCTX did not change in either group. In the P group, 5 vertebral fractures occurred in 5 subjects; in the F group, 1 vertebral fracture occurred in 1 subject. The P group lost a mean 0.48% height over the 4 years, and the F group 0.25%. Hyperosteoidosis was seen in 5/7 biopsies from F subjects (71%), but in 0/9 P subjects ($P = 0.005$).

We conclude that fluoride in doses equivalent to 20 mg/day stimulates bone formation when given with antiresorptive therapy. It produces significant increases in BMD, particularly in the spine, and this may be associated with a reduced rate of vertebral fracture. The possibility exists that doses of fluoride even lower than used in this trial may be effective at increasing BMD and reducing vertebral fracture, without inducing hyperosteoidosis.

P591-Tu

Strontium Ranelate Reduces the Risk of Vertebral Fractures in Osteoporotic Postmenopausal Xomen Whatever the Baseline Vertebral Fracture Status

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The anti-fracture efficacy of strontium ranelate 2g/day, a new orally active anti-osteoporotic agent with an innovative mode of action both increasing bone formation and decreasing bone resorption, has been recently reported. The SOTI study (1649 patients) demonstrated a significant decrease in the reduction of vertebral fracture risk in postmenopausal osteoporotic women by 41% over 3 years ($P < 0.001$). The TROPOS study (5091 patients) demonstrated the efficacy in reducing nonvertebral and hip fractures: in the intent-to-treat population over 3 years, a significant reduction of 36% in the relative risk of hip fracture was shown in high risk osteoporotic postmenopausal women ($P = 0.046$). A pre-determined analysis of pooled data of SOTI and TROPOS was performed (the study designs, centers, BMD and X-ray central reading centers were common to both studies). Among the whole pooled population, vertebral X-rays were performed yearly (semi-quantitative assessment) in 5082 women receiving strontium ranelate 2g/day orally or placebo plus calcium/vitamin D in both groups during 3 years. There was no difference between groups for baseline characteristics of age 74 ± 6.2 years; lumbar BMD T score −3.0 ± 1.6; femoral neck BMD T score −3.0 ± 0.7 (mean ± SD). Among those 5082 women, 2605 had no prevalent vertebral fracture (1320 in strontium ranelate group versus 1285 in placebo), 1110 had one prevalent vertebral fracture (533 versus 577) and 1365 had at least 2 prevalent vertebral fractures (682 versus 683). Whatever the baseline vertebral fracture status, a significant reduction in the incidence of patients experiencing a vertebral fracture was demonstrated in the intent-to-treat population over 3 years with a reduction of the relative risk by: (1) 48% in 2605 women without prevalent vertebral fracture (RR = 0.52, 95%CI [0.40; 0.67], $P < 0.001$). 87 patients in strontium ranelate group and 161 in placebo had a vertebral fracture during the study, (2) 45% in 1110 women with one only prevalent vertebral fracture (RR = 0.55, 95%CI [0.41; 0.74], $P < 0.001$), 70 patients in strontium ranelate group and 130 in placebo, (3) 33% in 1365 women with at least 2 prevalent vertebral fractures (RR = 0.67, 95%CI [0.55; 0.81], $P < 0.001$), 184 patients in strontium ranelate group and 252 in placebo. In conclusion, strontium ranelate 2g/day orally is a new anti-osteoporotic treatment effective in reducing the risk of vertebral fracture in osteoporotic postmenopausal women whatever the prevalent vertebral fracture status.

P592-Su

Calcium-sensing Receptor Mediates Strontium-ranelate-induced Osteoclasts Apoptosis

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Strontium ranelate is a new compound proposed in the treatment of postmenopausal osteoporosis to reduce vertebral and hip fracture risk in women. Strontium ranelate both

stimulates bone formation and decreases bone resorption, resulting in a rebalance of bone turnover in favor of bone formation. As Ca, extracellular strontium (Sr), is a full agonist of the calcium sensing receptor (CaR). However, to date, there were no direct proofs of the role played by CaR in Sr effects on osteoclastic bone resorption and osteoclast (OCs) apoptosis. Firstly, the present study used bone cells isolated from wild-type or CaR knock-out mice (as negative control) and confirmed by immunohistochemistry and confocal microscopy that OCs express CaR. Secondly, the present study demonstrated the role of CaR in strontium ranelate effects using mature rabbit OCs: (1) increasing Sr concentrations, up to 25 mM, stimulate OCs apoptosis by 40% (Hoechst staining). Western blot analysis using anti-caspases 3 and 9 antibodies as well as specific caspases inhibitor peptides (Z-VAD-fmk, Z-LEHD-fmk) clearly demonstrate that the caspases cascade is involved in Sr effects inducing OCs apoptotic cell death. (2) For the first time, we established with the immunocytochemical method[1] that increasing Sr directly triggers a transient translocation of NFkB into osteoclastic nuclei. Temporal regulation of NFkB activity regulates expression of numerous genes involved in cell survival as well as in cellular response to inflammation and stress. This effect is maximal at 30 min, where 50% of OCs show a nuclear localization of NFkB. (3) Both Sr-induced NFkB nuclear translocation and OCs apoptosis were blocked by the phospholipase C inhibitor (U73122, 10 mM), indicating that a G-protein-coupled receptor could be related to Sr effect on mature OCs. (4) Moreover, in mature rabbit OCs infected with CaR dominant negative (rAAV technology), the Sr-induced NFkB nuclear translocation was abolished, and the Sr-induced OCs apoptosis was significantly reduced by galactosidase-transfected cells (i.e. controlβ 50% compared to infected cells). This approach unequivocally demonstrates that Sr acts at least in part through CaR to enhance the OCs apoptosis, confirming many converging proofs. Further studies need to be carried out in order to specify the role played by NFkB signaling in these cellular events and whether this pathway is involved in strontium ranelate effects on bone resorption.

1 Komarova, JBC 2003.

P593-Mo

Experimental Comparison of Bone-Invigorative Herbal Medicine and Kidney-Invigorative Herbal Medicine in Treating Rat Osteoporosis by Ovariectomy

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The Chinese traditional herbal medicine (CH) has got great progress in the treatment of osteoporosis. According to its theory, it is believed that "bone" includes the anatomical bone, marrow, and cartilage, the bone-invigorative herb (BIH) seems to exert an influence on bone metabolism directly, and that "kidney" includes the anatomical kidney, the partial endocrine system and the immune system, contains a distillate of the human body. The "kidney" dominates and controls bone and marrow and plays a key role in growth and formation of bone. Thus, the strong "kidney" can nourish bone and make it flourish, but the weak "kidney" makes bone perish. The kidney-invigorative herb (KIH) seems to exert an influence on bone metabolism indirectly. In previous studies, these CH increased calcium and phosphorus in serum and bone density. The present study was undertaken to investigate different mechanisms of BIH and KIH in treating osteoporosis induced by ovariectomy. 60 female Wistar rats were randomly divided into 2 × 3 groups (10/group), 2 normal control group (NC), 2 ovariectomized group (OVX), and 2 OVX-treated with the CH (COVX). The COVX administered orally with BIH at 5 ml/kg/day (300 mg/kg) was marked BCOVX, and the COVX administered orally with the KIH at 5 ml/kg/day (300 mg/kg) was marked KCOVX. All rats were killed after 90 days. The serum ALP, IL-6, estradiol, bone IGF-1, and bone mineral density (BMD) were measured, and bone histomorphometry was performed. The level of the estrogen receptor messenger RNA (ER mRNA) of bone was detected by quantitative real time-PCR. Histomorphologically, it was observed that OVX showed a reduction of bone mass compared with NC. The trabecular bone area in COVX increased by 65% in comparison with OVX and also femur bone density (BMD) enhanced ($P < 0.05$). Immunohistostaining showed a positive reaction for IGF-1 in specific areas of the cytoplasm in osteoblasts and osteocytes. The image analysis indicated that the positive area increased, whereas the gray degree decreased in femurs of COVX. Immunoradioassayly, it was showed that the KIH increased level of estradiol ($P < 0.01$), ALP ($P < 0.01$), and IGF-1 ($P < 0.05$) decreased IL-6 ($P < 0.01$) of serum in KCOVX in comparison with the OVX. The BIH did similarly without raising serum estradiol. Molecularly, ER mRNA level showed a sharp decrease in OVX and BCOVX but increase in KCOVX in comparison with NC. The KIH may play a hormone-like action in treating osteoporosis.

P594-Tu

What is the Basis for the Distinct Differences in the Therapeutic Efficacy of Alfacalcidol and Plain Vitamin D?

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The respective roles of plain vitamin D and active analogs in prevention and therapy of osteoporosis are poorly understood by most physicians, and there is urgent need to clarify the underlying biological and pharmacological differences and the rationale for a differentiated use of the one or the other. Vitamin D produced in the skin or from nutritional intake will be completely hydroxylated in the liver to 25-OH-D. There is no regulation of this first step of activation. The second step, however, the renal 1\alpha-hydroxylation is strongly feedback-regulated. In states of sufficient 1\alpha,25-(OH)2-D, the 1\alpha-hydroxylase will be downregulated, and no further activation towards 1\alpha,25-(OH)2-D will take place. Accordingly, treatment with plain vitamin D is a nutritional supplementation effective in vitamin D deplete patients with normal kidney function. In vitamin D replete patients or renal insufficiency, no biological effect can be expected. Alfacalcidol (1\alpha-OH-D) is a synthetic active analogue of vitamin D which is already hydroxylated at the crucial 1\alpha-position what physiologically would take place in the kidney only after hepatic hydroxylation in patients with D-hormone deficiency. After oral intake and intestinal absorption, 1\alpha-OH-D will be automatically hydroxylated in the liver to 1\alpha,25-(OH)2-D, that is, alfacalcidol is a prodrug to calcitriol (D-hormone). The major difference to plain vitamin D is the fact that by this kind of activation the abovementioned feedback regulation of the final renal activation is bypassed by a direct activation in the liver. There is evidence that a smaller part of 1\alpha-OH-D will be activated by a 25-hydroxylase expressed by osteoblasts, i.e. locally in bone tissue. Accordingly, a localized autocrine or paracrine effect in bone tissue can be achieved when using alfacalcidol in addition to systemic D-hormone effects. The average daily dose of alfacalcidol is 0.5–1.0\μg. No toxic side effects have been described. The calcium supplementation during alfacalcidol therapy should be adjusted to dietary calcium intake. In general, daily calcium supplements should not exceed 500 mg. We conclude that alfacalcidol is an active hormonal therapy. This view is supported by an own study comparing head-to-head alfacalcidol and plain vitamin D in patients with established glucocorticoid-induced osteoporosis and by recent meta-analyses comparing plain vitamin D and active analogs in postmenopausal and glucocorticoid-induced osteoporosis.

P595-Su

The Effect of Oral Transfer Factor on Development of Osteopenia in Ovariectomized Rats

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Transfer factor (TF) is a dialysate of homogenized porcine leucocytes (Imunor®, S&D Pharma, Slovak Republic). It contains a mixture of low-molecular, biologically active substances as peptides and degradation products of nucleoproteins. The aim of the study was to evaluate the TF effect on bone resorption markers and bone mineral density using the model of post-menopausal osteoporosis in ovariectomized rats.

Methods: Adult female Sprague–Dawley rats (270 ± 10 g) were subjected to bilateral ovariectomy (OVX) and sham operation (SHAM). After the surgery, the animals were divided into four groups: SHAM controls, untreated OVX rats, OVX rats treated with 5 mg/kg of TF orally in two application forms: once a week (TF-1) and 3 times a week (TF-2). For a period of 8 weeks, the animals were on a diet containing 7.5 g Ca/kg, 6.5 g P/kg and 600 IU vitamin D3/kg diet. The weight of the pellet diet of the ovariectomized rats was restricted to the food weight consumed by the control sham-operated animals. Pyridinoline (Pyr), deoxypyridinoline (Dpyr) and creatinine were determined by a high-performance liquid chromatography (HPLC) method in urine samples collected over a 24-h period. Bone mineral density (BMD) of the whole body, femur and tibia were detected using DEXA. The whole body BMD of rats was determined before ovariectomy and at the end of study on day 56.

Results: Both urinary Pyr and DPyr were significantly higher in OVX rats compared to SHAM controls on day 28 of the study. TF moderately decreased the values of Pyr and DPyr in urine. Whole body BMD was significantly higher in OVX rats treated with both TF application forms compared to OVX controls. The bone mineral density of femur and tibia were significantly increased only when TF was applied 3 times a week (TF-2).

Conclusion: Our results show that orally applied transfer factor provides a protective effect on bone density in ovariectomized rats.

P596-Mo

The Rate that Family Physicians Prescribe Osteoporosis Medications to their Patients: Canadian Quality Circles (CQC) Project

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The CQC Project was designed to improve family physicians' adherence with the new Canadian osteoporosis (OP) guidelines (2002) and consists of four phases that include recruitment, baseline (BL) data collection, inter-

vention using Quality Circles, and follow-up (FU) data collection. 52 physicians were recruited as members in 7 Quality Circles. Members collected baseline (BL) and follow-up (FU) data on separate patients via chart reviews and the completion of the CQC form that captures data on OP diagnosis, risk factors, and treatment. 1505 BL and 1359 FU patient charts were selected at random. All patients were women 55 years of age and older and attended at least 2 visits to the member's practice in the previous 24 months. Individual and QC data were collated in profiles and provided to the members. The QCs then met to discuss the profiles, identify and analyze problems, recommend solutions, and participate in an OP workshop. The primary focus of the workshop was to assess postmenopausal OP and risk factor identification. FU data collection occurred after the intervention and was used to provide feedback and to gauge overall progress. Our analyses examined the percent difference in the number of times that family physicians prescribe OP medications to their patients who were thought and not thought to have OP. The medications examined included etidronate, alendronate, risedronate, raloxifene, and hormone replacement therapy (HRT).

The table shows the percent difference (BL%, FU%) in the use of medications following the intervention. For patients thought to have OP, the largest positive changes occurred with risedronate and alendronate, the more potent bisphosphonates; negative changes occurred with etidronate, a less potent bisphosphonate; HRT, a second line treatment for OP; and raloxifene.

In conclusion, the overall use of OP medications modestly increased following the Quality Circles intervention; however, the results suggest that more effective therapies were given to those with OP.

Table

Medication	Patients with osteoporosis	Patients without osteoporosis
Etidronate	−5% (28%, 23%)	−2% (8%, 6%)
Alendronate	3% (19%, 22%)	3% (3%, 6%)
Risedronate	6% (20%, 26%)	1% (2%, 3%)
Raloxifene	−2% (5%, 3%)	−1% (2%, 1%)
Hormone replacement	−0% (4%, 4%)	1% (8%, 9%)
All medications	3% (72%, 75%)	2% (22%, 24%)

P597-Tu

Treatment of Charcot arthropathy with oral bisphosphonate

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Introduction: Charcot Arthropathy is a devastating consequence of diabetes associated with sensory neuropathy. Standard treatment relies upon prompt diagnosis, immobilization of the affected area and intravenous bisphosphonate infusions. We describe two patients who were unable to receive intravenous bisphosphonates and were treated with oral bisphosphonates.

Case 1: A 57-year-old woman with type 2 diabetes of 17 years duration. She presented with painful swelling of the left foot. She had advanced neuropathy affecting the lower limbs. There was no evidence of localized sepsis, and a triple phase isotope bone scan confirmed increased uptake in the region of the tarso-metatarsal region. Plain radiographs and MR imaging confirmed Charcot arthropathy. She was treated with oral risedronate 30 mg o.d. for 2 months and her foot immobilized in an Aircast boot. She made a prompt clinical recovery, and markers of bone turnover showed a prompt and persisting response to oral bisphosphonate therapy.

Case 2: A 41-year-old man with type 1 diabetes of 26 years duration. He presented with painful swelling of the right forefoot. He had advanced neuropathy affecting the lower limbs and a previous digital amputation in the affected foot. There was no evidence of localized sepsis, and a triple phase isotope bone scan confirmed increased uptake in the region of the 1st–3rd metatarsal head region. Plain radiographs and MR imaging confirmed Charcot arthropathy. He was treated with oral alendronate 70 mg o.d. for 2 months and his foot immobilized in an Aircast boot. His foot improved quickly, and his markers of bone turnover were suppressed well after clinical cure had occurred.

Conclusion: Oral bisphosphonate therapy can be used to treat Charcot arthropathy in patients unable to receive intravenous therapy because of difficult venous access. The response in terms of clinical improvement and bone turnover markers was similar in both patients.

P598-Su

Comparison of Raloxifene and Bisphosphonates on Adherence, Health Outcomes and Treatment Satisfaction in Postmenopausal Asian Women

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Objective: To evaluate adherence with raloxifene compared to daily dosing bisphosphonates in Asian postmenopausal women at increased risk of osteoporotic fractures.

Methods: In this 1-year, observational, non-interventional study conducted in Asia (Hong Kong, Malaysia, Pakistan, Philippines, Singapore, Taiwan), 984 postmenopausal women (mean age ± SD: 67.1 ± 8.7 years) were treated with raloxifene 60 mg/day (n = 707; 72%) or the bisphosphonates alendronate 10 mg/day (n = 206; 21%) or risedronate 5 mg/day (n = 71; 7%) during their normal course of care. Patients were assessed at baseline, 6 and 12 months.

Results: The baseline characteristics of age, race, education, menopausal status and baseline fractures were comparable between the raloxifene and bisphosponate groups. More women on raloxifene completed the study (50.2%) compared to those on bisphosphonates (37.5%; $P < 0.001$). Patients took raloxifene for a longer period (median: 356 days) than bisphosphonates (348 days; $P < 0.05$). At 6 months, significantly fewer raloxifene patients (4.3%) changed medication compared to bisphosphonate patients (12.2%; $P < 0.01$). Inconvenient dosing was reported as a primary reason for changing medication in 19 (6.9%) bisphosphonate patients compared to 0 raloxifene patients. Percent compliance was comparable between raloxifene patients (6 months: 94.8%; 12 months: 95.2%) and bisphosphonate patients (6 months: 94.9%; 12 months: 93.3%). Patients indicated a positive feeling about being on raloxifene compared to bisphosphonates (10 out of 11 items on the compliance questionnaire favoured raloxifene at 6 months); e.g., 43.8% of bisphosphonate patients stated that the drug was "complicated to take" compared to 3.2% of raloxifene patients ($P < 0.0001$; 6 months). More raloxifene patients indicated that the treatment affected them positively compared to bisphosphonate patients at 6 months ($P < 0.0001$) and 12 months ($P < 0.01$), and more raloxifene patients were satisfied with the medication than bisphosphonate patients at 12 months ($P < 0.01$). The proportion of new, self-reported fractures of any nature was lower in patients on raloxifene (2.1%) compared to those on bisphosphonates (5.4%; $P = 0.058$).

Conclusions: Asian postmenopausal women at increased risk of osteoporotic fractures showed a greater propensity to remain on raloxifene compared to bisphosphonates. The women on raloxifene exhibited lower discontinuation rates, better health outcomes, and higher treatment satisfaction.

P599-Mo

The Efficacy of Risedronate for Japanese Women with Osteoporosis

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The aim of this study is to evaluate the efficacy of oral risedronate in Japanese women with primary osteoporosis. Two-hundred twenty-nine patients (avg. 71.0 years of age) at 5 hospitals had taken a daily oral dose of 2.5 mg of risedronate at least 6 months. This dose is half that used in Caucasians, however, previous study demonstrated that the optimal daily dose for Japanese is 2.5 mg (J Bone Miner Metab, 22: 120, 2004). Of 229 patients, 87 (38.0%) patients had prevalent fracture at the start of treatment.

Incidences of new fracture occurred in 12 patients (vertebral: 5, non-vertebral: 7). Of 12 patients, five patients (41.7%) recognized the incidence of new fracture within 6 months after the start of treatment. Bone mineral density at lumbar spine (LS-BMD) increased at 6 and 12 months after the start of treatment 4.6% and 5.3%, respectively. Sixty-eight percent of patients increased LS-BMD more than 2% at 12 months. Bone resorption marker (urinary N-telopeptide of type I collagen, NTX) decreased at 3, 6, and 12 months after the start of treatment were 33.0%, 30.2%, and 28.8%, respectively. According to the guideline of Japan Osteoporosis Society, the meaningful cut-point (minimum significant change) for urinary NTX was determined as a decrease from baseline of 35%, and the high risk of vertebral fracture was also determined as more than 54.3 nmol BCE/mmol Cr. According to the guideline, 79.0% of patients with high risk of fracture showed minimum significant changes in urinary NTX within 6 months. A change from baseline at 6 months in urinary NTX predicted the changes from baseline at 12 months in LS-BMD with sensitivity 74.5% and specificity 46.2%. Positive predictive value indicated that probability of 83.3% that LS-BMD would be increased more than 2% at 12 months if urinary NTX decreased below the cut point at 6 months. Negative predictive value indicated that probability of 33.3% that LS-BMD would not be increased more than 2% at 12 months if urinary NTX did not decrease below the cut point at 6 months.

In conclusion, oral risedronate at the daily dose of 2.5 mg significantly increased LS-BMD and decreased urinary NTX in Japanese women with osteoporosis in short-term. Daily oral risedronate (2.5 mg) was effective therapy for Japanese women with osteoporosis.

P600-Tu

Improvement of Peridental Bone Density and Quality with Bisphosphonate. A pQCT Analysis of Regional and Threshold Clustered Data

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The quality of peridental bone supporting teeth and/or prostheses stability can now be quantified with pQCT technology. Moreover, bone tissue may be improved with

certain bisphosphonate treatments. In order to test such hypothesis, we describe the preliminary results of an open, prospective assay. Subjects under treatment for periodontal disease and with evidence of jaw osteopenia gave consent to receive treatment with oral pamidronate in safe anti-osteoporotic doses. Patients (n = 17; fem 82%, age range 24–61 years) received 200 mg/day pamidronate in gastro-protected capsules (Gador SA, Argentina) and calcium supplementation during 6 months. They were assessed by a pQCT 3000-D system (Stratec-Germany) according to Capiglioni, R et al. (*Diagnóstico 1998, 7:898*) and for bone quality (from type I: high dense tissue, to type IV: severe osteopenic tissue), according to Roldán et al. (*JBMR 2001,16 (S1):S244*). In addition, small cortical and medullar sections (mean 543 mm^2) were studied by threshold analysis in order to observe if pamidronate was equally active in portions of tissues with different bone volume (from vBMD). Results were compared by Student's t tests. Whole section vBMD did not change significantly. Clustered type I tissue vary +0.3% (P = n.s.), type II was +0.9% (P = 0.08, close to significant), type III was +5.3% ($P <$ 0.003), while type IV areas tend to decrease −2.4% ($P <$ 0.09). Threshold analysis shows that at cortical bone areas above 700 mg increases (less porosity?). Results show a reduction of osteopenic areas with additional improvements at medium dense cortical areas (type III tissue). This observation requires future randomized controlled trials supporting the use of bisphosphonate in osteopenic peri-dental bone.

P601-Su

Mechanically Induced Dental Resorption Halted with Short-Term Oral Pamidronate. A Preliminary Clinical Study

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Abrupt changes in regional loadings and/or orthodontic trauma may activate macrophages, thus promoting an acute resorption of teeth roots. These changes may be irreversible and challenge teeth stability. We demonstrated in a group of adult patients with teeth resorption evidenced by panoramic radiography that 200 mg/day of disodium pamidronate capsules halted the resorption phase completely avoiding teeth mobility or losses up to 9 months (*Montangero V et al. Osteology 2000, 3:287*). In this second report, we extended the sample to 28 subjects (age range 22–70), who agreed to participate in the study following protocol instructions. This observation was open, prospective and longitudinal. A placebo-controlled group was not allowed in our facilities due to the risk of losing teeth. Patients were healthy and had not received previous medication. In most cases, dental root resorption was a radiological finding, being post-orthodontic trauma reported in few. Each patient received pamidronate + calcium supplementation (up to 800 mg/day) and was followed with radiology every 3 weeks during 12 months (9–14 range). Peripheral QCT (XCT-3000-D, Stratec-Germany) and single photon emission CT (SPECT) were also performed when available. Halting of root resorption was observed in 100.0% of the sample after 6 ± 1 months of starting therapy. Ten subjects (36.0%) optionally disrupted the treatment 1 month after achieving steady radiograph. All cases showed no further resorption, stability and total functional of dental pieces. pQCT and SPECT also show evidence of peridental changes in pamidronate-treated patients. Tolerability was good in all cases. Present data should be considered preliminary, and randomized controlled trials are required to confirm the positive results hereby attributed to pamidronate therapy. Partially granted by Gador SA, Buenos Aires.

P602-Mo

In Vitro Pharmaceutical and Biological Evaluation of Zoledronate-Loaded Calcium Phosphate Ceramics: An Innovative Strategy for the Local Treatment of Bone Pathological Resorption

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Calcium phosphate biomaterials, which exhibit physio-chemical properties closed to those of bone, have been successfully used as osteoconductive bone substitutes and contemplated as carrier for therapeutic agents. Bisphosphonates have a strong affinity for calcium phosphate crystals that compose the bone biological apatite. We aimed at using this affinity to develop a bone drug delivery system (DDS) associating zoledronate and a calcium-deficient apatite (CDA). In vitro pharmacokinetic and biological evaluation of this bone bioactive DDS was performed using primary rabbit osteoclasts and MC3T3-E1 osteoblasts.

31P solid state nuclear magnetic resonance spectra of zoledronate-loaded CDA showed the peak characteristic of the calcium phosphate matrix, along with an additional downfield resonance in the expected range for zoledronate (10–20 ppm). 14C labeled zoledronate-loaded CDA exhibits a kinetic release of 2.2 (±0.2) 10^{-5} M in the culture medium. In osteoclastic formation assay, zoledronate solution (10^{-6} to 10^{-10} M) reduced the number of TRAP, vitronectin receptor, and F-actin ring positive cells as well as the formation of pit resorption in a dose-dependent manner. Zoledronate-loaded CDA was found to decrease osteoclastic resorption and appeared almost equipotent to 10^{-6} M zoledronate. Extractive solution of zoledronate-loaded CDA exhibited dose-dependent effect on the reduction of osteoclastic resorption. Finally, zoledronate-loaded CDA

did not affect osteoblastic viability and ALP activity. Our data therefore demonstrate that CDA is effective for loading and release of bioactive zoledronate. Our findings suggest that such hybrid material could allow to increase efficiency of bisphosphonate by being locally delivered and could be of interest as an alternative to systemic treatments. Today, further experiments are under investigation to evaluate the ability of our hybrid material to prevent in vivo pathological bone resorption in ovariectomy-induced osteoporosis models.

This work was supported by the French Ministry of Research (ACI technologie pour la santé), Fondation de l'avenir pour la recherche médicale appliquée, CNRS (Matériaux Nouveaux-Fonctionnalités Nouvelles), and Novartis Pharma AG.

P603-Tu

Restoration and Maintenance of Bone Density with Alendronate in Children with Osteoporosis

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Children and adolescents of normal stature may develop osteoporosis (OP), [lumbar bone mineral density *Z* score LzBMD, ≤ -1.5 by DXA], often with fractures (fx), from genetic or acquired diseases. There are no approved therapies in children for OP. We report our long-term experience in 129 children and adolescents (68 girls;61 boys) with oral alendronale (o-aln) for Rx of OP. Patients ranged in age from infancy through late adolescence when the diagnosis of OP was made, and most frequently had idiopathic osteoporosis ($n = 61$) followed by corticosteroid-induced osteoporosis ($n = 19$). No patients reported were growth-hormone- or vitamin D-deficient. At initiation of o-aln, the median (25th and 75th percentile) LzBMD = -2.1 (-2.7;-1.4) and most had ≥ 1 atraumatic fx before o-aln. Patients were divided into 2 groups: those who finished a course of o-aln and those that remain on it. o-aln dose was 10 mg qd/70 mg qw in patients >30 kg and was 5 mg qd/35 mg qw $\leq$30 kg. 45 patients ("stopped") had an initial LzBMD = -1.8 (-2.4;-1.3) and were treated for 672 (513; 959) days with o-aln. At its discontinuation, the delta (\Delta) LzBMD achieved = +1.7 (+1; +2.2), and it differed ($P < 0.001$) from initial LzBMD. In 40 patients with sufficient follow-up data (6 months), the LzBMD = -0.2 (-0.9; +0.33), 410 (353; 797) days after discontinuing o-aln. The \Delta score did not differ between the LzBMD at the time of discontinuation or most recent value. All patients treated with o-aln and had therapy "stopped" had cessation of their fx within the 1st 6 months. None have resumed fx. 5 patients discontinued o-aln after a few months; 1 was due to esophageal symptoms. 83 patients ("active") with an initial LzBMD = -2.2 (-2.8;-1.5) are being treated in similar manner with o-aln for 685 (351; 1022) days [Nov 04]. The initial LzBMD in this group < ($P = 0.038$) vs. "stopped" group. At the most recent study in the "active" group, LzBMD was 0.94 (0.7; 1.03), a value different than that at initiation. The \Delta LzBMD achieved = +3 (2.3; 3.7) > the \Delta LzBMD at the time of discontinuation of o-aln in the "stopped" group ($P < 0.001$). In conclusion, a wide range of children and adolescents were diagnosed with OP and fx prior to presentation. o-aln led to a significant, quick increase in LzBMD and cessation of fx. Patients taken off of o-aln have maintained LzBMD for >1 year. Short courses of o-aln appear safe and efficacious in normalizing LzBMD, stopping fx, and maintaining bone density values once stopped.

P604-Su

Adherence with Bisphophonate Treatment for Osteoporosis in UK Patients

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Background: Adherence is a collective term for both drug persistence and compliance[1]. Studies have demonstrated that non-adherence to bisphosphonate therapy for osteoporosis adversely influences BMD and consequently risk of fracture[1,2,3]. Simplification of administration regimens has been used in an attempt to improve adherence.

Objective: A major component of adherence is the time from initiation of treatment to the completion/discontinuation of treatment (persistence)[1]. This research aims to review persistence levels with available (weekly versus daily) bisphosphonate regimens, using a UK database (DIN-LINK).

Method: DIN-LINK collates medical information from a sample of approximately 100 GP practices in Great Britain. Information was extracted from this database to assess persistence in patients starting a new course of bisphosphonate therapy over the 12-month period between October 2002 and October 2003. Persistence was measured over 1 year as the length of continuous therapy, with cessation defined as an interval in excess of 1.5 times the expected duration of the prescription[4].

Result: The proportions of patients persisting over time (after 6 and 12 months) are: 53%/43%/54% and 35%/22%/39%, with all/daily/weekly bisphosphonates, respectively.

Conclusion: Adherence with bisphosphonate therapy is poor. Within 1 year of initiation, approximately two thirds of all patients cease treatment, the majority stopping during the first 6 months. Weekly dosing has led to better adherence but remains suboptimal, with an annual persistence rate of still only 39%. Less frequent dosing may lead to better adherence, optimizing therapeutic outcomes[1]. Further research is needed to corroborate these results with data from alternative sources. Additionally, comparison internationally or with other chronic conditions is warranted to

understand the reasons for poor adherence and suggest possible solutions.

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P605-Mo

Effect of Alendronate on Bone Mineral Density and CA-P Metabolism in Senile and Postmenopausal Osteoporosis

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Aims: The aim of this study was to evaluate effect of alendronate on bone mineral density (BMD) in senile\postmenopausal osteoporosis and the possible influence of Ca-P metabolism disturbances on this effect.

Methods: The study included 38 senile\postmenopausal high turnover osteoporotic women (mean age 63.2 + 1.1). Alendronate (Fosamax®) 70 mg was prescribed weekly. Everyone received Ca (1 g per day). If serious hypocalcemia occurred or developed during the study period, alfacalcidol (alfaD3TEVA®, 0.5–0.75 mcg daily) was taken in addition. Control group consisted of 20 osteoporotic women and received 1 g Ca per day. The mean age, basal BMD in femoral neck, Ward's trigonum, trochanter, lumbar vertebra and biochemical did not differ significantly from the studied group. At baseline and 12 months, BMD was measured by dual energy X-ray absorptiometry (DXA, LunarProdigy). Biochemical parameters (Ca, Ca^{2+}, P, parathyroid hormone content and alkaline phosphatase activity of blood, Ca and P excretion, urine deoxypyridinoline) were measured every 3 months up to 1 year.

Results: In control group, BMD was not changed significantly in all estimated regions. Ca and Ca^{2+} of blood, Ca excretion tended obviously to rise. Other biochemical parameters were not changed. In alendronate group, BMD increase in all regions was observed in 1 year (from −2.46 + −0.11 to −2.26 + −0.11 SD in the neck, $P < 0.01$; from −3.02 + −0.09 to −2.92 + −0.08 SD in Ward's trigonum, $P < 0.05$; from −1.87 + −0.16 to −1.53 + −0.14 SD in great trochanter, $P < 0.01$; from −3.35 + −0.15 to −2.98 + −0.13 SD in lumbar spine, $P < 0.001$). Ca and Ca^{2+} of blood decreased (from 2.45 + −0.02 to 2.32 + −0.01 mmol/l, $P < 0.01$; and from 1.24 + −0.01 to 1.20 + −0.01 mmol/l, $P < 0.05$, correspondingly), urine deoxypyridinoline decreased not significantly. Decrease of serum P was observed unexpectedly (from 1.20 + −0.07 to 1.00 + −0.04 mmol/l, $P < 0.01$), and furthermore, parathyroid hormone tended to rise.

Conclusion: Alendronate induced BMD increase in senile and postmenopausal high turnover osteoporosis. We did not reveal significant correlations between BMD and biochemical parameters (r was less than 0.4; $P > 0.05$), but observed disturbances of Ca-P metabolism in some patients may be important in long-term results and should be corrected properly.

P606-Tu

Effects of Alendronate Treatment on Serum Levels of Osteoprotegerin and Total RANKL in Women with Postmenopausal Osteoporosis

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Several studies have reported that antiresorptive effect of biphosphonates is due to osteoclastogenesis inhibition. In this context, OPG-RANKL system is considered a critical element in osteoclast differentiation and activation. However, the effect of alendronate on these cytokines is not clearly established.

Aims: To determine the effects of alendronate treatment (70 mg once a week) on serum concentrations of OPG, total RANKL and biochemical markers of bone turnover in untreated women with postmenopausal osteoporosis.

Patient and methods: We selected 47 postmenopausal women (mean age 63 ± 7 years) with densitometric criteria of osteoporosis (T score ≤ -2.5 SD). We determined (basal and 3 months) basic anthropometric parameters, biochemical markers of bone turnover, serum OPG (osteoprotegerin ELISA KB 1011 Immundiagnostik AG Bensheim, Deutchland), total RANKL (total RANKL ELISA K 1016 Immundiagnostik AG, Bensheim, Deutchland) and BMD (DXA; Hologic QDR 4500) in lumbar spine (LS) femoral neck (FN) and total hip.

Results: Serum levels of OPG (146.2 ± 77.3 vs. 141.8 ± 79 pg/ml) and total RANKL (771 ± 1294 vs. 662 ± 1054 pg/ml) showed no significant changes at the third month of treatment ($P = 0.2$ and $P = 0.7$ respectively). Furthermore, we found a significant decrease of serum concentrations of total alkaline phosphatase (76.2 ± 11.9 vs. 64.2 ± 11.7 UI/L; $P = 0.001$), bone alkaline phosphatase (14 ± 7 vs. 4.2 ± 4 μg/ml; $P = 0.001$), TRAP (3 ± 0.6 vs. 2.5 ± 0.5 UI/L; $P = 0.002$) and osteocalcin (2.3 ± 1.6 vs. 1.08 ± 1.1 ng/ml; $P = 0.002$). We found no significant changes in vitamin D and PTH levels.

Conclusions: The treatment with alendronate in women with postmenopausal osteoporosis causes no significant changes in serum concentrations of OPG and total RANKL at short-term. These findings suggest that inhibitor effect of alendronate on bone remodeling is achieved by other pathways.

P607-Su

Effects of Alendronate on Bone Mineral Density and Bone Metabolic Markers in Patients with Liver Transplantation

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Objective: Orthotophic liver transplantation (OLT) is associated with osteoporosis which is characterized with bone loss and high bone turnover. We aimed to evaluate the effects of alendronate in bone mineral density (BMD) and bone turnover markers in patients with OLT.
Material and methods: In the prospective, controlled, open study with 24 months of follow up, 98 patients (mean age: 40.9 ± 11.8) with OLT were randomized to receive alendronate 70 mg weekly or no alendronate, calcium 1000 mg daily and 0.5 mcg calcitriol daily provided to all patients. Lumbar spine (LS) and hip (total and neck) BMDs were measured at the onset and repeated at 6-month intervals by dual-energy X-ray absorptiometry. Spinal radiographs were obtained to assess vertebral fractures at the same time. Additionally, serum osteocalcin, urinary deoxypyridinoline (DPD), serum parathyroid hormone (PTH) and biochemical parameters were determined every 3 months.
Results: Compared with control group, alendronate produced significantly greater increases in BMD of the LS (5.1% vs. 0.4%, $P < 0.05$ at 12 months, 8.9% vs. 1.4%, $P < 0.05$ at 24 months), femur neck (4.3% vs. −1.1%, $P < 0.05$ at 12 months, 8.7% vs. 0.6%, $P < 0.05$ at 24 months) and total femur (3.6% vs. −0.6%, $P < 0.05$ at 12 months, 6.2% vs. 0.3%, $P < 0.05$ at 24 months). In the alendronate group, osteocalcin and urinary DPD decreased significantly at 6th month, with no further change during the 2-year period, by −35.6% and −63.0%, on average, respectively ($P < 0.05$ vs. controls). In the control group, a significant increase in biochemical markers of bone turnover was observed during the 24 months in comparison to baseline values ($P < 0.05$). After the operation, the mean serum PTH levels were increased in all patients without differences between groups. No changes in the other biochemical parameters were observed in both groups. The weekly dosage of alendronate was well tolerated, and no severe side effects occurred.
Conclusion: This is the first report indicating that alendronate treatment was able to significantly increase BMD in patients with OLT. This increase was significantly higher in patients treated with alendronate, Ca and vitamin D when compared with patients treated with Ca and vitamin D alone.

P608-Mo

Cost-Effectiveness of Risedronate Therapy in Postmenopausal Women with Varying Risk of Osteoporotic Fractures: A Swiss Analysis

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Objective: Osteoporosis and its related fractures are a major source of illness and cost in Switzerland. The incidence of hospitalizations related to osteoporotic fractures increases sharply with age. This study used computer modeling to assess the economic impact of risedronate therapy in postmenopausal osteoporotic women with different risk profiles.
Methods: A fracture-incidence-based Markov model of osteoporosis with patients' transition across states was used to estimate cost per any fracture averted, cost per hip fracture averted and cost per QALY gained. Model inputs were specific to Switzerland and included fracture and mortality rates, lengths of stay for acute and rehabilitation care and intervention cost including acquisition cost for 35 mg of Actonel, office visits and monitoring of BMD. Health utilities and relative risk reductions were taken from published studies. Cost and outcomes were discounted at 3%. A population of 1000 women with osteoporosis and one prevalent fracture was modeled with 5 years of risedronate therapy starting at 70 years. The analysis was repeated for populations with an additional history of maternal hip fracture, a history with any fracture since the age of 50 or both. The analysis assumed that risedronate was effective only during therapy without any residual effect. A discontinuation rate of 50% was assumed.
Results: In the base case population, cost per QALY and cost per averted hip fracture were CHF 52,449 and CHF 80,286, respectively. In the presence of history of any fracture, a maternal hip fracture or both, costs per QALY decreased to CHF 42,568, CHF 39,128 and 30,849, respectively, and cost per hip fracture averted decreased to CHF 65,343, CHF 59,003 and CHF 46,724, respectively. Cost per any fracture averted was CHF 38,554 in the base case analysis and CHF 29,871, CHF 28,046 and CHF 21,040 in the presence of a history with any fracture since the age of 50, a maternal hip fracture and presence of both risk factors, respectively.
Conclusions: Risedronate is a cost-effective intervention in women with postmenopausal osteoporosis. Cost per unit of benefit gained varies according to the baseline risk profile.

P609-Tu

A New Mechanism of Action for Bisphosphonates: A N-BP-Induced Endogenous ATP-Analog (APPPI) Causes Mitochondria-Mediated Apoptosis

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Bisphosphonates are currently the most important class of antiresorptive drugs used for the treatment of diseases with excess bone resorption. Bisphosphonates can be divided into two pharmacological classes with distinct molecular mechanisms of action. Nitrogen-containing bisphosphonates (N-BPs), such as zoledronic acid, inhibit bone resorption and cause apoptosis of osteoclasts by preventing post-translational modification of GTP-binding proteins with isoprenoid lipids. Non-nitrogen-containing bisphosphonates (non-N-BPs), such as clodronate, do not inhibit protein isoprenylation but can be metabolically incorporated into non-hydrolyzable analogs of ATP that accumulate within osteoclasts, resulting in induction of osteoclast apoptosis by inhibiting the mitochondrial adenine nucleotide translocase (ANT) (1,2).

In the present study, however, we describe a new plausible mechanism of action on N-BPs. Recently, we found that N-BPs induce production of a novel intracellular ATP-analog (ApppI) in vitro in mammalian cells, including osteoclasts. ApppI production is a unique effect of N-BPs, which results from inhibition of FPP synthase in the mevalonate pathway and consequent accumulation of isopentenyl diphosphate (IPP). The present data strongly suggest that ApppI also induces mitochondria-mediated apoptosis by inhibiting mitochondrial adenine nucleotide translocase. Potentially, N-BPs may also induce the accumulation of dimethylallyl diphosphate (DMAPP, the structural isomer of IPP) in the mevalonate pathway and thus lead to production of the ATP-analog. The present data really show that N-BPs also induce the accumulation of DMAPP and therefore the production of ApppD. Studies for role of ApppD in action on N-BPs are in progress.

These findings provide evidence of a new mechanism of action for nitrogen-containing bisphosphonates. Among these, especially very potent bisphosphonates, such as zoledronic acid, represent a third class of bisphosphonates which combines inhibition of both isoprenylation and ANT as mechanisms of action.

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P610-Su

Persistence to Bisphosphonate Treatment in Actual Clinical Practice

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Persistence to osteoporosis treatment is important given the chronic nature of the underlying condition. The objective of the study was to evaluate the predictors of persistence to alendronate or risedronate treatment.

The data were obtained from two databases containing medical records of general practitioners in the UK: the General Practice Research Database and The Health Improvement Network research database. Patients who started alendronate or risedronate treatment were identified (i.e., first prescription at least 6 months after start of follow-up). Patients with Paget's disease were excluded. Life-table analyses were used to determine the percentage of repeat prescribing in the 3 months after the expected end of treatment with a prescription. Time-dependent Cox regression was used to determine the predictors of treatment discontinuation.

A total of 12,372 patients initiated alendronate or risedronate treatment after start of follow-up. The average age at start of treatment was 69.0 years, and the percentage of women was 83.0%. The study population included 10,584 alendronate and 1788 risedronate users (54.6% daily and 45.4% weekly tablets). The percentage of patients that continued treatment for at least 1 year was 63.6% and 45.5% for 3 years. Treatment discontinuation was most likely to occur after the first prescription (after this, 72.6% continued treatment for another year and 52.8% for another 3 years). Predictors for treatment discontinuation included age, sex, dosing frequency and concomitant use of calcium and/or vitamin D (i.e., prescribing by the GP with the bisphosphonate). Patients aged 65 to 79 years were less likely to discontinue treatment compared to patients aged 50 to 64 (ratio of discontinuation rates of 0.84 [95% confidence interval 0.78–0.91]). For patients aged 80+, this ratio was 0.90 (0.82–0.99). Users of weekly bisphosphonate were also less likely to discontinue than daily users (ratio of 0.87 (0.80–0.94). Concomitant use of calcium or vitamin D (as recorded in these databases) was low (23.1%) but improved treatment persistence (ratio of discontinuation rates of 0.88 (0.81–0.95)).

In conclusion, medication persistence improved following the introduction of weekly bisphosphonate formulations.

P611-Mo

Intravenous Zoledronate for Osteoporosis Therapy

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The intravenous route for the administration of bisphosphonates in osteoporosis has proved valuable where the oral route is poorly tolerated or contraindicated. Pamidronate is now widely used in this role. However, zoledronate offers advantages over pamidronate because of the persistence of its effects on bone mineral density (BMD) and bone turnover for at least a year after administration. We have investigated using zoledronate as an alternative to pamidronate in patients having IV therapy to reduce the number of therapy episodes and cost of treatment. 44 patients (36 female, 8 male) of average age 67 years have been treated. Baseline measurements were made of BMD, urine NTX, urine calcium and serum calcium, creatinine and vitamin D. Patients were given calcium and vitamin D supplements if there was evidence of deficiency. The indications for therapy were intolerance to oral bisphosphonate (18), malabsorption including short bowel syndrome and celiac disease (6), declining BMD on oral bisphosphonate (3), severe upper GI disorder (6), unable to swallow medication (1), changing from IV pamidronate (4), other (6). 29/44 of the patients had been on alternative antiresorptive therapy within the previous 12 months. Average baseline spine *T* score was −3.12 (SD 1.18) and femoral neck *T* score −2.52 (SD 1.15). Six patients experienced bone pain following treatment, prolonged in one case. Four patients had a significant flu like illness. One patient was delirious for 36 h, 1 patient reported dry mouth and a salt-like taste and 1 metallic taste and 1 patient developed hypocalcemia. Patients were monitored with 3 monthly NTX and yearly BMD. In 18 patients with 2 sets of BMD data, available average spine BMD rose 4% from 0.776 g/cm^2 to 0.801 g/cm^2 with a decline in 1 patient only. Average femoral neck BMD rose from 0.586 g/cm^2 to 0.607 g/cm^2. In patients with pre and post therapy data, average pretreatment NTX was 50 SMV/Creat with an average of 43% decrease following zoledronate. All patients had a normal creatinine prior to and 1 year post therapy with an average increase of 1 μmol/l. Intravenous zoledronate appears a safe alternative IV bisphosphonate to pamidronate with favorable changes in BMD and bone markers supporting its use in osteoporosis. The side effect profile may limit the use of the drug. We have found no evidence of renal impairment post therapy.

P612-Tu

Effects of Combined Agent of Alendronate and Calcitriol (MAXMARVIL®) on Bone Metabolism in Korean Postmenopausal Women: Multicenter, Double-Blind, Randomized, Placebo-Controlled Study

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Objective: To evaluate the effect of combined agent of 1,25-dihydroxyvitamin D3, 0.5 μg and alendronate, and 5 mg on the bone metabolism in postmenopausal women.
Design: A randomized, double-blind, prospective 6-month clinical trial.
Patients and measurements: A total of 217 postmenopausal women with osteoporosis were enrolled with randomized assignment of 199 patients to each treatment group (C group of combined drug, D group of 1alpha-hydroxyvitamin D3, 1.0 μg). None of the patients were vitamin-D-deficient assessed by serum 25(OH)D level and had received any drug affecting bone metabolism before being enrolled in our study. Serum biochemical assays including serum calcium and 24 h urinary calcium excretion and bone turnover markers i.e., bone-specific alkaline phosphatase (bs-ALP) and urine N-telopeptide (NTx), were performed at baseline and after 3 and 6 months of treatment. Bone mineral density (BMD) of L1 to L4 and femur was measured by dual energy X-ray absorptiometry (DXA) at the initial assessment and after 6 months of treatment.
Results: In the C group, the BMD of lumbar spine increased up to 2.42 ± 0.5% from baseline after 6 months ($P < 0.05$). On the other hand, the change of BMD in the D group was 0.28 ± 0.5% after 6 months. There was no significant difference of femoral BMD between C and D groups. The levels of the bs-ALP and NTx were significantly decreased in the C group than the D group: −22.04 ± 3.9% vs. −11.42 ± 2.8% ($P < 0.05$) and −25.46 ± 5.2% vs. 1.24 ± 6.2% ($P < 0.001$), respectively. Interestingly, there was significantly less hypercalciuric effect in the C group ($P < 0.05$).
Conclusions: Our study demonstrated that the combination of 1,25-dihydroxyvitamin D3 and alendronate is quite effective and has the advantage of decreasing the hypercalciuric effect of 1,25-dihydroxyvitamin D3 for the treatment of postmenopausal osteoporotic women.

P613-Su

Zoledronate Reduces Early Acute Bone Loss at the Hip Following Spinal Cord Injury

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Bone loss occurs rapidly following spinal cord injury (SCI) and predominantly affects the lower limbs. To date, there are limited data on strategies to prevent this bone loss and reduce future fracture risk.

All patients aged ≥18 with acute spinal cord injury (complete or incomplete injuries), who were within 3 months of injury, were invited to participate in this open label study. The active treatment was a single infusion of 4 mg IV zoledronate. All patients received standard care. Bone mineral density (BMD) was measured at baseline and at 3 months by dual energy X-ray absorbitometry (Hologic QDR-Delphi) at the lumbar spine and hip. BMD was compared by paired *t* tests and analysis of covariance.

12 patients (5 controls and 7 zoledronate) were recruited and randomized. The 2 groups were well matched for baseline characteristics such as age, sex distribution, baseline BMD and duration since injury.

The results are summarized in Table 1 below:

* = significant difference from baseline $P \leq 0.025$.

= significant difference between baseline and three month BMD $P < 0\ 0001$.

There were no significant differences either within or between groups at the lumbar spine. There were significant losses at both hip sites in the control group and at the total hip in the zoledronate group. Total hip BMD at 3 months was significantly lower in the control group than the active treatment group. 5 out of the 7 participants in the active treatment group experienced an acute phase reaction not associated with any long-term complications.

These data confirm that BMD is rapidly lost following spinal cord injury at the hip and femoral neck. They suggest that a single infusion of zoledronate reduces this bone loss at the hip. Further work is required to ascertain if this effect is maintained and has an effect on fracture rates.

Table 1

	Zoledronate group	Control group
% change lumbar spine BMD	−0.23	−4.87
% change total hip BMD	−1.49*,#	−7.38*,#
% change femoral neck BMD	−1.05	−6.14*

P614-Mo

Biphosphonates Therapy of Osteoporosis in Postmenopausal Women with Calcium Oxalate Nephrolithiasis

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Menopause is associated with increased bone resorption, increased urinary calcium excretion, which both increase the risk for the development of calcium-containing kidney stones and osteoporosis. Bisphosphonates can be very powerful inhibitors of bone resorption and at the same time the inhibitors of formation and aggregation of calcium oxalate crystals. Group of observation included 47 postmenopausal women aged 51–72 years (mean age 58.5 ± 5.5) with history of calcium oxalate renal stone formation. Osteoporosis was diagnosed by measurement of bone mineral density in the lumbar spine (L1–L4), proximal femur and distal arm using dual energy X-ray densitometer QDR-4500 (Hologic) as a $T < -2.5$. US examination was used for diagnosis or exclusion of current nephrolithiasis. No patients had a history of renal staghorn calculi formation. Urinary calcium excretion was assessed by 24 h urinary collection, and hypercalciuria was recognized as excretion of Ca >7.0 mmol/24 h. Levels of serum intact parathyroid hormone, 25-(OH) vitamin D, osteocalcin, calcium and phosphate were measured before start of therapy. 4 women had levels of intact parathyroid hormone higher than normal (from 8,2 to 14,5 pmol/L), however, refused surgical correction or had been considered unfit for surgery. None of the patients had hypercalcemia. All patients received therapy with oral 70 mg alendronate once in a week, supplemented with 625 mg of calcium carbonate and cholecalciferol 200 ME, at the end of a meal two times a day. After 2 years of therapy, every patient demonstrated increase of bone mineral density at lumbar spine and proximal femur from 5.5% to 15.7%, mean + 7.9 ± 2.5, no one episode of renal colic or renal stone formation was registered.

Conclusion: This observation showed that alendronate supplemented with calcium carbonate is not associated with increased risk of renal stone formation in postmenopausal osteoporotic patients and can be used for the therapy of osteoporosis in some patients with a history of nephrolithiasis. However, further studies are necessary to determine the ability of alendronate to prevent renal stone formation.

P615-Tu

Clinical Experience with Pamidronate Treatment in Children with Spastic Quadriplegic Cerebral PalsyA Retrospective Review

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The safety and efficacy of IV pamidronate to treat low bone mineral density (BMD) in children with spastic quadriplegic cerebral palsy (SQCP) have been evaluated and reported by our group ((1) The purpose of this study is to report on our expanded experience with IV pamidronate in children with severe cerebral palsy, including longitudinal bone density evaluations for up to 4 years after treatment. Seventeen non-ambulatory skeletally immature patients with SQCP, low bone density, and a history of previous fracture(s) were treated with a 3-day dosing cycle

of intravenous pamidronate at 3- to 4-month intervals for 1 year (five dosing cycles). BMD was measured by dual energy X-ray absorptiometry (DXA) in the lumbar spine and/or lateral distal femur (LDF) in all subjects before and during treatment. Post-treatment BMD was measured 12 subjects (nine subjects >1 year and six >2 years). At the end of treatment, BMD in the metaphyseal region of the LDF was increased compared with pre-treatment BMD by 66% + 55% (mean ± SE) and increased 48% + 41% in the lumbar spine. All subjects tolerated the treatment regimen without significant untoward effects. Post-treatment changes in BMD were variable. Both late decreases and continued increases in BMD were observed after treatment. Fourteen of 17 subjects sustained at least one fracture in the year before starting treatment; only two fractures occurred after treatment was initiated during a cumulative follow-up of over 40 patient–years. A 1-year course of pamidronate proved to be a safe and effective treatment for low BMD in children with SQCP. A sustained preservative effect on BMD was observed in some patients for greater than 2 years beyond treatment.

Reference:

1. Henderson RC, Lark RK, Kecskemethy H, Miller F, Harcke HT, Bachrach SJ. Bisphosphonates to treat osteoporosis in children with quadriplegic cerebral palsy: a randomized, placebo-controlled clinical trial. J. Pediatri. 141: 644–51, 2002.

P616-Su

Treatment of Established Primary Osteoporosis in Men with Risedronate Versus Etidronate: 1-Year Results of a Prospective Study

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Male osteoporosis represents an important public health problem. Only a few therapeutic trials have been performed in men. Most experience is available on therapy with alendronate. Other bisphosphonates have received limited attention in men.

This prospective, open label, randomized and active controlled clinical study compared the effects of oral risedronate (5 mg daily) and etidronate (400 mg daily for 2 weeks every 3 months) on bone mineral density (BMD) and fractures and biochemical markers of bone turnover. All men received 1000 mg calcium and 800 IU colecalciferol. After 12-month treatment, a significant increase of bone mineral density about 4.7% in risedronate patients and 2.9% in etidronate patients was observed ($P < 0.05$). The corresponding changes in total hip BMD were 2.2% and 2.0% and in femoral neck 2.1% and 2.3% for the risedronate and etidronate groups, respectively. The incidence rate of patients with new vertebral fracture was 3.3% for the risedronate group and 1.6% for the etidronate groups. The risk of a new fracture in the case of a prevalent fracture was 11% in the risedronate group and 14% in the etidronate group. Bone resorption markers decreased significantly after 3 months in the risedronate group ($P < 0.01$) and only after 12 months therapy in the etidronate group ($P < 0.01$). Biochemical parameters of bone formation decreased only in the risedronate patients ($P < 0.01$). Both therapies were well tolerated.

Risedronate as well as etidronate produced an increase in BMD. Over the time of 12-month treatment, no significant difference was observed between both bisphosphonates in their effects on bone mineral density, fractures and biochemical parameters. In spite of this fact, risedronate seems to be slightly more efficient.

P617-Mo

Bisphosphonate Release Following Cessation of Long-Term Treatment of Children with Osteoporosis

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Bisphosphonates (BPs) are taken up selectively by the skeleton, act at the surface and suppress bone resorption and are embedded in bone where they are biologically inert. Pharmacokinetic studies extending up to 1.5 years after intravenous infusions in humans indicated that BPs have a long residence time and they are slowly released, presumably with resumption of bone remodeling in areas here BP has been embedded. There is no direct information, however, about the release of BPs in patients who received long-term continuous therapy. Such information, apart from its obvious significance in the management of osteoporosis is of primary importance for young patients who are treated with BPs and can be obtained by measuring urinary excretion of BPs, the only route of elimination. We are studying the excretion of BPs in children with osteoporosis of different etiology (juvenile, RA, OI) following cessation of long-term treatment with daily BPs. We report here the first results in 6 children treated with daily oral pamidronate. Treatment had been given for 4 to 10 years, and pamidronate excretion was measured 3 to 13 years following cessation of therapy for a maximal observation period of 19 years. Pamidronate was detectable in the urines of patients up to 8 years following cessation of treatment, while it was undetectable in a patient who had received treatment for 6 years and was measured 13 years after stopping pamidronate. These results provide the first direct evidence of long-term release of BP and illustrate that this can persist for years after cessation of therapy. The activity of this released BP is currently unknown but may account for the stabilization of BMD and fracture rates after stopping BP treatment of osteoporotic women. In addition, they suggest caution in the selection of girls for BP treatment as currently there is only anectodal information about potential effects of BPs in fetal development.

P618-Tu

Once-Monthly Dosing of Oral Ibandronate is at Least as Effective as Daily Dosing in Postmenopausal Osteoporosis: 1-Year Results from Mobile

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Objectives: Administering oral bisphosphonates less frequently than weekly may help to overcome current adherence issues in postmenopausal osteoporosis (PMO) (1,2). Ibandronate, a potent, nitrogen-containing bisphosphonate, is effective and well tolerated when administered daily or intermittently with an extended between-dose interval (3). The MOBILE study is comparing the efficacy and safety of once-monthly oral ibandronate dosing regimens with the established daily dosing regimen (3-year vertebral fracture risk reduction: 62% (3)) in PMO.

Methods: MOBILE is a 2-year, randomized, double-blind, phase III, non-inferiority study. A total of 1602 women (55–80 years old; ≥5 years since menopause) with PMO (lumbar spine [L2–L4] BMD *T* score < −2.5 and ≥−5) are receiving oral ibandronate either daily (2.5 mg) or monthly as 100 mg (2 × 50 mg, administered on 2 consecutive days), 100 mg or 150 mg (single day). Participants are also receiving daily calcium (500 mg) and vitamin D (400 IU) supplements plus placebo medication to maintain the blind.

Results: After 1 year, lumbar spine (L2–L4) BMD increased by 4.3%, 4.1% and 4.9% in the 50 + 50 mg, 100 mg and 150 mg groups, respectively, versus 3.9% in the daily group (per protocol analysis). All once-monthly regimens were proven statistically non-inferior, and the 150 mg regimen statistically superior ($P = 0.002$), to the daily regimen (primary analysis). Substantial increases in proximal femur BMD were also observed in all treatment groups. Compared with the daily group, a larger proportion of women in the 100 mg and 150 mg groups achieved target increases in lumbar spine and/or total hip BMD (above baseline, and/or 6% and 3%, respectively). Similar and substantial reductions in serum CTX (sCTX) were observed in all treatment arms (Table). A significantly greater proportion of women in the 150 mg group compared with the daily group achieved target decreases in sCTX (30%, >50%, >70%).

Conclusions: At the studied doses, once-monthly oral dosing of ibandronate is at least as effective as an established daily dosing schedule in PMO.

1. Recker RR, et al. J Bone Miner Res 2004; 19 (Suppl. 1): S172
2. Cramer JA, et al. J Bone Miner Res 2004; 19 (Suppl. 1): S448
3. Chesnut CH, et al. J Bone Miner Res 2004; 19: 1241–9.

Table
Change (%) from baseline in sCTX (pre-dose)

Month	2.5 mg daily IBN	50 + 50 mg monthly IBN	100 mg monthly IBN	150 mg monthly IBN
3	−53.6	−50.0	−53.2	−66.1
6	−63.5	−60.7	−63.2	−73.4
12	−67.3	−62.8	−66.7	−75.8

P619-Su

Comparison of Analgesic Efficacy of Pamidronate and Human Calcitonin in Vertebral Osteoporotic Fracture Pains: A Double-Blind Study

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Our aim was to compare the analgesic efficacy of pamidronate and synthetic human calcitonin in intravenous infusion for recent painful benign vertebral compression in a randomized prospective double-blind study.

Twenty-seven patients aged 49 to 85 years, with recent (<3 months) painful benign non-traumatic vertebral compression, were included in the study. They received either pamidronate (1 mg/kg) or synthetic human calcitonin (1.5 mg) as an intravenous infusion. Pain and functional disability (EIFEL index) were evaluated before infusion and 4 and 30 days afterwards.

The pain score assessed on a visual analogue scale at D0 was 5.94 ± 2.47 in patients treated with pamidronate and 6.27 ± 2.50 in patients treated with calcitonin ($P = 0.74$), at D4 4.8 ± 2.80 with pamidronate versus 3.9 ± 2.68 with calcitonin ($P = 0.37$), and at D30 3.6 ± 3.13 with pamidronate versus 3.10 ± 2.76 with calcitonin ($P = 0.70$). Spinal function scores were 18.21 ± 3.17 at D0 in patients treated with pamidronate versus 17.23 ± 4.42 in patients treated with calcitonin ($P = 0.69$), and at D30 13.7 ± 5.36 with pamidronate versus 12.33 ± 3.22 with calcitonin ($P = 0.68$). We found no advantage of pamidronate over calcitonin in a single intravenous infusion for the treatment of painful recent benign vertebral compression. Since calcitonin is ten times less costly, its use should be preferred.

P620-Mo

Adherence to Treatment and Changes in Vitamin D Status in Hip Fracture Patientsparticipants of Post-surgical Treatment Program

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Hip fracture rate increases yearly by 1–3% in developed countries. Improvement of vitamin D status decreases hip fracture risk by 30%, alendronate leads to 50% fracture risk reduction.

Aims: To evaluate adherence to treatment in elderly participants of Post-Surgical Treatment Program (PSTP) during 1 year; to assess changes in the serum levels of calcium regulating hormones and bone mineral density (BMD) in this group.
Patients and methods: 125 consecutive elderly patients after surgical hip fracture correction were included in PSTP. Physical examination and laboratory evaluation were performed at baseline and during quarterly visits to the Metabolic Bone Diseases Clinic. All the patients received daily supplementation with 1500 mg of calcium carbonate and 800 IU of vitamin D. After improving vitamin D status, alendronate 70 mg/week was administered.
Laboratory evaluation: Intact PTH by IRMA, 25(OH)D3 by 125I-radioimmunoassay, routine biochemical tests.
BMD measurements were performed at the lumbar spine (LS), femoral neck (FN) and total hip (TH) with Lunar DEXA at baseline and after 1 year of antiresorbing therapy.
Results: There were 91 (73%) women, 34 (27%) men; mean age 72.68 ± 9.5. At baseline, 124 (99%) had inadequate vitamin serum D level; 27 (21.6%) were vitamin-D-deficient (25(OH)D3 <10 ng/ml).
Initial patients' compliance with prescribed supplements was 29 (23%). The time until improvement of vitamin D status (25(OH)D3 >18 ng/ml) and initiation of treatment with alendronate was 18 ± 6 months. Seventy one (57%) patients discontinued participation in the PSTP before starting alendronate, 65 (91%), due to non-compliance.
Alendronate was started in 54 (43%) patients; 46 (85%) completed 1-year treatment; 5 (9%) discontinued due to non-compliance, 3 (5.5%) due to adverse events.
Mean 25(OH)D3 serum level increased by 5.6 ng/ml (34%). BMD increased in LS by 5.2%, in FN by 4.8%, in TH by 2.7%, $P < 0.001$.
Conclusion: Majority of elderly hip fracture patients had inadequate vitamin D status; adherence to the prescribed calcium and vitamin D supplements and bisphosphonates was unsatisfactory even in hospital bound outpatient program.

P621-Tu

Monthly Oral Ibandronate is at Least as Effective as Daily Oral Ibandronate in Increasing Hip BMD in Postmenopausal Osteoporosis: 1-year Results from Mobile

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Objectives: Less frequent oral bisphosphonate dosing in postmenopausal osteoporosis (PMO) may further improve patient acceptability. Ibandronate, a potent, nitrogen-containing bisphosphonate, has proven efficacy and tolerability when given orally, either daily or intermittently (dosing interval of >2 months). In the MOBILE study, once-monthly and daily oral ibandronate were at least as effective in increasing lumbar spine BMD after 1 year (Table). Retrospective non-inferiority and superiority tests compared the observed changes in proximal femur BMD.
Methods: In MOBILE, 1602 women (55–80 years old; ≥5 YSM) with PMO (lumbar spine [L2–L4] BMD *T* score <−2.5 and ≥−5) are receiving 2-year treatment with 2.5 mg daily or 50 + 50 mg (single doses, consecutive days), 100 mg (single day) or 150 mg (single day) once-monthly oral ibandronate. Daily calcium (500 mg) and vitamin D (400 IU) supplements are also provided. Changes (%) in total hip, femoral neck and hip trochanter BMD were measured after 1 year. For all hip sites, margins of non-inferiority were calculated based on the observed difference between the placebo and daily ibandronate regimen in the BONE study.
Results: Substantial increases in proximal femur BMD were observed in all treatment arms at 1 year (Table). Increases in total hip and hip trochanter BMD provided by the once-monthly regimens were statistically non-inferior (margin: −0.60% and −0.72%, respectively) to those provided by the daily regimen (Table). In both cases, the 100 mg and 150 mg regimens were also statistically superior to the daily regimen (Table). Versus the daily regimen, non-inferior increases in femoral neck BMD were observed with both the 100 mg and 150 mg once-monthly regimens (margin: −0.44%; Table).
Conclusions: Once-monthly oral ibandronate is at least as effective as an efficacious daily oral ibandronate regimen in increasing lumbar spine and proximal femur BMD in PMO.

Table
Mean change from baseline (95% CI) in BMD after 1 year

	2.5 mg (n = 318)	50 + 50 mg (n = 326)	100 mg (n = 311)	150 mg (n = 320)
Lumbar spine	3.9	4.3 (−0.09, 1.12)*,†	4.1 (−0.42, 0.81)†	4.9 (0.38, 1.60)†,‡
Total hip	2.0	2.2 (−0.16, 0.70)†	2.7 (0.31, 1.18)†,‡	3.1 (0.64, 1.50)†,‡
Femoral neck	1.7	1.8 (−0.45, 0.63)	1.9 (−0.34, 0.76)†	2.2 (−0.04, 1.05)†
Trochanter	3.2	3.5 (−0.29, 0.97)†	3.9 (0.06, 1.33)†,‡	4.6 (0.82, 2.08)†,‡

*n = 328; †non-inferior and/or ‡superior vs. daily.

P622-Su

Strong Patient Preference for Once-Monthly Over Weekly Oral Bisphosphonate Dosing in Postmenopausal Osteoporosis

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Objectives: Recent studies demonstrate a strong patient preference for less frequent than daily oral bisphosphonate dosing in postmenopausal osteoporosis (PMO) (1,2). The influence of oral bisphosphonate dosing frequency on patient preference was further evaluated in a national cross-section survey. We report the outcomes of this study.
Methods: Women (aged ≥50 years) receiving weekly oral risedronate or alendronate for osteoporosis participated in face-to-face interviews at 20 sites across the USA. Participants were presented with a hypothetical scenario in which a new oral bisphosphonate was available that was similar to their existing medication except that it could be taken once monthly and the post-dose fasting period was 60 min (as opposed to the 30-min fasting period associated with their present medication). Participants were asked to express a preference for the once-monthly or weekly oral dosing schedule and the reasons for their preference. Preferences were assessed in the overall study population and by age group (50–64, 65–74 and 75+ years).
Results: Of the 393 women interviewed, 26 (7%) expressed no preference. Of the remaining 367 women, the majority expressed a preference for once-monthly (n = 247; 67%) over once-weekly (n = 120; 33%) dosing. Similar findings were reported in the three age groups examined, with the highest degree of preference for once-monthly dosing (n = 98/144, 68%) cited by the youngest age group (50–64 years). The most commonly stated reasons for preferring the once-monthly regimen were 'less frequent administration' and 'better fit with lifestyle'.
Conclusions: These findings suggest a strong patient preference for once-monthly over weekly oral bisphosphonate dosing. Consideration of patient preferences for less frequent bisphosphonate dosing regimens may help to ensure long-term therapeutic adherence in PMO.
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P623-Mo
Pamidronate Prevents Bone Loss and Decreased Bone Strength in Adult Female and Male Rats Fed an Isocaloric Low Protein Diet
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An isocaloric low protein diet is associated with decrease in bone mineral density and in bone strength as well as depressed somatotrop and gonadotrop axis. The negative bone balance is the consequence of increased bone resorption and decreased bone formation. Whether inhibition of bone resorption could prevent the low protein diet-induced bone loss and alteration of biomechanics is not known. We investigated the effects of the bisphosphonate pamidronate in 5.5-month-old female (for 19 weeks) or 6-month-old male rats (for 26 weeks) pair-fed a control (15% casein) or an isocaloric low-protein (2.5% casein). Pamidronate (0.6 mg/kg) was given subcutaneously 5 days/month for 17 weeks and for 22 weeks in female and male rats, respectively. Bone mineral density (BMD, g/cm^2), microarchitecture (trabecular bone volume, BV/TV; SMI, Index of connectivity) and bone strength (US, N) were measured at the level of the proximal and midshaft tibia. Urinary deoxypyridinoline excretion, serum osteocalcin and IGF-I were also measured. Values are means ± SEM, *$P < 0.05$ vs. control, $P < 0.05$ vs. low casein as evaluated by ANOVA. The increase in bone resorption in female rats (+100%) and in male rats (+33%) fed a low protein diet was prevented by pamidronate treatment. The reduced osteocalcin levels observed in rats fed a low protein diet were further decreased in both female (−34%) and in male (−30%) rats treated with pamidronate. The bone turnover decrease induced by pamidronate resulted in a prevention of bone strength reduction, of trabecular bone loss, of microarchitecture deterioration and of BMD decrease induced by the isocaloric low protein diet. Even a significant increase of these parameters was observed in male rats. Similar effects were observed at the level of midshaft tibia. Significant decrease of plasma IGF-I was observed in rats fed a low protein diet independently of pamidronate treatment. In conclusion, inhibition of increased bone resorption in rats fed an isocaloric low protein diet fully prevented bone loss and decrease of bone strength.

Table

	BMD	Ultimate strength	BV/TV	SMI
Control	0.2661 ± 0.003	155.5 ± 11.1	5.52 ± 0.80	2.88 ± 0.18
Low casein	0.2394 ± 0.007*	113.6 ± 4.5*	1.60 ± 0.41*	3.41 ± 0.15*
Low casein + pamidronate	0.2809 ± 0.009°	231.6 ± 18.1*,°	7.84 ± 0.74*,°	2.96 ± 0.0.07

P624-Tu
Favorable Safety Profile of Once-Monthly Oral Ibandronate in Postmenopausal Osteoporosis: 1-year Results from Mobile
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Objectives: Oral bisphosphonates in postmenopausal osteoporosis (PMO) are often discontinued due to adverse events (AEs). Adherence to therapy may be improved by reducing the opportunity for post-dose AEs through less frequent dosing. Oral ibandronate, administered daily or intermit-

tently with an extended dosing interval (>2 months), is well tolerated, with a similar safety profile to placebo (1). The MOBILE study is evaluating once-monthly oral ibandronate dosing in PMO. One-year safety and tolerability data are reported herein.

Methods: MOBILE is a 2-year, randomized, double-blind, phase III, non-inferiority study involving 1609 women with PMO (aged 55–80 years, ≥5 years since menopause, lumbar spine [L2–L4] BMD *T* score <−2.5 and ≥−5). The safety and tolerability profile of 100 mg [2 × 50 mg, administered on 2 consecutive days], 100 mg [single day] and 150 mg [single day] once-monthly oral ibandronate is being compared with the established 2.5 mg daily oral regimen. All participants are receiving daily calcium (500 mg) and vitamin D (400IU).

Results: A similar overall incidence of AEs, treatment-related AEs and treatment-related AEs leading to withdrawal was reported in the four treatment arms after 1 year (Table). Less than 8% of patients in each treatment group experienced a serious AE; only six treatment-related serious AEs and two serious AEs leading to withdrawal were reported (Table). A similar frequency of upper GI AEs was observed across the treatment arms (Table).

Conclusions: The safety and tolerability profile of monthly and daily oral ibandronate in women with PMO is similar. This is noteworthy, as daily oral ibandronate has shown a similar safety and tolerability profile to placebo in prior clinical studies.

1. Chesnut CH, et al. J Bone Miner Res 2004; 19: 1241–9.

Table
Overall incidence (*n* [%]) of AEs after 1 year

	2.5 mg daily IBN	50 + 50 mg monthly IBN	100 mg monthly IBN	150 mg monthly IBN
Any AE	273 (69.1)	264 (66.7)	268 (67.7)	277 (69.9)
Any DR AE	119 (30.1)	106 (26.8)	130 (32.8)	129 (32.6)
Any DR AE LT withdrawal	29 (7.3)	20 (5.1)	25 (6.3)	23 (5.8)
Any SAE	19 (4.8)	27 (6.8)	31 (7.8)	28 (7.1)
Any DR SAE	2 (0.5)	2 (0.5)	2 (0.5)	0 (0)
Any DR SAE LT withdrawal	1 (0.3)	1 (0.3)	0 (0)	0 (0)
Special interest AEs Upper GI AEs	71 (18.0)	63 (15.9)	86 (21.7)	67 (16.9)

DR = drug related; SAE = serious AE; LT = leading to.

P625-Su

Urinary Excretion and Efficacy of Oral Alendronate in Patients with Crohn's Disease and Osteoporosis

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The absorption of bisphosphonates from the gut is poor. The question arises whether the absorption of these agents and their bioavailability is further jeopardized by the gut's local inflammatory changes in patients with Crohn's disease, at high risk for osteoporosis, thus potentially affecting clinical outcome. To address this question, we investigated whether oral alendronate was adequately absorbed in patients with Crohn's disease and osteoporosis as defined by a *T* score < −2.5 at the lumbar spine and/or the femoral neck. Urinary excretion of alendronate and biochemical parameters of bone turnover were evaluated 3 and 6 months after start of treatment with oral alendronate at a dose of 10 mg/day in 19 osteoporotic patients with relatively stable Crohn's disease, 12 of whom had an intestinal resection. Thirteen patients had been previously treated with glucocorticoids and 5 were currently using them. The average 24 h urinary excretion of alendronate was 0.5–0.6% of the dose administered, a figure comparable to that reported in osteoporotic patients without gut pathology. The urinary excretion of alendronate was not influenced by disease localization, by gut resection or use of glucocorticoids. Urinary NTx/Cr decreased by 60% of its baseline value at 3 months after start of treatment plateauing thereafter. Bone alkaline phosphatase decreased maximally by 50% of its baseline value at 6 months. Our data suggest that, in patients with Crohn's disease, alendronate is adequately absorbed from the intestine and retained in the skeleton. This is confirmed by adequate suppression of biochemical markers of bone turnover. These data hold significant implications for the clinical management of patients with Crohn's disease and osteoporosis.

P626-Mo

Favorable Renal Safety Profile of Intravenous Ibandronate Injections in Postmenopausal Osteoporosis: 1-year Results from DIVA

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Objectives: Due to renal tolerability concerns, current intravenous (i.v.) bisphosphonates are administered as prolonged infusions. An i.v. bisphosphonate with a reduced dosing time and favorable safety profile could be advantageous. Recent studies highlight the favorable renal safety profile of ibandronate, a potent, nitrogen-containing bisphosphonate, when administered as a rapid (15–30 s) i.v. injection in postmenopausal osteoporosis (PMO). In the DIVA study, 2 mg q2mo and 3 mg q3mo i.v. ibandronate injections were at least as effective and similarly well tolerated as an established daily oral ibandronate regimen

(2.5 mg; 3-year vertebral fracture risk reduction: 62% (1)) in 1395 women with PMO after 1 year (2). Renal tolerability was also assessed.

Methods: Women with baseline serum creatinine >2.3 mg/dL were excluded. Renal adverse events were continuously monitored. Serum creatinine concentrations were measured at baseline and every 4 months thereafter in the q2mo arms and every 3 months thereafter in the q3mo arms. Creatinine clearance was estimated at the same time points using the Cockroft–Gault formula. Clinically, relevant changes in serum creatinine were defined as an increase from baseline of >0.5 mg/dL (if baseline creatinine <1.4 mg/dL) or >1 mg/dL (if baseline creatinine >1.4 mg/dL) or a 2-fold increase during treatment.

Results: A total of 1382 women were included in the 1-year safety analysis. Estimated baseline creatinine clearance was <90 mL/min in all and <60 mL/min in ~50% of participants. The incidence of renal adverse events was low and similar across the treatment groups (2%, 3% and 2% in the daily, 2 mg q2mo and 3 mg q3mo arms, respectively). No cases of acute renal failure were reported. Only 6 participants had clinically relevant changes in serum creatinine, none of which were considered treatment-related. The proportion of participants with any decrease in creatinine clearance (at any time point) was similar among the treatment groups: 14.2%, 14.1% and 17.4% in the daily, 2 mg q2mo and 3 mg q3mo arms, respectively. The mean annual change in creatinine clearance was <1 mL/min in all treatment groups, comparable with historical data from patients treated with placebo.

Conclusions: Intermittent i.v. ibandronate injections have a favorable renal safety profile in PMO. No differences were detected versus an approved daily oral ibandronate regimen.

1. Chesnut C, et al. J Bone Miner Res 2004; 19: 1241–9.
2. Recker R, et al. ACR 2004.

P627-Tu

Effect of 3- and 5-year Treatment with Risedronate on the Bone Mineralization Density Distribution of Trabecular Bone in Human Iliac Crest Biopsies

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In addition to bone microarchitecture and bone mineral density (BMD), bone material properties including the degree and distribution of mineralization, are important contributors to bone strength and fracture risk. In postmenopausal osteoporotic subjects, risedronate reduces fracture risk while concomitantly preserving bone microarchitecture and increasing BMD. In this analysis, we compared the effects of up to 5-year treatment with placebo or risedronate on the mineralization density distribution of trabecular bone via quantitative backscattered electron imaging (qBEI). qBEI is a validated technique with outcomes of mean mineralization, homogeneity of mineralization and degree of primary mineralization that are highly correlated with mineralized tissue mechanical properties at the material level. We examined a subset of iliac crest bone biopsies obtained from a clinical trial in women with postmenopausal osteoporosis. These included paired biopsies at baseline and after 3-year treatment with placebo (n = 8 pairs) or risedronate (5 mg/day; n = 10 pairs) and a third biopsy from 8 of these 10 subjects after 5-year risedronate treatment. Three-year risedronate therapy significantly increased the mean degree (+4.8%) and homogeneity (+14.8%) of mineralization and significantly decreased the low mineralized bone (−40.6%) compared to baseline. With 5-year risedronate therapy, this increase in mean mineralization was maintained, but homogeneity decreased towards baseline level. Three-year placebo treatment resulted in a smaller increase in mean degree of mineralization and had no effect on homogeneity of mineralization or amount of low mineralized bone. The mean level of mineralization with 3- and 5-year risedronate treatment was similar to historical control data from normal subjects. The increase in homogeneity of mineralization at 3 years is consistent with antiresorptive activity and reduction in bone turnover. The decrease in homogeneity of mineralization from 3 to 5 years with no change in degree of mineralization is not fully understood but may be due to a slow mineralization of bone resulting from the initial filling of the remodeling space or to a direct effect on the bone matrix as a result of the unique physical chemical properties of risedronate. These findings demonstrate that, in subjects with postmenopausal osteoporosis, 5-year treatment with risedronate increases degree of mineralization of trabecular bone to normal control levels without producing hypermineralization.

P628-Su

Effects of 3- and 5-year Treatment with Risedronate on the Bone Mineral Maturity/Crystallinity and Collagen Cross-links as a Function of Trabecular Surface Activity

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In addition to bone microarchitecture and BMD, bone material properties including the degree and distribution of

mineralization, mineral maturity/crystallinity, and collagen cross-links are important contributors to bone strength and fracture risk. Quantitative backscatter electron imaging (qBEI) analysis of human iliac crest biopsies revealed that up to 5-year treatment with risedronate increased the degree of mineralization of trabecular bone to normal control values. In the present study, the effects of up to 5-year treatment with risedronate on trabecular bone mineral maturity/crystallinity and collagen cross-link ratio (pyr/deH-DHLNL) as a function of trabecular surface activity (forming vs. resorbing) and depth (to 40 um) from the trabecular surface was examined by means of Fourier Transform Infrared Imaging. The iliac crest biopsies included pairs at baseline and after 3-year treatment with placebo (n = 8 pairs) or risedronate (5 mg/day; n = 10 pairs), and a third biopsy from 8 of these 10 subjects after 5-year risedronate treatment. At forming surfaces, the baseline and 3-year placebo groups exhibited mineral maturity/crystallinity and collagen cross-link ratio indices similar to those reported for untreated osteoporotic subjects and higher than those reported for premenopausal controls. Risedronate (3- and 5-year), however, significantly reduced mineral maturity/crystallinity and collagen cross-link ratio compared to baseline and placebo at all depths to 40 μm. The values of the 5-year risedronate group were similar to those of premenopausal normal controls. At resorbing surfaces, there were no differences among baseline, placebo, and risedronate (both 3- and 5-year) groups in mineral maturity/crystallinity or collagen cross-link ratio at any depth. The values were similar to those in untreated osteoporotic subjects and higher than those in normal controls. The anatomical locations and time points for these differential effects are consistent with mineralization data (via qBEI) and may result at least in part from direct effects on the bone matrix due to the unique physical chemical properties of risedronate. This reversal and normalization of osteoporosis-related changes in bone material properties at formation surfaces may be one of the contributing factors to bone strength and to risedronate's anti-fracture efficacy.

P629-Mo

Head-to-head Clinical Trials Comparing Osteoporosis Treatments: Design Considerations

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Properly conducted clinical trials provide the highest level of evidence of treatment efficacy, and head-to-head trials are the ideal way to compare efficacy and tolerability of agents. Although fracture is the optimal measure of efficacy, the need for huge sample sizes and other complexities of trial design makes it unlikely that head-to-head fracture trials will be performed, and alternatives should be considered.

The sample size in head-to-head fracture trials is influenced by, not only the expected incidence of fractures, but by other factors as well, including the analysis objective, for example, an equivalence or non-inferiority trial versus a superiority trial. Because both active treatments may reduce the overall incidence of fracture and the difference between treatment groups may be relatively small (compared to the difference between either active agent and placebo), sample sizes will be considerably larger than in a placebo-controlled trial.

In a superiority trial, with a power of 80% and a fracture rate of 1.2% among participants in one group, 235,000 participants would be required to show a difference of 10% in efficacy; 18,080 would be required to show a difference of 30%. Even if the fracture rate in one group was as high as 15%, 14,700 participants would be needed to demonstrate a difference. In an equivalence trial, sample sizes in the 30,000 to 40,000 range are required. Comparing fracture incidence in smaller studies is misleading and fundamentally meaningless because the power to detect a real difference is negligible. Non-significant differences are almost certainly due to chance. In this regard, meta-analysis may be valuable, since the procedure pools all available data to derive the most precise estimate of effect of an agent.

As an alternative, head-to-head trials could examine endpoints that are well-validated intermediate endpoints for fracture events, including BMD and bone turnover. Such trials can be considerably smaller and more quickly completed than fracture trials, and a large number of studies have documented the strong, graded association between fracture incidence and these surrogates.

P630-Tu

Fracture Risk and Gastrointestinal Effects of Bisphosphonates: Limitations of Observational Data in Comparing Treatments

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Recent studies using medical claims data suggest that differences in fracture risk, gastrointestinal (GI) event profiles, and associated resource utilization may exist among bisphosphonates (BPs). Our objective was to use principles of evidence-based medicine to evaluate and rate these studies in the context of other evidence. We evaluated recent administrative claims analyses utilizing recommendations for evaluation of evidence to compare with findings from other types of studies. Prior reviews of evidence were also consulted. The evidence-based medicine hierarchy was used to rate evidence.

In the hierarchy of evidence, administrative claims data have the least validity for comparing two or more agents; results of head-to-head clinical trials have the greatest validity. The

primary, and insurmountable, difficulties with analyses of claims data are that patients were not randomly assigned to treatment and patients and physicians were not blinded to treatment assignment. As a consequence, there is no way to assure that the groups were comparable. Many potential sources of bias exist in analyses of such data, and matching on age or other variables may not effectively control all bias. For example, patients may have been warned to watch for certain adverse events, which could increase reporting and apparent associations with BP use even if a true cause–effect relationship does not exist. The risk of future fracture may differ widely between groups, because severity of disease information (bone mineral density, fracture history) is not available in claims data. A classic example of limitations of non-trial data is that of hormone replacement therapy, which, in observational studies, appeared to prevent cardiovascular disease but was later proven in a large randomized controlled trial to increase risk.

In contrast to some observational studies, randomized trials have not demonstrated significant differences in GI events between different BPs or between BP and placebo. Observational studies of fracture outcomes are also inconsistent with results from randomized trials. Randomized clinical trials are the highest level of scientific evidence. Analyses of medical claims and other studies without randomization and blinding should be viewed with caution as they represent a much lower level of evidence, and the results are not adequate to address a cause–effect relationship.

P631-Su

Facts-international: Greater Increases in BMD with Alendronate Once Weekly Compared to Risedronate Once Weekly

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Objective: This 12-month, multicenter, international, double-blind trial was designed to evaluate efficacy and tolerability of alendronate once weekly (ALN) as compared to risedronate once weekly (RIS) for treatment of osteoporosis in postmenopausal women.

Methods: Women with osteoporosis (T score < -2.0 at either lumbar spine, trochanter, total hip or femoral neck) were enrolled at 75 centers in 27 countries. Patients were randomized to treatment with either active ALN 70 mg weekly + RIS placebo weekly or active RIS 35 mg weekly + ALN placebo weekly. Measurements included spine and hip BMD, markers of bone turnover (BSAP and urinary NTx), and adverse experience reporting for assessment of tolerability.

Results: A total of 936 women were randomized; mean age 64 years (range 43–90); 79% were Caucasian. 75% had a BMD T score < -2.5 at any site or a wrist, spine or hip fracture after age 45. ALN produced greater increases in BMD than did RIS at 12 months at all sites measured (hip trochanter 3.6% ALN vs. 2.7% RIS, $P < 0.01$; lumbar spine 4.6% ALN vs. 3.8% RIS, $P < 0.01$; total hip 2.7% ALN vs. 2.0% RIS, $P < 0.001$; femoral neck 2.3% ALN vs. 1.7% RIS, $P < 0.05$). Greater reduction in bone turnover was seen with ALN compared to RIS: NTx decreased 58% with ALN compared to 47% with RIS at 12 months ($P < 0.001$); BSAP decreased 45% with alendronate compared to 34% with RIS at 12 months ($P < 0.001$). Decreases in BSAP and NTx were significantly greater with ALN compared to RIS at 3, 6 and 12 months ($P < 0.001$). Overall tolerability was similar for the 2 agents. Percentage of patients reporting an upper GI AE was similar (20% ALN, 20% RIS).

Conclusions: This study demonstrates that alendronate once weekly provides greater increases in BMD at both hip and spine sites than does risedronate once weekly and that alendronate decreases bone resorption to a greater degree than does risedronate. Similar tolerability was noted with alendronate and with risedronate. A similar study conducted in the US (JBMR 2004) demonstrated similar results.

P632-Mo

Differences in Early Dynamics of Serum Bone Markers in Women with Postmenopausal Osteoporosis Treated by Alendronate or Risedronate

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Background: Bone biochemical markers are useful tools for monitoring biphosphonates treatment and are also considered markers of bone quality. The exact dynamics of serum bone markers after initiation of biphosphonates therapy are unclear, and early changes of serum bone markers have not been evaluated.

Materials and methods: 70 post menopausal women afflicted with osteoporosis have been recruited (mean age 63.2 ± 1.4), randomized and treated weekly with alendronate 35 mg (AL) or risedronate 35 mg (RE). At the baseline, patients were clinically evaluated with a bone mineral density (by DEXA at L1–L4 and proximal femur), spine X ray with vertebral morphometry and determination of blood bone markers (CTX and BAP) at 0, 15, 30, 60 and 90 days. Bone markers serum changes from the beginning of the treatment were calculated using the following formula: [(post basal value − basal value) / (basal value)] × 100. Changes from baseline inter and intra group were analyzed by ANOVA.

Results: Patients treated with AL had a progressive decrease of CTX in the first 90 days (15 days—20.6%, 30 days—39.3, 60 days—51%, 90 days—56%), while those on RE reached quickly the NADIR after 15 days of therapy (15

days—57%, 30 days—59.3, 60 days—61%, 90 days—59%). The difference on CTX among the two groups was statistically significant at 15 and 30 days ($P < 0.05$). In patients treated with AL serum levels of BAP were significantly reduced after 60 days, while in those on RE, BAP was significantly reduced at 30 and 60 days ($P < 0.05$).
Discussion: In conclusion, Ris reduces bone turnover more rapidly than AL. The onset of drug action may be important, especially in those with high fracture risk.

P633-Tu

Effect of Aminobiphosphonates on Activation and Differentiation of t Gamma Delta Lymphocytes: An in Vivo Study

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Introduction: Bisphosphonates are powerful inhibitors of bone reabsorption, used for the treatment and prevention of osteoporosis (OP) and other diseases characterized by an increased bone remodeling.
In vivo studies showed that aminobiphosphonates activate T γδ lymphocytes. The expression of CD27 e CD45RA antigens defines 4 subsets of human T γδ lymphocytes: naive $CD27^+$ $CD45\ RA^+$ lymphocytes central memory (CM) $CD27^+$ $CD45RA^-$ express the receptors of homing for limph-nodes and miss immediate effectors functions; lymphocytes effectors memory (EM) $CD27^-$ $CD45RA^-$ and terminally differentiated (TEMRA) $CD27^-$ $CD45RA^+$ express homing receptors for inflammation sites and have immediate effectors functions.
Aim of the study. Aim of the study was to identify aminobiphosphonates effects on 4 subsets of T γδ in peripheral blood of OP patients on treatment with oral or infusive therapy. The used medications were: alendronate, risedronate, neridronate, zoledronate.
Materials and methods: 35 patients have been studied and alternatively randomized. Each patient underwent DEXA L1−L4 and at femoral neck, spine X rays with morphometric measurement. To identify the subsets of lymphocytes T γδ , lymphocytes from peripheral blood were isolated and analyzed using citofluorimetry with three fluorences. Monoclonal antibodies used are: anti Vδ2-FITC, anti CD27PE and anti CD 45 RA PE Cy-5.
Results: Patients treated with alendronate ($n = 11$): age 65 ± 10, *T*-score L1−L4 −2.7 ± 0.8, T-score femural neck −2.93 ± 0.8. Patients treated with risedronate ($n = 11$): age 62 ± 9, *T*-score L1−L4 −2.18 ± 0.5, *T*-score femoral neck −2.2 ± 0.7. Patients treated with zoledronate ($n = 7$): age 64 ± 8, *T*-score L1−L4 −2.54 ± 0.8), *T*-score femoral neck −2 ± 0.8. Patients treated with neridronate ($n = 9$): age 63 ± 5, *T*-score L1−L4 −2.6 ± 0.8, *T*-score neck −2.4 ± 0.6.

Table

CASI	NAIVE	CM	EM	TEMRA
AL (11)	↓ 72%	↓ 63%	↑ 72%	↑ 90%
RE (11)	↓ 45%	↓ 45%	↑ 63%	↑ 72%
N (6)	↓ 66%	↓ 66 %	↑ 66%	↑ 66%
ZOL (7)	↓ 85%	↓ 100%	↑ 100%	↑ 100%

Discussion: All drugs used were able to activate Tγδ lymphocytes and differentiate them in effectors subpopulations.
Alendronate and zoledronate showed a more prominent action inducing a reduction of the naive and CM and an increase of EM and TEMRA. Our data demonstrate that aminobiphosphonates induce the differentiation of T γδ lymphocytes in the effectors phenotype, able to induce cytotoxic responses.
We suppose that biphosphonates may induce T γ9Vδ2 lymphocytes to mediate the antireabsorbitive activity.
Dieli F, Gebbia N, Poccia F, Caccamo N, Montesano C, Fulfaro F, Arcara C, Valerio MR, Meraviglia S, Di Sano C, Sireci G, Salerno A.-Induction of gammadelta T-lymphocyte effector functions by bisphosphonate zoledronic acid in cancer patients in vivo. Blood. 2003 Sep 15;102(6):2310–1.

P634-Su

In Vitro Effects of Bisphosphonates on Gamma Delta Cell Activation and Differentiation

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Bisphosphonates (BPs) are drugs that inhibit bone resorption used for the treatment of osteoporosis and other metabolic bone diseases in which osteoclast number and bone resorption increase and bone mass decreases. BPs are analogs of endogenous pyrophosphate (POP). A new potential mechanism of these compounds was recently reported. These drugs were shown to induce expansion of human Vγ9Vδ2 T cells in peripheral blood, in vivo and in vitro, raising the possibility that N-BPs may induce Vγ9Vδ2 T cells to exert anti-resorptive activity. Furthermore, mevalonate pathway inhibition also results in metabolites accumulation, such as isopentenyl pyrophosphate (IPP) and dimetyl-allyl pyrophosphate (DMAPP), both able to stimulate Vγ9Vδ2 T cells.
Expression of CD27 and CD45RA antigens defines four subsets of human γδ T lymphocytes (5). Naive CD27+ CD45RA+ and central memory (CM) CD27+CD45RA− cells express lymph node homing receptors, abound in lymph node, and lack immediate effector functions. Conversely, effector memory (EM) CD27−CD45RA− and terminally differentiated (TEMRA) CD27−CD45RA+ cells, which express receptors for homing to inflamed tissue, are poorly represented in the lymph nodes while abound at sites of inflammation and display immediate effector functions.
The objective of this study was to evaluate the in vitro effects of aminobisphosphonates on Vγ9Vδ2 T cell subsets derived from peripheral blood of healthy subjects.

Compounds: Alendronate, neridronate, risedronate, zoledronate BrHPP was used as positive control. All drugs were tested at 100 nM final concentration.
Cell preparation and in vitro culture: Peripheral blood mononuclear cells (PBMCs) were isolated by centrifugation of heparinized peripheral blood over Ficoll–Hypaque gradients. 5 × 105 cells were cultured in 24-well plates at 37°C in humidified atmosphere (5% CO2) in the presence of drugs. Medium used throughout was complete RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS), 2 mM glutamine, 20 nM Hepes and 100 u/ml penicillin, streptomycin and gentamycin. At the third day of culture, 20 u/ml of human recombinant interleukin-2 (rIL-2) were added to wells. Vγ9Vδ2 expansion and differentiation were evaluated after 1 week of culture. For subset identification, PBMCs were isolated and analyzed using 3-color flow cytometry. Monoclonal antibodies used included: anti-Vδ2 FITC, anti-CD27 PE and anti-CD45RA PE Cy5.

P635-Mo

The Impact of Background OP Meds on Baseline Fracture Risk from the Horizon PFT with Zoledronic Acid

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The HORIZON PFT (Pivotal Fracture Trial), is a multinational, randomized, double-blind, placebo-controlled clinical trial to evaluate zoledronic acid (Zol) in reducing hip and vertebral fracture risk in postmenopausal women age 65–89 with osteoporosis. The novel study design stratifies by use of concomitant osteoporosis medications. A resulting question is if the patient groups differ in terms of fracture risk due to the use of background osteoporosis medications. Investigators at 230 clinical centers worldwide enrolled 7765 postmenopausal women with femoral neck BMD < −2.5 or between −1.5 and −2.5 with at least one moderate or two mild vertebral deformities. All patients were randomized to receive 3 annual doses (5 mg IV over 15 min) of Zol or placebo, plus daily supplements of calcium (1000–1200 mg) and vitamin D (400–1000 IU). Stratum I patients were not taking any other background osteoporosis medication. Stratum II patients were allowed use of osteoporosis background medication, including HRT, SERMS, tibolone, or calcitonin. Bisphosphonates, PTH, Fluoride and strontium were excluded.

Baseline characteristics including age, BMD and existing vertebral fracture very similar for both strata with the exception that the vast majority of Stratum II patients were recruited from North America/Oceania and Western Europe. Using a risk model developed from the SOF database, the probability of fracture over 3 years for: vertebral, hip and non-spine clinical fractures was estimated (Table 1). Numerically, the range of fracture probabilities is similar between strata, falling within the range of that seen across regions.
Despite differences in the use of osteoporosis background medications between strata, the baseline characteristics and fracture risks are remarkably similar.

Table
Probability of fracture over 3 years (%)

	Vertebral (1)	Hip (2)	Non-spine (3)
Overall	13.7	3.5	16.8
Stratum 1	14.3	3.6	17.2
Stratum 2	11.6	3.1	15.5
North America	12.9	3.5	16.3
Latin America	14.2	3.9	17.4
Western Europe	13.6	3.6	16.7
Asia	13.8	3.0	16.6
Eastern Europe	14.4	3.4	17.2

Assuming (1) 20%, (2) 15%, (3) 10% risk reduction.

P636-Tu

Ibandronate Improves Osseointegration of Cementless Metal Implants to a Similar Extent whether given Continuously or as a Single Equivalent Cumulative Dose

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Objectives: Osseointegration – defined as the direct structural and functional connection between bone and the surface of a load-carrying implant – determines the secondary stability and long-term survival of uncemented total joint replacements. Bisphosphonates increase the early bone formation rate and osseointegration around cementless metal implants and improve periprosthetic bone mineral density. The potent nitrogen-containing bisphosphonate, ibandronate, can reduce the time to osseointegration of hydroxyapatite-coated implants. However, as continuous dosing of bisphosphonates may result in suboptimal adherence to therapy, less frequent dosing is desirable. This study examined whether a single injection of ibandronate at an equivalent dose to continuous daily injections was effective at improving the osseointegration of metal implants.
Methods: Titanium or hydroxyapatite-coated titanium implants were inserted into the medullary canal of each femur (through the intercondylar notch of the knee) of 60 female Sprague–Dawley rats using a press-fit technique. Following surgery, animals were randomly assigned to 27 days treatment with subcutaneous ibandronate 25 μg/kg/day,

an equivalent single dose of ibandronate 675 μg/kg or NaCl 0.9%. After 27 days, animals were sacrificed, and femoral specimens harvested. Following lateral radiography, specimens were prepared for quantitative histomorphometry using standard techniques. Histomorphometry was performed on the first metaphyseal cross-section behind the epiphysis. The osseointegrated implant surface (OIS), i.e. the percentage of the implant surface in direct contact with surrounding bone, was determined using image analysis.

Results: Both single and continuous daily ibandronate injections significantly improved OIS compared with the control for hydroxyapatite-coated titanium implants. No significant differences were observed between the two active treatments (Table). No significant improvement in OIS was observed with uncoated titanium-only implants.

Conclusion: Hydroxyapatite appears to assist the anti-resorptive and pro-osteoblastic effect of ibandronate. A convenient single injection of ibandronate is as effective as continuous daily dosing in improving osseointegration and consequent stabilization of hydroxyapatite-coated metal implants.

Table

Treatment	Mean OIS (SD), %
NaCI 0.9% (control)	29.4 (15.1)
IBN 25 μg/kg/day × 27 days	46.1 (21.4)*
IBN 675 μg/kg/day (one dose)	45.3 (24.1)*

IB *n* = ibandronate; *$P < 0.05$ vs. control.

P637-Su
Incidence of Influenza-like Symptoms with Intermittent Intravenous Ibandronate Injections: 1-year Results from DIVA

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Objectives: Influenza-like illness (ILI) is a commonly experienced side effect of intravenous (i.v.) and high dose oral bisphosphonate dosing. In prior clinical studies, a low incidence of transient and mild ILI was observed following the i.v. administration of ibandronate (0.5 mg, 1 mg and 2 mg q3mo) by rapid (15–30 s) injection. In the DIVA study, 2 mg q2mo and 3 mg q3mo i.v. ibandronate injections were at least as effective as an established daily oral ibandronate regimen (2.5 mg; 3-year vertebral fracture risk reduction: 62%[1]) in 1395 women with postmenopausal osteoporosis (PMO) after 1 year[2]. A detailed analysis of ILI was performed.

Methods: To overcome likely inconsistencies in the reporting of ILI, a constellation of 33 potential ILI adverse event (AE) terms, collectively termed 'influenza-like symptoms', was prospectively defined. These included both specific reported terms (e.g. ILI, acute-phase reaction (APR), myalgia, arthralgia) and more general reported terms (e.g. headache, dizziness, fatigue, feeling hot). Consistent with the typical characteristics of ILI, only those events occurring within 3 days of dosing and with a duration of <7 days were included in the analysis.

Results: A total of 1382 women were included in the 1-year safety analysis. As expected, a higher incidence of 'influenza-like symptoms' was reported in the i.v. arms than the oral arm (Table). However, the incidence of ILI, APR, myalgia and arthralgia was low. In addition, influenza-like symptoms mostly occurred with the initial administration only (>80% of affected patients reported no repeat symptoms), were generally mild to moderate in intensity, transient in nature and resolved without symptomatic treatment. Withdrawals due to influenza-like symptoms were also low (0.4–2.6%).

Conclusions: Intermittent i.v. ibandronate injections (2 mg q2mo, 3 mg q3mo) are associated with a low incidence of ILI in PMO.

1. Chesnut C, et al. J Bone Miner Res 2004;19:1241–9.
2. Recker R, et al. ACR 2004.

Table
Incidence of ILI* (*n* [%]) after 1 year

	2.5mg daily oral IBN (n = 465)	2mg q2mo i.v. IBN (n = 448)	3mg q3mo i.v. IBN (n = 469)
Any influenza-like symptom (ILS)	18 (3.9)	64 (14.3)	45 (9.6)
ILI	3 (0.6)	15 (3.3)	15 (3.2)
APR	0 (0)	2 (0.4)	2 (0.4)
Myalgia	1 (0.3)	14 (3.1)	6 (1.3)
Arthralgia	1 (0.2)	5 (1.1)	6 (1.3)

P638-Mo
Less Frequent Oral Bisphosphonate Dosing Improves Therapeutic Persistence in Postmenopausal Osteoporosis

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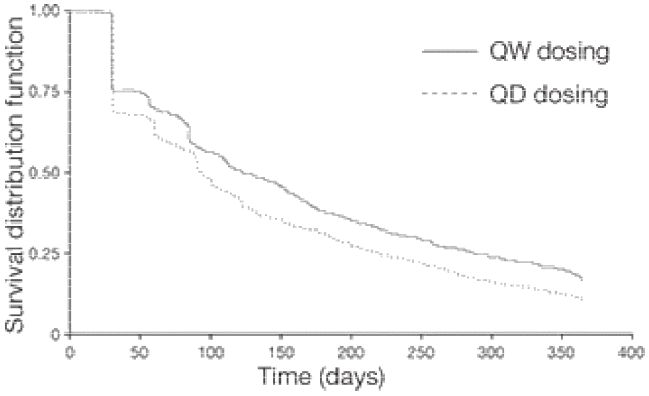
Objectives: Less frequent than daily oral bisphosphonate dosing schedules are predicted to improve therapeutic adherence in postmenopausal osteoporosis (PMO). This study explored persistence with daily (qd) and weekly (qw) oral bisphosphonates.

Methods: Women aged >45 years with PMO starting a prescription for alendronate (35 or 70 mg qw or 5 or 10 mg qd) or risedronate (35 mg qw or 5 mg qd) between October 2000 and December 2002 were identified from six US health plans (four commercial, two Medicaid). Prescription data were then collected from administration claims databases. Persistence was defined as the time from when the first prescription was filled to the last recorded prescription of the index drug, plus the supply (in days) of drug in the last prescription. Non-

persistence constituted a gap of >30 days or change from the index drug (to a different dosage or different drug). The association between treatment cohort and non-persistence was examined using a Cox proportional hazards model.

Results: One-year follow-up data were obtained for 2291 women (qw, n = 1293; qd, n = 998; mean age: 62 years). Persistence rates were 52.7% and 42.1% at 3 months and 16.4% and 10.2% at 12 months in the qw and qd cohorts, respectively. The mean duration of persistence with bisphosphonate therapy during the first year of treatment was longer with qw dosing (168 vs. 140 days, respectively; Figure). The qw regimen was associated with a lower risk of non-persistence versus the qd regimen at 12 months (HR 0.80; 95% CI: 0.72–0.89; $P < 0.001$). Women who switched from qd to qw dosing ($n = 243$) showed improved persistence (201 days) versus those who did not (n = 755; 152 days). Switching to the qw regimen was also associated with a significantly lower risk of early treatment discontinuation (HR 0.69; 95% CI: 0.58–0.82; $P < 0.0001$).

Conclusions: Less frequent than daily oral bisphosphonate dosing is associated with improved persistence. However, adherence with weekly oral bisphosphonates remains suboptimal. Less frequent than weekly dosing schedules, such as once monthly, may further improve therapeutic adherence in PMO.



P639-Tu

Dose- and Time-Dependent Apoptosis of Human Sarcoma Cells by Ibandronate and Clodronate in Vitro

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Objectives: Although the treatment of sarcomas is associated with high survival rates (~80%), there are still a significant number of non-responders who go on to develop metastases and finally die. Bisphosphonates are known to induce apoptosis in a number of cell types, including osteoclasts and breast cancer cells. The direct effects of bisphosphonates on primary bone tumors are not resolved. We evaluated the direct effects of clodronate (CL) and ibandronate (IB) on human sarcoma cells in vitro.

Methods: Two human osteosarcoma cell lines (SAOS 2 and 791 TM HOS) and two human Ewing's sarcoma cell lines (RD-ES and TC 71) were treated with CL (2 mM, 1 mM–10^{-7} M) or IB (10^{-3} M–10^{-7} M) for 24, 48 and 72 h. Cell apoptosis was evaluated by fluorescent flow cytometric analysis: quantification via FACS and PI staining. After 72 h staining with trypan blue, dead cells were counted.

Results: CL-treated SAOS cultures show after 72 h incubation in concentrations of 2 mM and 10^{-3} M 12.6% and 8.7% apoptotic cells, respectively. CL-treated HOS cultures show after 48 h incubation in the range of 2 mM and 10^{-3} M <10% apoptotic cells and after 72 h incubation at 10^{-3} M 10–25% apoptotic cells. Ewing's sarcoma cell cultures (RD-ES cells) treated with CL show after 72 h incubation at 2 mM 10–25% apoptotic cells, while TC 71 cells show at 2 mM <10% apoptotic cells. 10^{-3} M IB solution shows after 48 h <10% and after 72 h 10–25% apoptotic SAOS cells. HOS cells show after 48 h in the range of 10^{-3} M 10–25% and at 10^{-4} M <10% apoptopic cells; after 72 h, apoptotic cells increased to >40% at both IB concentrations. RDES cells incubated with IB showed at 10^{-3} M (5.1%)–10^{-5} M (12.3%) clear parts of the culture in the S-phase of the cell cycle. TC 71 show at concentration of 10^{-3} M and 10^{-4} M IB 10–25% and at 10^{-5} M and 10^{-6} M IB <10% apoptopic cells, respectively.

Conclusions: CL and IB have time- and dose-related direct effects on primary bone tumor cells. Both bisphosphonates induce apoptosis and inhibit cell proliferation. However, a much better effect was observed with IB at lower concentrations. If similar results are observed in vivo, use of IB as an adjuvant to existing chemotherapeutic protocols may be indicated.

P640-Su

Site-specific Variations in Therapeutic Effects of Salmon Calcitonin–Nasal Spray (CT–NS) on Bone Quality (BQUAL) (Trabecular Microarchitecture (TMA) and Bone Quantity (BQUANT) (BMD): Results from the Quest Study

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Reduction in osteoporotic (OP) fracture risk in response to certain anti-resorptive therapies may be due less to

effects on BQUANT-BMD than on BQUAL-TMA. To explore this hypothesis, the 2 year QUEST study in 91 postmenopausal (PM) OP women assessed the effects of CT–NS+calcium (Ca) vs. placebo (P)+Ca on BMD (DXA) and TMA (high resolution MRI) at multiple skeletal sites.

Although no significant change through 2 years was noted in BMD (spine/hip/distal radius) in CT–NS or P groups, regardless of the change in BMD, a consistent and significant improvement or preservation in TMA through 2 years was noted in the CT–NS group at distal radius and hip (T2*), with consistent and significant deterioration noted in these parameters in the P group (example: at distal radius +2.0% appBV/TV, +1.7% app trab #, −2.3% app trab spacing in CT–NS, compared to −9.1%, −6.9%, +12.9% in P; changes significant within and between groups, $P < 0.001–0.05$).

As well, in the Ca only P group, significant deterioration was noted in parameters of TMA at distal radius and hip even in women gaining or showing no change in BMD at spine, hip or distal radius (example: in women in the P group gaining or showing no change in BMD at the total hip, a significant ($P < 0.005$) deleterious +10.0% increase for the MRI-T2* measurement at the lower trochanteric site).

These results suggest (1) a beneficial effect of the anti-resorptive therapy CT–NS on TMA regardless of its effect on BMD, (2) a potential loss in TMA in PM-OP women receiving only calcium even when BMD is stable or increasing and (3) a possible independent effect of anti-resorptive therapies on BQUANT-BMD and BQUAL-TMA.

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P641-Mo

The Effect of High Calcium Fortified Milk Supplementation on Biochemical Markers of Bone Resorption and Formation in Premenopausal Women

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Females between the ages of 20 and 35 years may still have the opportunity to increase their peak bone mass. In adolescent girls, there was an increase in bone mineral deposition with milk consumption but no effect on turnover, while another study showed increased calcium retention due to increased milk intake and increased calcium absorption resulting in decreased bone resorption. The purpose of this study was to compare the effect of high calcium skim milk (ANLENE TM) and high calcium skim milk with added vitamin K1 on markers of bone formation/resorption in premenopausal women.

Women between 20 and 35 years of age were supplemented with two servings of high calcium skim milk (1000 mg extra calcium per day) or high calcium skim milk with added vitamin K for 16 weeks. An unsupplemented group of women was used as controls ($n = 26$/group for all three groups). Bone density was assessed at baseline, and markers for bone turnover and resorption (osteocalcin, CTx, P1NP) were measured at baseline, weeks 2, 12 and 16. Plasma vitamin K and undercarboxylated osteocalcin were measured in the control and group supplemented with vitamin-K-fortified milk at weeks 0 and 16, while parathyroid hormone and vitamin D levels were assessed in all women at weeks 0 and 16. Habitual food and calcium intake was assessed. Repeated measures ANOVA over weeks 0 to 16 was done for all markers. A split plot model with group in the main plot and week and week * group interactions as the sub plot was used. Measurements were considered to be significantly different if $P < 0.05$.

Baseline values for age, body mass index and bone density did not differ between groups. Plasma vitamin K1 levels increased in the vitamin-K1-fortified milk supplemented group (0.271 to 0.762 μg/L, $P < 0.05$), while levels for undercarboxylated osteocalcin decreased over 16 weeks (9.68 to 4.46 ng/mL, $P < 0.05$). Plasma CTx and osteocalcin reduced significantly in both supplemented groups as compared to control over 16 weeks (CTx >30% and OC >15%). PTH and vitamin D levels did not change significantly in the groups over 16 weeks.

High calcium milk supplementation significantly reduced bone resorption and turnover in younger women over 16 weeks. The addition of vitamin K1 strengthened the effect of added calcium. High calcium milk supplementation in younger women may still have an impact on attainment of peak bone mass by reducing bone turnover and loss.

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P642-Tu

Acidic Protein Fractions from Whey or Milk Limit Bone Loss in the Female Ovariectomized Rat

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Bovine milk has been shown to contain bioactive components with anti-resorptive activity in bone and may also contain growth factors that affect bone growth. Earlier studies showed that bovine whey proteins suppressed bone resorption in female ovariectomized rats. A novel osteotropic activity was subsequently identified in the whey basic protein fraction, but there is some evidence that other whey components may also have bone bioactivity.

In an initial study, we investigated whether acidic protein fractions (AF) isolated from bovine milk or whey protein concentrate (WPC) could prevent bone loss and/or bone resorption in mature female rats. Six-month-old female rats were ovariectomized (OVX) or left intact (sham). The OVX rats were randomized into 4 groups. One group remained

the control (OVX), while three groups were fed various whey acidic protein fractions (AF) as 0.3% (w/w) of the diet for 4 months. Femur bone mineral density indicated that one of the whey AFs limited bone loss due to OVX. Plasma C-telopeptide of type I collagen decreased significantly in all groups, but the decrease was more substantial in all the AF groups. Biomechanical data showed that the acidic fractions may affect bone architecture.

In a follow-up study, we extended our observations by investigating whether alternative acidic protein fractions (AF) isolated from WPCs made with either rennet or mineral acid could prevent bone loss in female rats. We also compared the impact of these acidic protein fractions with that of basic (high isoelectric point) protein fractions isolated from both milk and rennet WPC. Six-month-old female rats were OVXed or left intact. The OVX rats were then randomized into five groups. One group remained the control (OVX), while the remaining four groups were each fed one of the test fractions as 0.3% (w/w) of the diet for 4 months. Bone mineral density of the spines and femurs indicated that the AF prepared from mineral acid WPC limited bone loss due to OVX. Biomechanical data suggested that both the AFs (from either mineral acid or rennet WPC) may affect bone collagen and mineralization. This is the first report that acidic whey protein fractions isolated from bovine milk may inhibit bone loss due to OVX in vivo.

Funded by Fonterra Cooperative Group Ltd.

P643-Su

Dose–response of Hesperidin, a Citrus Flavonoid, on Bone Mass in Young Intact Rats

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A sub-group of flavonoids, flavanones, is present in our diet almost exclusively in citrus fruits. Hesperidin is the main flavanone found in orange fruit (~2 g/kg), and we have previously reported its ability to affect bone mass and strength in intact and ovariectomized rats. The aim of the current study was to establish the minimum effective dose of pure hesperidin (Hp) which could stimulate bone formation in 3-month-old intact rats. The dose–response was carried out on 60 young intact Wistar rats, with a time duration of 3 months. The animals were assigned to 6 groups of 10 rats each. One group received a soy-protein-free, semi-purified standard diet (SH), while the others were fed the same diet but with added hesperidin at various doses: 0.125% (SH1); 0.25% (SH2); 0.5% (SH3); 1% (SH4); and 2.5% (SH5) of the diet. Throughout the study period, rats kept growing with the same pattern of body weight evolution in each group and, on day 85, no difference in body composition among groups was detected. At necropsy, femoral mineral density (g/cm^2) was significantly increased in SH2 and SH3 (+5% vs. SH; $P < 0.05$) groups with the same magnitude, this effect was even stronger in SH4 and SH5 groups, when compared to the controls (+6.1% vs. SH; $P < 0.01$). This impact was achieved in both total and metaphyseal compartments. Diaphyseal femoral density was also increased by Hp consumption starting from the 0.25% dose upwards (SH2: +4.1% vs. SH; $P < 0.05$; SH4: +5.2% vs. SH; $P < 0.01$). This was paralleled with an enhanced femoral failure load. No differences were observed in BMD or femoral strength, with the lowest dose (0.125%). Plasma osteocalcin concentrations were unchanged in all groups, while urinary deoxypyridinolin excretion was significantly decreased in the group from 0.125–2.5% (SH1: −13.3% vs. SH), indicating a slow down of bone resorption. Plasma hesperetin concentration (aglycone form) increased in a dose-dependent manner, each dose inducing a significant difference in plasma level when compared to the lowest dose. A strong correlation with hesperetin intake (24 h) was noted ($R^2 = 0.98$) and with urinary DPD excretion ($R^2 = 0.88$). In conclusion, hesperidin consumption improved both femoral strength and mineral density. This appeared to be linked with a decreased catabolism. The effective dose was 0.25% hesperidin, corresponding to 1.76 μM plasma hesperetin when measured during the post-absorptive period.

P644-Mo

Zinc Increases the Effects of Protein Supplements on Serum IGF-I and Bone Turnover in Frail Elderly

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Protein malnutrition is frequent in elderly. It is associated with decreased bone formation, increased resorption and bone loss in relation with depressed somatotrop axis. Zinc modulates IGF-I production. To investigate whether the effects of protein supplement could be amplified by zinc, 61 frail elderly were given an oral 20 g protein supplement and calcium (550 mg), with or without 30 mg/day of zinc for 4 weeks. Hospitalized subjects with high risk of malnutrition were selected according to the day mini-nutritional assessment. Plasma IGF-I (μg/l), CrossLaps (ng/ml), osteocalcin (μg/l) and albumin (g/l) were measured before and after 1, 2 and 4 weeks of protein repletion. Functional performance was assessed using activity of daily living score. Significance of differences was evaluated using an unpaired Student's t test or with an ANOVA. At baseline, mean (±SEM) age of the subjects were 83.6 ± 1.3 in protein/zinc group and 86.4 ± 1.3 in protein controls (NS); plasma IGF-I was 82.6 ± 9.0 and 73.3 ± 8.3 (NS), and albumin 31.0 ± 0.8 and 30.4 ± 0.8 (NS), respectively. Protein supplements with or without zinc significantly increased plasma IGF-I ($P <$ 0.05). In patients receiving zinc, IGF-I reached a plateau

significantly earlier than in controls (48.2 ± 14.3 vs. 22.4 ± 4.7%, $P < 0.05$) by 1 week. Patients receiving protein/zinc reduced bone resorption as indicated by a decrease in serum CrossLaps (−21.0 ± 6.7 vs. 23 ± 18.2%, $P < 0.05$), significant already by 1 week. Bone formation as reflected by osteocalcin was increased in both groups (16.4 ± 7.0 and 21.5 ± 9.0%, $P < 0.05$) as compared to baseline. Activity of daily living score was improved in both groups (27.0 ± 3.1 and 18.3 ± 4.7%, $P < 0.05$) but was significantly increased by zinc in the subgroup with albumin lower than 30 mg/L (25.7 ± 8.1 vs. 11.9 ± 6.5%, $P < 0.05$). Zinc supplement positively influenced the kinetics of IGF-I recovery and decreased bone resorption upon protein repletion. Combination of protein and zinc appears to be superior to protein alone on plasma IGF-I, bone resorption and activity of daily living in frail elderly.

P645-Tu

Effects of Phytoestrogens and their Metabolites, Equol, on Estrogenic Activity and Osteoclast Formation

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Eqoul is a nonsteroidal estrogen of the isoflavone phytoestrogen and exclusively a product of intestinal metabolism of its precursor, daidzein (DDZ). Although there are many convincing data on the bone-sparing effects of soy isoflavones such as genistein (GTN) and DDZ, the effect of equol on bone remains elusive. In this study, we compared the estrogenic activity of equol with other isoflavone derivatives in several in vitro studies, such as competition binding assays with ERá or ERβ protein, the mRNA expression of estrogen target gene, pS2, and ER-dependent transcriptional gene expression assay in several cell lines by co-transfection of ERá or ERβ cDNA with estrogen-dependent reporter plasmid. Furthermore, we examined the effects of phytoestrogens on the RANKL-induced osteoclast formation in the presence or absence of estrogen.
Phytoestrogens; GTN (genistein), DDZ, eqoul, *O*-Dma (*O*-desmethyl-angolensin). Relative binding affinities (RBAs) of phytoestrogens are 100–1000-fold lower than that of E2, and they bound more stronger to ERβ than to ERá. The phytoestrogens stimulated the transcriptional activity of both ER subtypes in co-transfection analysis using MCF-7, MC3T3E1 and 293 embryonic kidney cells. The phytoestrogens induced the cell proliferation 2–6-fold higher in MCF-7 cell, but not in MC3T3E1 cell line. The RANKL-induced osteoclast formations were inhibited with E2 (10^{-8} M–10^{-5} M), GTN (10^{-6} M–10^{-5} M) and equol (10^{-7} M–10^{-5} M), but not with others metabolites. In the presence of E2, equol acted as an estrogen antagonist in several in vitro experiments. It inhibited the suppressive effect of E2 on osteoclast formation. When we compared the activity of equol with its precursors in several in vitro experiments, equol has more potent estrogenic activity than other isoflavones and high concentrations of equol can suppress OC activity.

P646-Su

Dietary Intake of Key Bone Health Nutrients in Swiss Elderly Women—Results from EVANIBUS

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As part of an ongoing study into osteoporosis in Swiss elderly women (evaluation of nutrient intakes on bone ultrasound; EVANIBUS), the influence of dietary intake on bone health indices is being examined in a total of 401 ambulatory women from Lausanne. Since few dietary methodologies exist for populations within Switzerland, it was considered imperative to develop a Swiss FFQ. Assessment of dietary intake is a critical component in the determination of the effect of nutritional factors on osteoporosis. For the assessment of average long-term dietary intake in large numbers of individuals, food frequency questionnaires (FFQ) have emerged as a particularly useful tool, as they give a better approximation of usual long-term dietary intake than short-term records and can be self-administered. The FFQ used has been specifically developed, validated and tested for reproducibility in a population of Swiss elderly women.
Dietary intake of 401 Swiss women (mean age 80.4 years, mean weight 62.7 kg, mean BMI 24.9kg/m2) was assessed with the validated FFQ. A dietician prior to completing the FFQ advised each subject on the FFQ methodologies, and contact was made after the women had filled in the FFQ to ensure compliance and competency. During their visit, the 401 women also filled in the MNA (Mini Nutritional Assessment), a general health and physical activity questionnaire, a urine sample was taken, and each woman had a measurement of calcaneal bone mass using broad band ultrasound attenuation (BUA-Achilles, Lunar Corporation). As shown in the Table, the nutrients of main concern for optimum bone health were lower than the French RDA. Of particular interest were the sub-optimal intakes of protein and energy and key micronutrients including calcium and magnesium. These data illustrate the extent to which dietary inadequacy is frequent in an elderly Swiss population. Further analysis of the associations between these nutrients and bone health indices collected during the study are currently underway.

Table

Nutrients	RDA	Mean	SD	Range	% of FFQs below RDA
E (kcal)	1700-2000	1544.1	447.7	531–3131	78
Prot (g)	60.7	65.3	19.9	21–134.3	46
Ca (mg)	1200	983.1	388.7	147–2480	77
Mg (mg)	360	287.7	93.1	84.1–627	83
K (mg)	3000	2761.4	874.6	820–6308	67

P647-Mo

Long-term Changes on OPG-RANKL System, Hormonal Profile and Bone Mineral Density in Patients with Postmenopausal Osteoporosis Patients Treated with Raloxifene

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Previous in vitro studies suggest that the antiresorptive effect of raloxifene (a selective estrogen receptor modulator) might be mediated by changes in several cytokines involved in the bone remodeling process. In this context, the OPG-RANKL system is considered a key component in the osteoclastogenesis regulation. In addition, the treatment with raloxifene leads to changes in hormonal profile that can be related with its extra-osseous effects. However, the long-term effects of this treatment are not adequately established in osteoporotic patients.

Aims: To determine the effects of raloxifene treatment on serum concentrations of OPG, RANKL, IGF system, biochemical markers of bone turnover, hormonal profile and BMD in untreated women with postmenopausal osteoporosis.

Subjects and methods: We selected 47 postmenopausal women (mean age 63 ± 7 years) with densitometric criteria of osteoporosis (T score ≤ -2.5 SD). We determined at baseline, 3, 6 and 12 months anthropometric parameters, biochemical markers of bone turnover, serum levels of IGF-I, IGFBP-3, OPG (OPG ELISA, BIO-MEDICA-GRUPPE Wien, Austria) RANKL (sRANKL ELISA BIO-MEDICA-GRUPPE Wien, Austria) and ultrasensitive estradiol (E2) (DSL-39100 3rd Generation Estradiol LAUGHS, Diagnostic System Laboratories, Inc, Texas, USA). BMD (DXA; Hologic QDR 4500) in lumbar spine (LS) femoral neck (FN) and total hip was measured basal and 12 months after raloxifene (60 mg/day) treatment.

Results: BMD in LS increased significantly (1.8%) at the 12th month of treatment ($P = 0.04$). The biochemical markers of bone turnover (total alkaline phosphatase, bone alkaline phosphatase, osteocalcin, TRAP, urine CTX) decreased significantly from the third month of treatment. Serum levels of OPG decreased at the third month of treatment ($P = 0.001$) and returned to basal levels at 6th and 12th month. There were a high percentage of undetectable serum RANKL levels (>60%) in all visits. E2 serum levels increased significantly since the third month ($P < 0.008$). We also found a significant and sustained reduction in IGF-1 levels since the third month ($P < 0.01$).

Conclusions: The treatment with raloxifene in osteoporotic postmenopausal women leads to a initial decrease in serum levels of OPG that might be attributed to the inhibitor effect on bone remodeling. The changes in IGF-I could be related to the extra-osseous effects of this drug. The measurement of serum RANKL does not add any additional information.

P648-Tu

Effect of Raloxifene on Clinical Fractures in Asian Women with Postmenopausal Osteoporosis

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Osteoporosis is becoming a major public health problem in Asian countries, with a projected rapid increase in osteoporotic fractures, particularly in China, as urbanization increases. The purpose of this post-hoc analysis of fracture results was to assess the effect of raloxifene treatment on incident clinical fractures in 2 studies of the treatment of postmenopausal osteoporosis in Asian women in Japan and China. Two randomized placebo-controlled clinical trials were conducted comparing 12 months of treatment with either placebo, raloxifene 60 mg (RLX60) or raloxifene 120 mg (RLX120) (Japan only) once-daily in postmenopausal women with osteoporosis. A total of 488 women completed the trials: 284 in Japan and 204 in China. Baseline characteristics for women in each trial were: (mean ± SD) age (year), 64.8 ± 6.3 vs. 65.3 ± 6.0; BMI (kg/m^2), 21.8 ± 2.8 vs. 23.0 ± 2.9; and prevalent vertebral fractures, 26.4% vs. 13.7%, for Japanese and Chinese women, respectively. New clinical vertebral and nonvertebral fractures were confirmed by radiographic review or clinical reports by investigators at each research facility. New clinical vertebral fractures were significantly reduced in the combined raloxifene-treated groups ($n = 289$) vs. placebo ($n = 199$) ($P = 0.002$) and for RLX60 ($n = 194$) vs. placebo ($P = 0.01$). The combined raloxifene-treated groups, as well as RLX60, also had a significantly lower incidence of any new clinical fracture ($P = 0.001$ and $P = 0.01$, respectively). In conclusion, raloxifene 60 mg daily for 1 year resulted in a significant reduction in clinical vertebral fractures and all clinical fractures in Asian postmenopausal women with osteoporosis.

Table

Fractures	Placebo (N = 199) n (%)	RLX60 (N = 194) n (%)	RLX60 RR* (95% CI)	RLX120 (N = 95) n (%)	RLX pooled (N = 289) n (%)	RLX pooled RR* (95% CI)
Clinical vertebral	7 (3.5%)	0	NA	0	0	NA
Nonvertebral	5 (2.5%)	2 (1.0%)	0.41 (0.08–2.09)	0	2 (0.7%)	0.28 (0.05–1.41)
Any clinical	12 (6.0%)	2 (1.0%)	0.17 (0.04–0.75)	0	2 (0.7%)	0.11 (0.03–0.51)

* RR = relative risk vs. placebo group (95% CI).

P649-Su

Change of Biochemical Bone Markers after Tibolone in Korean Postmenopausal Women

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To investigate the patterns of biochemical bone markers in postmenopausal women with tibolone, urinary deoxypyridinoline (DPD), N-telopeptide of type I collagen (NTX), and serum osteocalcin (OC), bone-specific alkaline phosphatase (BSAP) were measured. From July 2002 to January 2004, a total of 239 postmenopausal women were enrolled in the present study, and 117 healthy premenopausal women with regular menstruation were served as control. The study population was grouped into the tibolone group and the non-tibolone group. The women in the tibolone group have received tibolone 2.5 mg for more than 3 months. The biochemical bone markers of all women were assayed. Results were analyzed with Student's t test and Fisher's Exact Test as appropriate. The urinary DPD of the non-tibolone group was higher than those of the tibolone and premenopausal groups (5.51 ± 2.47 vs. 3.36 ± 1.02 and 4.01 ± 3.86 nM/mM, $P < 0.05$, respectively). The urinary NTX of the non-tibolone group was also higher comparing to the tibolone and premenopausal groups (48.71 ± 11.54 vs. 33.70 ± 17.43 and 33.70 ± 17.43 BCE/mmol, $P < 0.05$, respectively). However, there were no significant differences in the concentrations of serum BSAP and OC among the three groups. In conclusion, the urinary DPD and NTX might be a sensitive indicator of bone metabolism and also be useful to monitor the treatment efficiency in postmenopausal women undergoing tibolone treatment.

P650-Mo

Impact of the Women's Health Initiative (WHI) Trial on Osteoporosis Prevention

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In July 2002, widespread media coverage was given to publication of the WHI trial results, which indicated that HRT in healthy postmenopausal women is not justified, on a risk-to-health benefit basis, for the primary prevention of several chronic diseases (including osteoporosis). The media focus on the breast cancer risk associated with HRT engendered a public perception that HRT was associated with a high absolute breast cancer risk. Consequently, the ensuing rapid decrease in HRT use among post-menopausal women is largely attributed to direct media influence. This paper draws attention to the associated implications of these events for osteoporosis prevention. During the period July–Nov 2002, our mean monthly dual-energy X-ray absorptiometry (DXA) spine scan rate for women (40–75 years, potential secondary osteoporosis conditions excluded) increased 99% compared to that for the preceding 12-month interval (239 vs. 120; $P < 0.01$). Subsequently, (Dec 2002–Jun 2003) the mean monthly scan rate rapidly decreased to within 4% (125; NS) of pre-WHI levels. Our results are also consistent with a 54% increase, during the same period, in national Medicare DXA reimbursements for women with established osteoporosis. The latter data do not include women having non-reimbursable DXA scans and thus underestimate true activity. In Aug 2002, the highest relative percentage (62.3%), for 14 months (and all months since), of women presenting for their first DXA scan occurred. There was also a significant mean age increase, compared to the pre-WHI period, for women having their first (60.1 vs. 59.0; $P < 0.01$) but not a second DXA scan (62.1 vs. 61.2; NS). Reviewing monthly Medicare data throughout 2003–2004 did not identify any clearly defined surge in DXA services. The national Pharmaceutical Benefits Scheme (PBS) statistics showed that post-WHI prescription rates for the two most commonly prescribed estrogen and estrogen–progestin drugs decreased 53 and 33% respectively, with no discernible "rebound" as of Oct 2004. Our findings suggest that many women on HRT and their physicians, concerned by the WHI findings, arranged a DXA scan to assess BMD before stopping HRT. Other evidence from PBS and Medicare data suggests that HRT discontinuance was not accompanied by a change to alternative osteo-protective medication. Given the magnitude of the HRT decrease, the findings of the WHI substantially decreased the number of post-menopausal women taking osteo-protective medication.

P651-Tu

A Twin Study of Hip Strength Associated with Hormone Replacement Therapy

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Hormone replacement therapy (HRT) has been shown to preserve or improve areal bone mineral density (BMD) and to prevent fractures in post-menopausal women. Studying the effects of HRT on indices of bone strength should help to explain how osteoporosis therapy prevents fractures. In a previous twin study, we reported that HRT users had 6.2% greater lumbar spine BMD than non-users but found no significant BMD difference at the proximal femur.

Our objective here was to estimate the within-pair difference in measures of bone strength of the proximal femur using hip structural analysis (HSA) in female twin pairs discordant for HRT use. Forty-one twin pairs (16 monozygotic, 25 dizygotic) were identified where one twin had more than 6 months of continuous HRT use, mean 71 months (range 6–276), and the other had no exposure to HRT. HSA parameters were evaluated from proximal femur densitometry scans at the narrowest segment of the femoral neck (NN), intertrochanteric (IT) and upper femoral shaft (FS) sites. All data were adjusted for age, height and weight. There were no significant within-pair differences in age, weight, height, dietary calcium intake, body mass index, fat mass or lean mass.

At the NN region, there were significant within-pair differences in HSA-derived parameters as follows comparing HRT users with non-users, respectively: cross-sectional moment of inertia (1.61 vs. 1.46 cm^4, $P = 0.003$), subperiosteal width (2.90 cm vs. 2.84 cm, $P = 0.018$), section modulus (1.02 cm^3 vs. 0.96 cm^3, $P = 0.016$) and estimated endocortical diameter (2.62 cm vs. 2.56 cm, $P = 0.031$). There were no significant differences in NN areal BMD or HSA-derived parameters including areal BMD at the IT or FS regions.

These results showing a 6.6% greater index of bending strength at the femoral neck in HRT users suggest that HRT in routine clinical use increases bone strength and reduces hip fracture risk independently of change in areal BMD.

P652-Su

The Influence of Melatonin on Prevention of Osteoporosis in Ovariectomized Rat by Rotating Magnetic Field

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The osteoporosis affects a hundred million persons in China, 90% of whom are postmenopausal women. The pathogenesis of osteoporosis is multifactorial. There is evidence that the menopause is associated with a decline in melatonin (MLT) secretion and an increased of pineal calcification. Animal data indicate that pineal MLT is involved in the regulation of calcium and phosphorus metabolism by stimulating the activity of the parathyroid glands and by inhibiting calcitonin release and inhibiting prostaglandin synthesis. The pineal gland may function as a fine regulator of calcium homeostasis. Since application of external magnetic fields has been shown to synchronize MLT secretion in animals and humans, it is reasonable to propose that treatment by magnetic fields may be beneficial for osteoporosis. Now, little is known about how MLT works in treatment of osteoporosis by magnetic field. The aim of this study was to confirm whether there is some positive relation during osteoporosis, MLT and rotating magnetic fields (RMF) to compare similarity and dissimilarity of effect about treatment of osteoporosis by RMF in day time and in night time. 24 female Wistar rats were divided into 4 groups (6 per group). 6 remained intact as normal control group (NC), 6 were ovariectomized (COVX), 6 were sharmly ovariectomized (SOVX), 6 were ovariectomized, treated by RMF in day time (OVXMD) and 6 were ovariectomized, treated by RMF in night time (OVXMN). The animals were kept in a room at 25°C, light/dark = 12/12. The OVXMD and OVXMN were treated under RMF (4000 GS, 200 R/min, 60 min/day, 14 days) in day time 7:00–8:00 and in night time 19:00–20:00 respectively. All of them were killed at 12 weeks after operation. The serum osteocalocin (OC), IGF-1, IL-1, IL-6 and MEL were tested by RIA. Total bone mineral density was detected before killed. The histomorphometric measurement and calculations of distal femur were observed by morphometry. The total bone density, trabecular thickness and trabecular number increased, OC and IGF-1 heightened, IL-1, IL-6 declined in OVXMD and OVXMN group. Level of change was significant in the OC ($P < 0.01$), IGF-1 ($P < 0.01$), IL-1 ($P < 0.05$), IL-6 ($P < 0.05$) of OVXMD more than in that of OVXMN. MEL of day time increases in OVXMD more than in OVXMN ($P < 0.01$). The treatment of RMF in day time changed the MLT rhythm of high level in night and low level in day and kept MLT high level during 24 h. The MEL is one of the key factors in treatment of osteoporosis by RMF.

P653-Mo

The Optimal Intensity for Exercise to Prevent from Decreasing Bone Mass in Ovariectomized Mice

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It is well known that the maintenance and improvement of bone mass need mechanical stress. Physical activity is one of the methods to keep bone mass. But, the optimum intensity for exercise is not established. Therefore, this study was investigated to determine the optimum intensity for

exercise to prevent from decreasing bone mass in ovariectomized mice model.
Fifty female ICR mice aged 12 weeks were used and assigned randomly to 5 groups. Four groups were ovariectomized (OVX) and the remaining was sham-operated (SHAM) under pentobarbital sodium. Three groups of OVX were run on a treadmill at 8 (A), 16 (B) and 24 m/s (C), 5 days/week for 10 weeks, respectively. Directly after running, the blood lactic acid level (LA) was measured. Mechanical strength of the left femur and tibia was measured by the three-point bending test. In statistical analysis, one-way ANOVA and post hoc test (Fisher's PLSD) were used to find the optimum intensity for exercise and LA to keep bone mass. A significance level of $P = 0.05$ was set. This study was carried out in accordance with the Guide for Animal Experimentation, Hiroshima University and the Committee of Research Facilities of Laboratory Animal Science, Hiroshima University School of Medicine.
Mechanical strength of the femur in B group was significantly higher than that in OVX group. Compared with all running groups, mechanical strength of the femur in OVX animals was low significantly. LA in A Group was lower than that in B and C groups significantly. LA values of B and C groups were about 3.0 mM.
This study suggested that exercise curbed bone loss, and this inhibitory was changed by the exercise intensity. The intensity of B group was most effective to keep bone mass in this study. It was expected that the intensity of A group was low and that of C was too high to curb bone mass. Average LA level in B mice was equivalent to maximal oxygen consumption 3.0 l/min in human. Though results of animals cannot be applied to human cases, it is clearly that the optimum intensity existed in the range of about this LA level.

P654-Tu

Effects of Treadmill Running Exercise on Trabecular Architecture During Remobilization after Suspension-induced Osteopenia in Young Rats: Three-dimensional Observation using Micro-CT

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Three-dimensional trabecular architecture was investigated in the femur of tail-suspended young growing rats, and the effects of treadmill running during remobilization were examined.
Eighteen 5-week-old male Wistar rats were tail-suspended for 2 weeks and then sacrificed directly after immobilization, after free remobilization (S + CON), or controlled physical exercise (S + TR) for 5 weeks. The exercise regimen consisted of treadmill running of 20 m/min, 1 h/day and 5 days/week. Bone mineral density (BMD) of total right femur was measured by dual energy X-ray absorptiometry. Three-dimensional trabecular bone architecture at the distal femoral metaphysis was evaluated using micro-computed tomography (micro-CT). Cortical width, total bone area and cross-sectional moment of inertia (CSMI) were calculated using the tomographic data of femoral diaphysis.
Tail suspension induced a significant decrease in femoral BMD, cortical width, total bone area and CSMI and marked deterioration of trabecular architecture. Femoral bone length was not significantly altered. After 5 weeks of free remobilization, femoral BMD, cortical thickness, hindlimb muscle weight and body weight returned to the age-matched control values; however, trabeculae were still thinner and less connected, and CSMI was slightly smaller. Rats in the S + TR showed a significant increase in trabecular thickness, number and connectivity at the distal femoral metaphysis and total femoral BMD when compared with S + CON rats. No significant difference was observed between S + TR and age-matched control in parameters of trabecular architecture and femoral BMD.
These results indicate that the suspension-induced trabecular deterioration persists after remobilization and treadmill running exercise during remobilization period could restore the integrity of trabecular architecture as well as bone mass at the femur in young growing rat.

P655-Su

Effects of Jump Exercise on Trabecular Architecture during Remobilization after Suspension-induced Osteopenia in Young Rats: Comparison with Treadmill Running

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High-impact exercise is considered to be very beneficial for bones. We investigated the ability of jump exercise to restore bone mass and structure after the deterioration induced by tail-suspension in young growing rats and compared with treadmill running exercise.
Twenty-one 5-week-old male Wistar rats were tail suspended for 2 weeks and then sacrificed after free remobilization (S + CON), jump exercise (S + JU) or treadmill running (S + TR) for 5 weeks. The jump exercise protocol was 10 times/day, 5 days/week and the jumping height was 40 cm. Treadmill running was performed at 20 m/min, 1 h/day and 5 days/week. Bone mineral density (BMD) of total right femur was measured by dual energy X-ray absorptiometry. Three-dimensional trabecular bone architecture at the distal femoral metaphysis was evaluated using microcomputed tomography (micro-CT). Cortical width, total bone area and cross-

sectional moment of inertia (CSMI) were calculated using the tomographic data of femoral diaphysis.
Rats in both S + TR and S + JU showed a significant increase in trabecular thickness, number and connectivity at the distal femoral metaphysis and total femoral BMD when compared with S + CON rats. No significant difference was observed between S + TR and S + JU in parameters of trabecular architecture, cortical geometry and femoral BMD. However, as compared with the age-matched control rats, trabecular thickness, cortical thickness and total femoral BMD were significantly higher only in S+JU rats and not S + TR rats. In the BMD analysis of subregions of femur, diaphyseal region showed this tendency.
These results indicate that high-impact and low-repetition exercise has beneficial effects on the recovery of suspension-induced osteopenia at the femur in young growing rat. The effect was the same as or even slightly greater than the treadmill running exercise.

P656-Mo

Intensity of Physical Activity is a Predictor of Bone Density Change in Premenopausal Women

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Introduction: High-impact exercise is known to be beneficial for bones. However, the optimal amount of exercise is not known. The aim of the present study was to evaluate the effects of the intensity of exercise on bone mineral density (BMD).
Methods: We performed a 12-month population-based trial with 120 women, from a cohort of 5161 women, aged 35 to 40 years, randomly assigned to an exercise or a control group. The exercise regimen consisted of supervised high-impact exercises three times per week and an additional home program. The intensity of the physical activity was assessed continuously with an accelerometer-based body movement monitor (Newtest ltd., Oulu, Finland), and the number of peak accelerations was analyzed at six acceleration levels (0.3–1.0 g, 1.1–2.4 g, 2.5–3.8 g, 3.9–5.3 g, 5.4–7.2 g and 7.3–9.2 g). BMD was measured from the hip, spine (L1–L4) and radius by dual-energy X-ray absorptiometry. The calcaneus was measured using quantitative ultrasound.
Results: Complete data on bone density measurements and physical activity recordings from 64 subjects were available and included in the pooled correlation and regression analyses. The physical activity at acceleration levels exceeding 3.9 g correlated positively with the BMD change in the hip area (Table). L1 BMD change correlated positively with the accelerations exceeding 5.4 g ($P < 0.05$) and calcaneal speed of sound with the accelerations at the level of 1.1–2.4 g ($P < 0.05$). The mean daily number of peak accelerations exceeding 3.9 g was 49.8/day (95% CI: 34.2–55.3) on all subjects ($N = 64$) and 77.9/day (95% CI: 54.3–101.5) on subjects in exercise group ($N = 34$). In the regression analyses, the baseline BMD was negatively associated with the BMD change at the hip.
Conclusions: Physical activity, measured as peak accelerations, is a significant determinant of BMD change. In the hip area, the threshold level for improving BMD is less than 100 impacts per day at acceleration levels exceeding 3.9 g. Effective levels of physical activity are reached during normal physical exercise in healthy premenopausal women.

Table
Correlation coefficients in the hip area

Variable	0.3–1.0 g	1.1–2.4 g	2.5–3.8 g	3.9–5.3 g	5.4–7.2 g	7.3–9.2 g
FN BMD	0.058	0.075	0.157	0.343**	0.402***	0.334**
TR BMD	0.186	0.170	0.187	0.347**	0.416***	0.397***
WT BMD	0.196	0.102	0.200	0.305*	0.375**	0.382**

FN = femoral neck, TR = trochanter, WT = Ward's triangle.

P657-Tu

Effect of Whole Body Vibration Exercise on Lumbar Bone Mineral Density, Bone Turnover, and Chronic Back Pain in Postmenopausal Osteoporotic Women Treated with Alendronate

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Background and aims: Exercise may enhance the effect of alendronate on bone mineral density (BMD) and chronic back pain in elderly women with osteoporosis. The aim of this study was to determine whether whole body vibration exercise would enhance the effect of alendronate on lumbar BMD, bone turnover, and chronic back pain in postmenopausal women with osteoporosis.
Methods: Fifty postmenopausal women with osteoporosis, 55–88 years of age, were randomly divided into two groups of 25 patients each: alendronate (5 mg daily, ALN) and alendronate plus exercise (ALN + EX) groups. Exercise consisted of whole body vibration exercise using a Galileo machine (Novotec, Pforzheim, Germany), with an intensity of 20 Hz, frequency of once a week, and duration of exercise of 4 min. The period of the study was 12 months. Lumbar BMD was measured by dual energy X-ray absorptiometry (Hologic QDR 1500W), urinary cross-linked N-terminal telopeptides of type I collagen (NTX) and serum alkaline phosphatase (ALP) levels were measured by enzyme-linked

immunosorbent assay and standard laboratory techniques, respectively, and chronic back pain was evaluated by face scale score at the baseline and every 6 months.
Results: There were no significant differences in baseline characteristics including age, body mass index, years since menopause, lumbar BMD, urinary NTX and serum ALP levels, and face scale score between the two groups. The increase in lumbar BMD and the reduction in urinary NTX and serum ALP levels were similar in the ALN and ALN + EX groups. However, the improvement of chronic back pain was greater in the ALN + EX group that in the ALN group.
Conclusions: The results of this study suggest that whole body vibration exercise using a Galileo machine appears to be useful to improve chronic back pain, probably by relaxing the back muscle in postmenopausal osteoporotic women treated with alendronate.

P658-Su
Bone Geometric Adaptation After a Radius Fracture: A pQCT Study
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Previous studies have described long bone response to fracture immobilization as similar to that experienced by aging, space flight or neurological injury, but there have not been any reports of bone geometric adaptation and volumetric bone density. Knowledge of the specific relative contribution of bone properties assists in understanding the mechanism of loss and can be used for the development of appropriate interventions. We used peripheral quantitative computed tomography (pQCT) and dual energy X-ray absorptiometry (DXA) to quantify bone and muscle response to fracture immobilization. Peripheral quantitative computed tomography (pQCT) is a safe and precise technique to differentiate cortical from trabecular bone and assess both bone geometry and density. We measured side-to-side differences in bone and muscle using a cross-sectional design in women aged 50 years and older who had previously sustained a fragility radius fracture (from 6 months–12 years post distal radius fracture). We measured the 4% and 30% sites of the radius using pQCT, DXA at the radius, grip strength and functional measurements. Thirty-one women (mean age 72.4 ± 9.7 years) were assessed. In the primary analysis, we compared fractured side to non-fractured side and did not control for hand dominance. We observed an increase in total cross-sectional area at the distal (4%) radius without a significant increase in density. At the midshaft (30% site), we observed a significant decrease in total area and less cortical bone on the previously fractured side. Grip strength was significantly lower on the fracture limb. We found significant relationships between grip strength and bone parameters. In a secondary analysis, we assessed outcome measures for dominant side fractures and non-dominant fractures separately. We observed a greater discrepancy between limbs with a non-dominant side fracture. This cross-sectional study suggests that a non-dominant fracture may have more bone loss and require more intensive post-fracture immobilization to maintain bone strength. Furthermore, this study suggests that the radius is a potential model to study bone response to immobilisation and pQCT is an important technology for clinicians and researchers to develop and assess site-specific exercise interventions to increase muscle and bone strength as part of evidence-based rehabilitation.

P659-Mo
Raloxifene Therapy Decreases the Risk of New Clinical Vertebral Fractures at 6 Months
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Raloxifene treatment (60 mg/day) significantly decreased the risk of new clinical vertebral fractures by 68% at 1 year in the Multiple Outcomes of Raloxifene Evaluation (MORE) trial [Arch Int Med 2002;162:1140–3], but the effects within the first year are unknown. In MORE, 7705 postmenopausal women were randomized to placebo or raloxifene at 60 or 120 mg/day. Scheduled vertebral radiographs were obtained at baseline, 2 years and 3 years. Other efficacy and safety endpoints were assessed at clinic visits held at 3 and 6 months and every 6 months thereafter. When patients reported symptoms suggestive of vertebral fracture at or between these clinic visits, radiographs were taken, and if a new adjudicated fracture was found, this was considered as a clinical vertebral fracture. The analyses included all randomized patients with a baseline and at least 1 post-baseline radiograph (n = 6828). One woman treated with raloxifene 60 mg/day (n = 2259) and 10 women in the placebo group (n = 2292) had a clinical vertebral fracture in the first 6 months, resulting in a 90% relative risk (RR) reduction [RR 0.10 (95% CI 0.01, 0.63)]. Similar results were observed with raloxifene 120 mg/day at 6 months. When the raloxifene groups were pooled, a significant (P = 0.034) decrease in clinical vertebral fracture risk [RR 0.20 (95% CI 0.03, 0.90)] was seen as early as 3 months. In summary, the risk of new clinical vertebral fractures is significantly reduced with 3 or 6 months of raloxifene therapy.

P660-Tu
Body Mass Index Affects the Functional Outcome after Hip Fracture in Elderly Women
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Low body mass index (BMI) is associated with high risk of both osteoporosis and fragility fractures, but its impact on

functional recovery after fractures is unknown. Our aim was to investigate the association between BMI and functional recovery in hip-fracture women. We evaluated a total of 580 Caucasian women admitted consecutively to our Division of Physical Medicine and Rehabilitation because of an original hip fracture. A total of 28 of the 580 women were excluded because their hip fracture was caused either by major trauma or by cancer affecting the bone. All of the 552 remaining women suffered from fractures that were either spontaneous or caused by minimal trauma. A total of 42 of these 552 women were excluded because they died or were transferred to other hospitals. The final study sample included 510 hip-fracture women. The functional evaluation was performed by using the Barthel index. In the 510 women, BMI was 22.8 ± 4.1 kg/m^2 (mean ± SD). BMI range was 12.5–38.3 kg/m^2. The median Barthel index score was 50 (interquartile range = 25) on admission to rehabilitation and 90 (interquartile range = 25) on discharge. The median increase in the Barthel index score resulting from rehabilitation was 35 (interquartile range = 15). After adjustment for seven prognostic factors (age, pressure ulcers, cognitive impairment, neurologic impairment, infections, femur bone mineral density and Barthel index score assessed on admission to rehabilitation), a significant negative association was found between BMI and both the Barthel index score assessed after rehabilitation ($R^2 = 0.559$; $P < 0.001$) and the change in the Barthel index score resulting from rehabilitation ($R^2 = 0.253$; $P < 0.001$). The results were similar when BMI was evaluated as either individual values or after categorization according to the World Health Organization criteria (underweight <18.5 kg/m^2, normal 18.5–24.9 kg/m^2, overweight 25–29.9 kg/m^2, obese 30 kg/m^2 and above). Our results show that BMI was negatively associated with functional recovery assessed by using Barthel index scores in a large sample of hip-fracture women. BMI may affect functional recovery after hip-fracture, apart from influencing hip-fracture risk: subjects with higher BMI and low hip-fracture risk may have poorer functional recovery in case of hip fracture. Conversely, subjects with lower BMI and high hip-fracture risk may have a better functional recovery in case of hip-fracture.

P661-Su

Effect of Raloxifene on Incidence of Invasive Breast Cancer in Postmenopausal Women Having a History of Prior Hormone Therapy Use: Results of the Core Trial

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Introduction: Raloxifene is approved for prevention and treatment of osteoporosis in postmenopausal women. Results from the Continuing Outcomes Relevant to Evista® (CORE) trial, a double-blind, placebo-controlled, 4-year extension of the 4-year Multiple Outcomes of Raloxifene Evaluation (MORE) osteoporosis treatment trial demonstrated that reduction in invasive breast cancer (BrCa) incidence, specifically estrogen receptor (ER)-positive invasive BrCa, continued beyond 4 years of raloxifene treatment, with a neutral effect on ER-negative BrCa in postmenopausal women with osteoporosis (OP) randomized in the MORE trial.

Objective: This pre-specified secondary analysis of MORE plus CORE data compared the effect of 8 years of RLX with placebo (Plc) on invasive breast cancer incidence in women reporting a history of prior hormone therapy (HT) use.

Methods: Of the 7705 postmenopausal women with OP who enrolled in MORE, 4011 chose to continue in CORE. Women choosing to not continue in CORE were censored at the end of MORE. Women randomized to RLX 60 or 120 mg/day in MORE were assigned to RLX 60 mg/day in CORE; those randomized to Plc in MORE were assigned to Plc in CORE. Information on prior HT use, defined as use of estrogen only or combined estrogen–progestin therapy prior to MORE randomization, was obtained from 7682 participants; 5111 in the RLX group and 2571 in the Plc group. Time to first invasive breast cancer (IBC) for the two therapy groups was compared by a Cox proportional hazards model.

Results: Previous HT use was reported by 2235 women and no previous HT use by 5447 women. In these women, the overall reduction in IBC incidence for the 8 years of MORE plus CORE was 66% (hazard ratio [HR] 0.34; 95% confidence interval [CI] 0.22–0.50). In the Plc group, incidence of IBC was 2.7% in those with prior HT use compared to 2.1% in those with no prior use (pv0.279). In women with a history of prior HT use, RLX significantly reduced IBC incidence by 71% (HR 0.29; 95% CI 0.14–0.59) compared to Plc. In women with no prior exposure to HT, a 64% reduction in incidence of IBC was seen in those receiving RLX (HR 0.36; 95% CI 0.22–0.59). Regardless of prior HT use, the magnitude of reduction in IBC incidence with RLX did not differ (interaction $P = 0.618$).

Conclusion: Compared to placebo, raloxifene significantly reduced the incidence of invasive breast cancer in postmenopausal women with osteoporosis having a prior history of hormone therapy use.

P662-Mo

Breast Cancer Risk Reduction Over 8 years in Postmenopausal Women with Osteopenia or Osteoporosis Receiving Raloxifene

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Introduction: Raloxifene is approved for prevention and treatment of osteoporosis in postmenopausal women. Results from the Continuing Outcomes Relevant to Evista® (CORE) trial, a double-blind, placebo-controlled, 4-year extension of the 4-year Multiple Outcomes of Raloxifene Evaluation (MORE) osteoporosis treatment trial demonstrated that reduction in invasive breast cancer (BrCa) incidence, specifically estrogen receptor (ER)-positive invasive BrCa, continued beyond 4 years of raloxifene treatment, with a neutral effect on ER-negative BrCa.
Objective: This pre-specified analysis examines raloxifene's effect (pooled 60 and 120 mg/d) on invasive BrCa incidence in postmenopausal women with osteopenia or osteoporosis over the 8 years of the MORE plus CORE trials.
Methods: MORE enrolled 7705 postmenopausal women $\leq$80 years of age; 4011 chose to continue in CORE. Women randomized to placebo in MORE were assigned to placebo in CORE. Those randomized to raloxifene 60 mg/day or 120 mg/day in MORE were assigned to 60 mg/day in CORE. Osteoporosis (n = 3836) was defined as having a vertebral fracture (VF) or total hip BMD T score ≤ -2.5 (NHANES III criteria) at MORE baseline; osteopenia (n = 3829) was defined as T score > -2.5 without VF. Hazard ratios and 95% CIs were calculated using Cox proportional hazards models.
Results: For women assigned to placebo, there was no difference between the osteopenia and osteoporosis groups in invasive BrCa incidence (36 vs. 22 cases, respectively; P = 0.16); however, invasive ER-positive BrCa incidence was greater in the osteopenia group than the osteoporosis group (30 vs. 14 cases, respectively; P = 0.04). For women assigned to raloxifene, invasive BrCa incidence was significantly reduced compared with placebo for the osteopenia (65% risk reduction; HR 0.35 [0.21, 0.58]) and osteoporosis groups (69% risk reduction; HR 0.31 [0.16, 0.60]). Invasive ER-positive BrCa was also significantly reduced in women assigned to raloxifene compared with placebo for the osteopenia (78% risk reduction; HR 0.22 [0.11, 0.42]) and osteoporosis groups (71% risk reduction; HR 0.29 [0.13, 0.67]). These reductions were similar between the osteopenia and osteoporosis groups (interaction P = 0.81 and 0.57 for invasive BrCa and invasive ER-positive BrCa, respectively).
Conclusion: Invasive BrCa and invasive ER-positive BrCa incidences were significantly, and similarly, reduced over 8 years in postmenopausal women receiving raloxifene who had either osteopenia or osteoporosis.

P663-Tu

Meta-Analysis of the Efficacy of Raloxifene on Reduction of Vertebral Fracture Risk

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Raloxifene (RLX) reduced the risk of vertebral fractures (VFx) in the Multiple Outcomes of Raloxifene Evaluation (MORE) trial. Six other RLX studies have included predefined baseline and post-baseline spinal radiographs. In an attempt to determine whether the RLX effect is consistent and to more accurately define the point estimate for fracture risk reduction, a meta-analysis was undertaken using all randomized, double-blind, placebo-controlled trials in postmenopausal women in which fracture data were available from prospectively scheduled spinal radiographs.
Three prevention studies (Jolly et al., 2003), two arms of the MORE trial (Ettinger et al., 1999), and three additional treatment studies (Morii et al., 2003; Zheng et al., 2003; Lufkin et al., 2004) were included in the analyses. A systematic review of the literature (MedLine, EMBASE) confirmed that all appropriate RLX studies had been identified. The raloxifene 60 mg/day (RLX60) dose and also the pooled 120 mg/day and 150 mg/day (RLX120/150) doses were considered. A logistic regression model was used to test for heterogeneity of treatment effect among the studies (treatment-by-study interaction significant), and, provided that heterogeneity was not present, estimate the treatment effect via the odds ratio (95% CI).
There was no evidence of treatment heterogeneity among studies assessing vertebral fractures for either RLX60 or RLX120/150 (interaction P values 0.30 and 0.15, respectively). The overall odd ratios (95% CI) were 0.60 (0.49, 0.74) for RLX60 and 0.51 (0.41, 0.64) for RLX120/150. These results show a consistent effect of raloxifene on the reduction of vertebral fracture risk across studies in several populations of postmenopausal women.

P664-Su

A Multi-disciplinary Patient Education Program Increases Patients Dietary Intake of Calcium-interim Analysis from a Randomized Prospective Trial

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Lifestyle factors such as exercise, use of tobacco and alcohol, and dietary habits are independent risk factors for osteopo-

rosis. Program aiming at individual life style factors and fall prevention programs have significant positive effects, however, only few randomized studies have been performed.
Aim: We hypothesized that dietary intake of calcium may be increased by a multi-disciplinary systematic education program in patients with osteoporosis.
Participants and design: One-hundred-and-sixty-eight patients aged 48 to 81 years (156 females and 12 males), who were recently diagnosed with osteoporosis and started on specific treatment, were randomized to either the "school" or "control" group. In the school group, patients attended four classes with 8–12 participants during 4 weeks. Teaching was performed by nurses, physiotherapists, dieticians, and doctors. The classes covered "facts on osteoporosis", "fractures and pain", "diet", "preventive measures", "balance and exercise", and "medical treatment". Teaching was designed to increase empowerment. In this interim analysis, we assessed the dietary calcium intake using a validated questionnaire at study entry and at 3 months. Intake of supplementation with calcium and vitamin D was not included in the analysis.
Results: At study entry, no differences in age (64.6 ± 7.6 years in the control group and 64.4 ± 9.8 years in the school group) or dietary calcium intake (943 ± 332 mg/day vs. 967 ± 375 mg/day) were seen. In the school group (n = 90), the patients increased their calcium intake with 50 (−150 to 250) mg/day (median (interquartil range)), while no change (−325 to 325) in dietary calcium intake was seen in the control group (n = 78). This difference was highly significant ($P < 0.01$ between groups).
Conclusion: Systematic education of patients with osteoporosis leads to a slightly, however significantly, increased dietary calcium intake. It remains to be demonstrated whether the systematic education program also increases compliance with pharmacological treatment and modifies other lifestyle factors.

P665-Mo
Urinary NTX, Serum CTX, Bone ALP and Osteocalcin Evaluation during 3 years of Risedronate Treatment
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Aim: The aim of this study was to investigate (1) the effect of risedronate on urinary NTX, serum CTX, Bone ALP and Osteocalcin during 3 years of treatment of early postmenopausal women.
Patients–Methods: 40 early postmenopausal women 48–53 years old with T score (lumbar DEXA) less than 2 SD and no prior metabolic disorder or spine fractures were divided in 2 groups: group A (n = 30) received 5 mg risedronate, 1 mg alphacalcidol and 1000 mg calcium bicarbonate for the first year and no alphacalcidol for the next 2 years, while group B received the same except risedronate. uNTX, sCTX, BALP, Osteocalcin (OC) were measured in 0, 6, 12, 24 and 36 months.
Results: uNTX decreased by 16.15%, 8.15%, 9.1%and 8.5% in group A (P value less than 0.0005), while it increased by 5.1% (P = 0.006), 10.74% (P = 0.002), 14.5% (P = 0.0005) in group B. sCTX decreased by 11.67% (P = 0.0005), 9.63% (P = 0.0005), 9.65% (P = 0.0005) and 9.5% (P = 0.0005), while it increased by 8.9% (P = 0.014), 13.3% (P = 0.0005), 16.5% (P = 0.0005) and 18.9% (P = 0.0005) in group B. Osteocalcin decreased by 6.2%, 8.5%, 8.96% and 9.2% (all P values = 0.0005) in group A, while it increased by 4.23% (P = 0.006), 2.75% (NS), 3.15% (NS) and 2.7% (NS)in group B. BALP decreased by 10.05%, 20.18%, 21.1%, and 21.3% (all P = 0.0005) in group A, while none significantly (NS) increased in group B. No secondary hyperparathyroidism was noted due to alphacalcidol administration during the first year.
Conclusion: Risedronate effectively decreases uNTX, sCTX, Osteocalcin and BALP as early as 6 months post treatment, and this effect is maintained until the end of the 36 months follow-up, without any values falling bellow normal which could mean frozen bone formation. Both Osteocalcin after the 6-month period and BALP for the 36-month period showed no significant increases in the untreated group and therefore cannot be as useful as uNTX and sCTX for the follow up of risedronate treatment.

P666-Tu
Patient Compliance with Raloxifene (daily), Alendronate (daily and weekly) and Risedronate (daily) in the Treatment of Postmenopausal Osteoporosis
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The effectiveness of medical treatment for osteoporosis depends on compliance with the assigned medication. We conducted a 1-year open-label, prospective, non-randomized observational study of patients age 60 years or greater to assess compliance with different osteoporosis medications prescribed in clinical practice. Demographic data, the reasons for intervention, concomitant diseases, educational status and disease knowledge were captured at baseline. Self-reported compliance data were collected and are expressed as the percentage of tablets actually taken vs. required during a month. Patients were classified as non-compliant if they missed more than 20% of daily or 50% of weekly medications. Discontinuation and reasons for discontinuation were also recorded. Of 6076 patients recruited in 10 European countries, Lebanon and South Africa, 5688 met eligibility criteria. Out of those, 63% were assigned to 60 mg daily raloxifene (RAL), 8.7% to 10 mg daily alendronate (AQD), 13.5% to 70 mg once weekly alendronate (AQW), 9.2% to 5 mg daily risedronate (RIS) and 5.5% to other treatments. Because of loss to follow-up or missing data at endpoint, 1076 (18.9%) patients were excluded from the completer analysis. The Table summarizes the percentages of those who were compliant, demonstrated low compliance at study end or discontinued before study end. The discontinuation was due to side effects in 3.8%, 5.8%, 7.0% and 4.4% of patients on RAL, AQD, AQW and RIS, respectively. For RAL, both discontinuation and discontinuation due to side effects was significantly lower than for AQW ($P < 0.005$). The mean percentage of tablets actually taken vs. prescribed was high for all four groups of patients on medication at study endpoint: 96% for RAL ($P < 0.0001$ vs. AQW and $P < 0.01$ vs. RIS), 95% for AQD ($P < 0.0001$ vs. AQW), 88% for AQW and 93% for RIS ($P < 0.0001$ vs. AQW). Generally, patients with awareness on health complications of osteoporosis had higher compliance. Our data indicate a high overall compliance with the prescribed osteoporosis medication use in the clinical practice. When compared to once weekly medications, greater compliance was seen with once daily medications. This study was supported by a research grant of Eli Lilly and Co.

Table
Percentage of patients on a level of compliance

	RAL (%)	AQD (%)	AQW (%)	RIS (%)
Compliant	80	78	65*	74
Low compliance	2.5	4.7	8.8	3.3
Discontinued	18	18	26**	22

*$P < 0.05$ vs. any other treatment, **$P < 0.05$ vs. RAL, AQD.

P667-Su

Raloxifene Increases Intestinal Calcium Absorption in Elderly Postmenopausal Women with Osteoporosis

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Decrease of intestinal calcium absorption with age has been implicated in the pathogenesis of bone loss in elderly women. The effect of raloxifene on intestinal calcium absorption was assessed in 39 elderly women (aged 65 years in average) with postmenopausal osteoporosis. After giving written informed consent, patients were assigned in a double blinded manner to receive raloxifene (60 or 120 mg/day) ($n = 27$) or placebo ($n = 12$) for 1 year. All patients received calcium (1 g/day) and vitamin D (800 IU/day). There were no differences between groups in age, years since menopause, body weight or calcium intake. Intestinal calcium absorption was measured using stable stroncium (Sr) as a marker (Sips AJ, et al. Clin Chem 1995;41:1446–50). The determination of strontium was performed using the atomic absorption spectrometry method, equipped with a graphite furnace (GF AAS).
The fractional absorption of Sr was calculated at 60 min (Fc60) after oral intake of stable strontium (2.5 mmol SrCl2) with a standardized meal. Results are expressed as mean ± SD. Changes from baseline were assessed by paired *t* test. After 1 year of treatment, fractional absorption of Sr increased significantly in the raloxifene group (baseline, 0.06 ± 0.02, after 1 year, 0.09 ± 0.05, $P < 0.001$) as compared with the placebo group (baseline, 0.07 ± 0.03, after 1 year, 0.07 ± 0.02, $P = 0.591$). Our results confirm that raloxifene increases intestinal calcium efficiency in calcium and vitamin D repleted elderly postmenopausal osteoporotic women (Zanchetta JR, et al. JBMR 2001;16 (Suppl 1): S535).

P668-Mo

Changes in BMD of the Spine, Hip and Hand in Patients with Rheumatoid Arthritis during 1 year Treatment with Anti-TNF (Oslo-Truro-Amsterdam (OSTRA) Collaborative Study)

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Introduction: Osteoporosis is a well-known feature of rheumatoid arthritis (RA). Cross-sectional studies have shown that patients with RA have a lower bone mineral density (BMD) than healthy controls. Disease activity is associated with loss of BMD in RA. Therefore, active treatment of RA patients may prevent loss of BMD in RA patients. The current most effective drugs in the treatment of RA are the tumor necrosis factor alpha (TNF-alpha) blocking agents, e.g. infliximab.
Objective: To investigate the changes in BMD of the spine, hip and hand in patients with rheumatoid arthritis (RA) during 1-year treatment with infliximab.
Methods: Patients with RA, 92 from Amsterdam and 10 from Oslo, who were treated with infliximab during 1 year

were included. All patients had active RA at the start of treatment. Disease activity was measured before every infusion by the disease activity score of 28 joints (DAS-28). The bone mineral density (BMD), spine and total hip (DEXA) and hand (DXR) was measured before the start of treatment and after 1 year of treatment with infliximab.

Results: The patient group consisted of middle aged (mean 55 years), mainly female (85%) patients. The median (range) disease duration was 11 (1 to 49) years, twenty-five (25%) patients used prednisone (median dose 7.5 mg). The disease activity measured by the DAS-28 decreased from 5.9 (1.5) at baseline to 3.7 (1.5) at 1 year. The BMD of the vertebral spine and hip showed small non-significant changes: +0.02 and −0.2% respectively (Table 1). The BMD of the hand decreased −0.8% compared to baseline; this decrease was statistically significant ($P < 0.05$) (Table 1).

Conclusion: The results of this study show that infliximab is capable of arresting generalized bone loss in RA patients as indicated by the neglectable non-significant change in spine and hip BMD (which is in contrast with the usually occurring bone loss in active RA). However, treatment with infliximab did not stop the localized bone loss at the hands.

Table 1
Changes in BMD of the spine, total-hip and hand

	Baseline g/cm^2	1-year g/cm^2	Difference	% change	P value
BMD spine (n = 102)	0.998 (0.18)	1.00 (0.20)	0.002	+0.2	0.973
BMD hip (n = 89)	0.843 (0.15)	0.840 (0.14)	−0.003	−0.2	0.624
BMD hand (n = 54)	0.497 (0.09)	0.493 (0.10)	−0.004	−0.8	0.016

P669-Tu

Changes in Markers of Bone Metabolism in Patients with Active Rheumatoid Arthritis during 1 year Treatment with Infliximab (Oslo-Truro-Amsterdam (OSTRA) Collaborative Study)

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Introduction: In a previous study, we investigated the changes in bone metabolism in RA patients during the first 6 weeks of treatment with infliximab, a very effective, relatively new, antirheumatic drug blocking TNF-alpha (1). We found a favorable response on markers of bone metabolism: bone resorption decreased, while bone formation increased.

Objective: To investigate the changes in bone metabolism in RA patients during 1 year treatment with infliximab.

Methods: Patients with RA, 62 from Amsterdam and 10 from Oslo, who were treated with infliximab during 1 year, were included. Infliximab (3 mg/kg) was administered at 0, 2 and 6 weeks and from then every 8 weeks (intravenous). Osteocalcin (formation), β-CTx (resorption), receptor activator of Nfkappab ligand (RANKL) and osteoprogesterin (OPG) were determined in serum at 0, 14, 30 and 46 weeks.

Results: The patient group consisted of middle aged (mean age 53 years), mainly female patients (83%). The median (range) disease duration was 10 (1 to 49) years, twenty-five (25%) patients used prednisone (median dose 7.5 mg). The mean DAS-28 decreased from 5.9 (1.5) at baseline to 3.7 (1.5) at 46 weeks. β-CTx decreased significantly compared to baseline during the first 14 weeks and remained stable at that level during the rest of the year. Osteocalcin was stable except for a slight increase at 14 weeks. RANKL showed a significant progressive decrease at all time points, whereas OPG remained stable (Table 1). This resulted in a favorable RANKL/OPG ratio. The change in β-CTx was related to the change in DAS-28 and CRP during the 0 to 14 weeks. No correlation between changes in disease activity and the other markers of bone metabolism was found.

Conclusion: We showed that during 1-year treatment with infliximab in patients with RA, β-CTx and RANKL decreased and that bone formation remains unchanged. The decrease in bone resoprtion is at least partly due to a change in disease activity. Therefore, we conclude that long-term infliximab treatment has a favorable effect on bone metabolism in RA.

	0 weeks	14 weeks	30 weeks	46 weeks
β-CTx ng/ml	0.207 (0.01–0.68)	0.156 (0.1–0.59)*	0.150 (0.1–0.56)*	0.151 (0.01–0.56)*
Osteocalcin ng/ml	17.5 (3.47–38.80)	19.0 (5.43–42.35)*	17.5 (4.08–47.22)	17.7 (5.08–51.15)
RANKL pmol/ml	1547 (218–23030)	1239 (78–9420)*	1065 (85–19640)*	949 (125–13312)*
OPG pmol/ml	4.7 (1.7–13.4)	4.5 (2.3–11.2)	4.5 (2.6–11.3)	4.2 (1.9–12.10)

P670-Su

Compliance and Effect of Bone Protective Treatment in Elderly Females: 5-year follow-up Study

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The prevalence of osteoporosis and related fractures and the associated morbidity and mortality continue to rise sharply. Many of the fractures are known to be associated with propensity to fall.

Five years ago, we carried out a study undertaking diagnosis, risk assessment and treatment of osteoporosis among the young elderly (70 to 75 years) female population. Of 418 women, 319 (76%) attended. They underwent bone mineral density assessment and measures of propensity to fall.

Women were considered at increased risk of fracture if *T* score for spine or hip was below −2.5 or if BUA was below 60 dB/MHz. They were randomized to HRT, bisphosphonates or calcium and vitamin D supplementation. After 5 years, 252 women (79%) were invited to attend for follow-up bone densitometry and BUA. Data were collected on compliance with treatment, falls and fractures.

Of 187 attending for follow-up, 70 were osteoporotic, 65 (93%) were commenced on treatment and 46 (66%) remained on treatment at 5 years (Table 1).

There were 14 patients suffering incident fragility fractures: 9 defined osteoporotic (4 who were on treatment and 5 not on treatment) and 5 non-osteoporotic. Almost 50% of patients reported at least 1 fall, and the majority of fractures were as a consequence of a fall.

Conclusion: Osteoporosis screening of women aged 70 to 75 years identifies 1/3 as being osteoporotic. GPs are willing to prescribe and patients willing to accept bone protective treatment, particularly calcium and bisphosphonates. After 5 years, 66% of those defined at risk in our population were compliant with some form of bone protective treatment. Identification of frequent fallers and effective interventions may further assist in prevention of fractures.

P671-Mo

Esophogeal Transit Time of the Film-coated Risedronate 35 mg Tablet in Osteoporotic Patients with Variable Degrees of Kyphosis

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Introduction: The major risk factors for "pill-induced esophagitis" are age-related esophageal dysmotility disorders and characteristics of the dosage form. Tablet shape and nature of coating can have a dramatic impact on esophageal transit time. Previous studies have shown excellent transit times in patients with postmenopausal osteoporosis and gastroesophageal reflux disease. It is unknown if the severity of kyphosis can affect transit time.

Objectives: This study investigated the relationship of esophageal transit times to severity of kyphosis using commercially available, film-coated 35 mg risedronate tablets ingested with 2 separate water volumes (50 mL and 120 mL). The volumes of water in this study are less than the recommended US package insert, which states that a minimum of 6–8 ounces (180–240 mL) should be ingested with the tablet. These volumes were chosen specifically to exaggerate any swallowing problems.

Methods: 23 osteoporotic subjects with variable degrees of kyphosis (2 men, 21 women) with a mean age of 72 years (range 55–83) participated in a single-center, open-label, crossover gamma scintigraphy study. The effective single dose provided to each subject from the radio-labeled formulation was 0.13 mSv achieved with technetium-99m sodium pertechnetate. Subjects were seated in front of a gamma camera for anterior imaging of the esophagus. The esophageal transit times (oropharynx to the upper margin of the stomach) of the radio-labeled tablet were measured from the images using a dedicated nuclear medicine computer (Nuclear Diagnostics Ltd, London).

Results: There was no observed delay in esophageal transit times associated with severity of kyphosis with either of the 2 ingested water volumes. The mean esophageal transit time of the film-coated radio-labeled tablet with 50 mL (mean: 15.6 s, range 4.0–116.0 s) and 120 mL (mean: 12.0 s, range 4.5–60 s) was rapid, and no subjects complained of symptoms suggesting dysmotility. There was no difference in mean esophageal transit time between the two administered fluid volumes ($P = 0.58$).

Conclusion: Severity of kyphosis does not affect the esophageal transit time of the commercially available 35 mg risedronate film-coated oval tablet. This is reassuring data for the physician treating kyphotic osteoporotic patients.

P672-Tu

Improving the Quality of Osteoporosis Management in Daily Clinical Practice. The Importance of Audit

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The beneficial effect of various pharmacological and non-pharmacological interventions in the prevention of future fractures in high-risk osteoporosis (OP) patients is well established. Secondary OP, primarily vitamin D deficiency, is common in elderly patients with fragility fractures (FF).

In order to elucidate whether local OP guidelines were followed for (1) identification of high risk patients, 92) examination program: screening for secondary OP and referral to DXA and (3) institution of relevant treatment, an audit focusing on these points was performed. All in-patient records were evaluated by the treating physicians ($n = 8$) and nurses. The 56-bed ward mainly receives rheumatological patients (20%) and elderly patients with recent FF (80%) requiring hospital rehabilitation. Among the risk factors registered were recent FF, falls risk and BMI < 19.

Increased focus on OP, including specific staff education and feedback of audit results, was introduced following the first audit.

Main Results: Table below (* in patients with fall risk). Fall risk and BMI < 19: approximately 65% and 30% of the patients, respectively.

Conclusion: At baseline, local OP guidelines were only followed sporadically. Compliance with guidelines regarding identification, examination program and treatment of OP patients improved following intervention, but further improvement can be achieved. For this purpose, continuing revision of guidelines, extended staff education and audits 3 times per annum are planned.

Table

	September 2003	January 2004	May 2004	December 2004
n with risk factors	38	37	38	27
Age (years)	61–100	58–98	56–101	61–96
DXA when relevant	20%	54%	30%	100%
Routine bloods	89%	97%	100%	100%
S-Ca++	45%	65%	79%	93%
S-25-OHD and S-PTH	24%	62%	84%	89%
Calcium/vitamin D	37%	70%	82%	93%
Bisphosphonate	5%	14%	13%	19%
Hip protectors*	0%	8%	3%	33%

P673-Su

Risedronate 15 mg/day for 2 years Exhibits an Excellent Safety Profile over a Wide Range of Renal Function

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Background: Postmenopausal osteoporosis and renal insufficiency become more prevalent with increasing age. Following oral administration, risedronate is primarily eliminated via the kidneys. Reduced drug clearance and consequent elevated serum levels could potentially increase the likelihood of adverse events.

Objectives: This analysis investigates the influence of renal function on the safety profile of risedronate 15 mg/day. This dose is 3 times the daily dosage approved for the prevention and treatment of postmenopausal osteoporosis in Europe and the US.

Methods: The analysis included patients enrolled in the placebo-controlled phase III osteoarthritis clinical trials. Patients were randomized to receive placebo (N = 622, female 70%: male 30%) or risedronate 15 mg daily (N = 609, female 71%: male 29%). For each patient, creatinine clearance was estimated using the Cockcroft–Gault methodology based on baseline serum creatinine, body weight and age. The incidence of adverse events was summarized for patients possessing a wide variety of renal function.

Results: The mean age (SE) of the risedronate-treated population was 61.6 (8.6) years and 61.9 years (8.8) for the placebo. The baseline range of creatinine clearance was 37.2 to 270.0 ml/min for the risedronate arm and 31.8 to 213.1 ml/min for placebo arm. The average duration of drug exposure was 104 weeks.

There was no observed relationship between AE incidence rate and baseline renal function in the two treatment groups (placebo: R^2 = 0.001; 15 mg risedronate: R^2 = 0.001). The AE incidence observed was also not statistically different between the treatment groups.

Conclusion: This analysis shows, based on phase III clinical trial experience, that risedronate 15 mg/day, which is 3 times higher than the usual dose for treatment of postmenopausal osteoporosis, demonstrates an excellent safety profile, similar to placebo, over a wide spectrum of renal function. This is clinically important considering the high concomitant prevalence of osteoporosis and renal insufficiency in the elderly population.

P674-Mo

Effect of Osteoporosis Treatments on Risk of Non-vertebral Fractures: A Review and Meta-Analysis of Intention-to-treat Populations

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Most osteoporosis treatments have proven efficacy in reducing the risk of vertebral fractures, whereas evidence is less straightforward for prevention of non-vertebral fractures. Conclusions as to the efficacy of a treatment should be based primarily on analyses of the original intention to treat (ITT) population rather than on a post hoc exploratory subgroup analyses. However, in many cases, non-vertebral anti-fracture efficacy has been derived by subsequent subgroup analyses. This abstract reviews the non-vertebral anti-fracture efficacy of several osteoporosis therapies using the stringent assessment of the ITT

population. Meta-analysis was additionally performed for therapies possessing more than one trial fulfilling entry criteria for review.
Inclusion for review included data on non-vertebral anti-fracture efficacy obtained from randomized, placebo-controlled, phase III clinical trials of at least 3-years' duration. Ten clinical trials met the criteria for review. Only two trials demonstrated statistically significant ($P < 0.05$) non-vertebral anti-fracture efficacy in the ITT population: risedronate (VERT-NA: RR = 0.60, $P = 0.020$ and HIP: RR = 0.80, $P = 0.030$) One trial showed borderline significance: strontium ranelate (TROPOS: RR = 0.84, $P = 0.050$). For alendronate and risedronate, a meta-analysis was also performed as these treatments had more than one trial with complete data. Meta-analysis showed significant reductions in the relative risk of non-vertebral fracture for both alendronate ($P = 0.012$) and risedronate ($P = 0.001$).
Risedronate is the only agent shown to demonstrate non-vertebral fracture efficacy in more than one trial. In a pooling of trial results, a meta-analysis showed that both alendronate and risedronate provide non-vertebral anti-fracture efficacy.

P675-Tu
Fragility Fractures in Orthopedics, Assessment and Treatment
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Purpose: To assess whether fragility fractures are being referred by orthopedics for assessment and treatment of osteoporosis at our institution.
Method and materials: Patients with distal radius fractures presenting to fracture clinic and hip fractures admitted in the hospital were followed up to assess whether treatment was initiated or referred for treatment and or investigation of osteoporosis.
This was assessed through their medical records after being discharged or six weeks post injury which ever was earliest.
Results: 100 patients were assessed with 50 hip fractures and 50 wrist fractures. Majority of patients were neither referred nor started on treatment.
Outcome: Orthopedic surgeons need to be made aware of the advantages of starting treatment or further investigation of fragility fractures. Orthopedic surgeons can play a major role in improving the treatment of osteoporosis and decreasing morbidity from this disease.

P676-Su
Bridging the literacy GAP
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Recent literature has highlighted the failure of the current guidelines implementation in Canada (1) and a persistent care gap in the treatment of osteoporosis (2). An active community education program has, however, been shown to be effective, in selective settings, in changing physician behavior and by enhancing physician–patient dialogue to result in more BMD orders (3). Improving patients' level of information has also been recommended as a practical efficacious means of enhancing adherence (4). Poor health literacy is increasingly recognized as a critical factor affecting patient–physician communication and health outcomes (5). 38% of Canadians between the ages of 56 and 65 and 53% of those over 65 have an inadequate (level 1) literacy level according to the International Adult Literacy Survey of 1996 (6). Optimal methods need to be identified to educate older adults on osteoporosis and enhance the medical dialogue on this condition and perhaps improve behavior. We have created an experimental theatrical community educational program on osteoporosis. A 45-min play on a patient's experience of the management of a fragility fracture, based on patients' interviews and on the Canadian Guidelines for the Management of Osteoporosis (7), has been presented in various communities by a senior's group in Quebec. The storytelling value of the theater, as a verbal and thus less threatening teaching method (8), may help older adults to better come to terms with the message and the troubling aspects of the doctor–patient relationship. Before and after evaluations have shown that 35% (59/165) of the patients had never asked their physician about osteoporosis or had never been questioned about it. After the play, 83% (115/138) stated they would take measures to know if they had osteoporosis and to be treated if needed.

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P677-Mo
Prevalence of Visual Impairment in Patients with either a First or Second Hip Fracture
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Introduction: Visual impairment is highly prevalent and commonly unreported in the elderly. It has been identified as a significant, independent predictor of hip fracture in the elderly 1, with even moderate impairment doubling the risk of this fracture. Despite available treatments, few patients receive a visual assessment post the primary hip fracture and 10% go on to fracture their second hip. This study compares the prevalence of visual impairment in both first and second hip fracture patients.
Methods: 80 consecutive patients within 1 week of a hip fracture were assessed for both distant and near visual acuity with a Snellan chart using uniocular vision and current corrective lenses. Visual acuity of 6/12 or less denoted visual impairment 2. A history of previous fracture was also recorded.
Results: 20 patients could not comply with test due to confusion or the unavailability of their corrective lenses. Out of the 60 (45f, 15m) which remained, for 14 patients, it was their second hip fracture in the past 5 years. Normal acuity was noted in 28% of first and 7% of second hip fracture patients. Mild to moderate impairment was present in 50% and 57% of these two groups. Moderate to severe impairment was present in 22% of the first hip fracture and 36% of the second hip fracture groups. 72% of first hip fracture patients were visually impaired and 93% of second hip fracture patients. Only two patients had a referral to an optician. There was a trend towards patients with a second hip fracture having worse visual acuity ($P = 0.087$).
Conclusion: Visual assessment should be carried out in all patients presenting with hip fracture. Failure to do this is a missed opportunity to reduce the subsequent high risk of a second hip fracture. All hip fracture patients with impaired vision should be urgently assessed by an optometrist.

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P678-Tu

A UK Economic Evaluation of the Effects of Calcium and Vitamin D in Reducing Falls and Fractures

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Introduction: Calcium and vitamin D have been shown to reduce hip fractures and more recently improve muscle function and reduce falls. It is thus postulated that prescribing calcium and vitamin D to care home residents (both residential and nursing) has the potential to reduce falls, reduce subsequent hip fractures and save lives. We undertook a modeling exercise to estimate the cost effectiveness and cost utility of calcium and vitamin D supplementation to all care home residents in the UK.
Methods: Data were derived from an extensive literature review and all available, appropriate Department of Health statistics. Population data were derived from the 2001 UK census data. Analysis was based on the population of all UK care home residents to receive supplementation (calcium 1 g and vitamin D 800 IU)*, with unit costs inflated to UK £2002/3 prices. One-way and multi-way sensitivity analysis were undertaken to test the robustness of the model. Parameters in the economic model included direct health care costs (increased supplementation, change in hospital bed days, change in treatment costs) and outcomes (fractures prevented, bed days saved, lives saved, quality adjusted life years (QALYs)).
Results: Calcium and vitamin D supplementation was found in the cost-effectiveness and cost-utility analyses to dominate. This means that supplementation is both cheaper and more effective than doing nothing to reduce injuries from falls. The net cost impact in the base case was a cost saving of £28.5 million (40.7 million Euro), 10,831 life years saved, 4076 fracture prevented, and 56,972 hospital bed days saved with a gain of 8684 QALYs. Individual cost savings amounted to £624 per fall prevented, £500 per hospital bed day saved, £7000 per hip fracture prevented and £3279 per QALY. Sensitivity analyses both one way and multi-way for treatment cost, falls and fracture reduction demonstrated the robustness of this result.
Conclusion: Calcium and vitamin D supplementation is a cost-effective intervention in reducing falls and fractures in the UK elderly care home population and should therefore be offered to this population routinely.
*Calcichew D3 Forte (market leader) prices as per the BPI.

P679-Su

A Comparison of Teriparatide and Calcitonin Therapy in Post-Menopausal Women with Osteoporosis

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Objective: To compare the effects of teriparatide and calcitonin treatment on changes in bone mineral density (BMD), biochemical bone markers, and safety in postmenopausal women with osteoporosis.
Method: This is a controlled, randomized study of teriparatide and calcitonin treatment for osteoporosis in Asian women from Hong Kong, Singapore, Philippines, Malaysia and Thailand. Patients with established osteoporosis (n = 104) were randomized to 6-month treatment with either teriparatide (20 μg/day subcutaneously, n = 47) or calcitonin (100 IU/day subcutaneously, n = 57). Calcium (≤500 mg/day) and vitamin D (200–400 IU/day) supplements were taken throughout the study.
Results: Patients were aged 70.6 ± 6.8 years (mean ± SD), weighed 54.3 ± 9.8 kg (mean ± SD), had 1.2 + 0.6 baseline fractures (mean ± SD) and were 22.8 ± 8.5 (mean ± SD) years post-menopause. Most (88.5%) of these patients had taken no prior medications for osteoporosis. Study completion rates were similar for both treatment groups (teriparatide vs. calcitonin, 83.0% vs. 71.9%). Teriparatide was associated with a mean 5.03% ± 4.77% increase in lumbar spine BMD (P < 0.0001, mean ± SD change from baseline), whereas lumbar BMD for patients on calcitonin was largely stable (mean change of 0.36% ± 4.12%, P = 0.16). Irrespective of treatment group, there were no statistically significant changes observed for hip or femoral neck BMD. Serum bone specific alkaline phosphatase increased by 55.9% (median change from baseline, range 75.6% to 821%) in patients taking teriparatide (P < 0.0001). This was more marked (P < 0.0001) than the 5.0% increase (median change from baseline, range 62.5% to 144.5%) observed with calcitonin (P = 0.24). Osteocalcin increased after teriparatide treatment by 156.15% (median change from baseline, range 51.8% to 900%, P < 0.0001), whereas it decreased in patients taking calcitonin (median change of 15.25%, range 65.7% to 131.1%, P = 0.028). Similar rates of adverse events were observed, with 70% of patients taking teriparatide reporting at least 1 adverse event, compared with 75% of calcitonin patients. Nausea and dizziness were the most common adverse events reported for both groups (teriparatide vs. calcitonin, 13.0% vs. 23.2%, 10.9% vs. 21.4% respectively). There were no clinically relevant changes observed in laboratory parameters.
Conclusions: The changes observed in bone markers in this study are consistent with osteoblastic stimulation by teriparatide and antiresorptive action of calcitonin.

P680-Mo
Teriparatide Effects at High Bone-Turnover Sites. Example of a Case Monitored Through Jaw Quantitative Tomography
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The effects of teriparatide (1–34 rhPTH) and most osteotropic agents have been evaluated by bone biochemical markers and bone mineral density by DXA. Both techniques reflect changes at the whole skeleton or whole scan section (planar). But, critical bone changes occur specifically in small portions of the evaluated section, being probably not detected. Abnormal changes may be triggered in fragile sites, and the effect of medication in those sites is not known. The human mandible is the site of higher metabolic turnover and may likely reflect the pharmacodynamic features of high remodeling sites. We here show teriparatide effects in a woman who gave consent to receive treatment and tomographic monitoring of her jaw. The female – aged 64 years with 17 years since menopause – had a DXA lumbar densitometric t score of −1.76 and other risk factors for fracture 4 years ago. Her denture was complete on the right side and edentulous on the left side. First, she was treated with HRT but, after developing a mammary dysplasia, was switched to ibandronic acid 2 mg every 3 months and then to daily 25 μg teriparatide s.c. for the last 6 months. A low radiation pQCT system (XCT 3000-D, Stratec-Germany) was used before and during teriparatide administration. Her jaw was scanned and classified in different bone quality types from I (dense) to type IV (severe osteopenic). Areas of same bone quality were clustered and estimated as a percent of the total bone scan surface. After 6 months, her lumbar DXA increased from −1.6 to −1.5 (same degree that with ibandronic acid but with 5-fold shorter treatment). At jaw, dense tissue type I increased +6.5% and type II 0.2%, while osteopenic tissue decreased type III −6.0%, and type IV −5.7%. Hence, we observed tissues of better quality only after 6 months of therapy. Bigger changes occurred at the dentate side suggesting that teriparatide can react positively in mechanically stimulated sites. It is here postulated that teriparatide can promote bone mass in high turnover sites showing a positive interaction by adapting to mechanical loadings. This deserves a randomized controlled trial confirmation.

P681-Tu
Unexpected Induction of Anabolic Genes following Single Low Dose Exposure of Osteoblasts to PTH
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Parathyroid hormone (PTH) can induce both catabolic and anabolic effects on bone in vivo, although the mechanism underpinning this effect remains poorly understood. While intermittent exposure to PTH is known to induce anabolic changes and continuous exposure causes catabolic effects, we have investigated whether the opposing responses to PTH exposure may be induced solely by varying agonist concentration. Changes in mRNA expression by quiescent SaOS-2 osteoblast-like cells were measured by quantitative

PCR (Biorad iCycler) following exposure to a range of concentrations of PTH 1–34 from 0–100 ng/mL for 8 h. Expression of receptor activator of NF-kappaB ligand (RANKL) increased in a dose-dependent manner, with the highest level of expression observed following induction with 100ng/mL PTH, in accord with previous findings. While osteoprotegerin (OPG) expression is known to decrease in response to higher concentrations of PTH, we found that very low concentration PTH (0.01–0.1ng/mL) significantly increased mRNA expression above vehicle ($P < 0.05$), and comparable results were observed in preliminary studies of OPG protein expression following the same induction regimen. Similarly, collagen-1 mRNA showed this biphasic expression, with 10–100 ng/mL PTH inducing inhibition of expression, while exposure to 0.01–0.1 ng/mL PTH resulted in an increase in expression over vehicle ($P < 0.05$). No significant differences in PTH1R expression were detected between PTH concentrations, and despite previous data describing increased osteoblast proliferation in vitro in response to low concentration PTH, no significant differences in ornithine decarboxylase were observed in this study. Reporter cell studies revealed that the half-maximal induction of the immediate early gene c-*fos* occurred at doses significantly higher than those causing increased gene expression of OPG and collagen-1. Half-maximal induction of cAMP similarly occurred at higher PTH concentrations than those inducing anabolic gene expression. The signaling mechanism behind the induction of OPG and collagen-1 expression following stimulation by low-dose PTH 1–34 therefore remains to be fully elucidated. These findings indicate that exposure of bone cells to varying concentrations of PTH may play a role in the observed opposing effects of PTH on bone mineral density.

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P682-Su

Early BMD Response at the Lumbar Spine to Teriparatide: Six-month Results from the EUROFORS Trial

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The objective of this analysis was to investigate the rate of early BMD response after 6 months of treatment with teriparatide (TPTD) in osteoporotic women. All women from the EUROFORS cohort who had evaluable baseline and 6-month follow-up BMD scans of the lumbar spine (LS) were included in this analysis. EUROFORS is a prospective randomized trial of 24 months duration, designed to investigate various sequential treatments of teriparatide over 2 years in 866 postmenopausal women with established osteoporosis. In the first year, all patients received open-label treatment with teriparatide 20 μg/day and supplements (500 mg/day calcium and 400–800 IU/day vitamin D). Participants were categorized into 3 distinct groups: osteoporosis treatment-naive patients (group 1), patients with adequate (group 2) or inadequate (group 3) clinical outcome to prior antiresorptive (AR) therapy. Inadequate clinical outcome to prior AR therapy was predefined as: (a) sustaining $\geq$1 new clinical fragility fracture(s) despite prescription of AR therapy during the 12 months prior to fracture; (b) *T* score $\leq$ 3.0 SD or (c) BMD decrease $\geq$3.5% at lumbar spine (LS), total hip or femoral neck $\geq$2 years after initiating AR therapy. Since the BMD response to TPTD at the hip requires a longer lag time, response categories in this 6-month analysis were defined based on LS BMD alone, using the protocol-defined threshold of 3.5% change from baseline as "least significant change" for grouping patients with response (gain of >3.5%), uncertain response and non-response (loss of >3.5%). The respective number (%) of patients in each category is shown in the Table for the 3 subgroups and the total study population.

Statistical comparisons of group 2 and 3 subsets by previously used AR drug(s) (alendronate, risedronate, etidronate, raloxifene, ET/EPT, calcitonin) were not performed because the numbers of non-responders were too small. In conclusion, the rate of women with an early BMD non-response to TPTD at the LS was small, regardless of the nature and outcome of prior antiresorptive treatment.

Table
Number (%) of patients per response category

	Group 1	Group 2	Group 3	Total
Non-response	2 (1.2)	4 (2.3)	25 (6.3)	31 (4.2)
Uncertain response	63 (36.8)	77 (43.8)	158 (39.8)	298 (40.1)
Response	106 (62.0)	95 (54.0)	214 (53.9)	415 (55.8)
Total	171 (100)	176 (100)	397 (100)	744 (100)

P683-Mo

The Safety and Efficacy of PTH in Women Receiving Hormone Therapy

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Full-length (1–84) parathyroid hormone (PTH) reduces vertebral fractures and increases bone mineral density (BMD) in postmenopausal women with osteoporosis (PMO). Because some PTH candidates may be receiving hormone therapy (HT; estrogen with or without progestin), we examined the efficacy and safety of PTH when added to HT. One hundred and eighty women (mean age, 58.8 years; mean years postmenopausal, 12.6) with PMO receiving stable regimens of bone-sparing doses of HT −2.0 were ≤ for ≥6 months and with lumbar spine (LS) BMD *T* scores g PTH (*n* = 90) or randomized to daily subcutaneous injections of 100 placebo (*n* = 90). All subjects received daily calcium/vitamin D supplementation (700 mg/800 IU). A total of 121 subjects (67 HT, 54 PTH + HT) completed 12 months of therapy.

Mean percent change in BMD (SD) from screening to Month 12.

Site Placebo + HT PTH + HT *P* value *.

Lumbar spine 1.15 (3.34) 7.10 (5.44) <0.001.

Total hip 1.19 (2.86) 1.27 (3.25) 0.881.

Femoral neck 0.42 (3.23) 1.40 (4.17) 0.113.

**P* values are from 2-way ANOVA.

Markers of bone turnover were increased consistently in the PTH + HT group at Month 12. Bone-specific alkaline phosphatase increased 166% ($P = 0.0008$) from screening in the PTH + HT group and decreased 68.3% in the HT group ($P = 0.617$). Urine pyridinoline cross-linked N-telopeptide increased 114% ($P < 0.0001$) in the PTH + HT group and slightly increased 16% in the HT group ($P < 0.6153$).

Adverse events (AEs) were reported by 91.1% and 95.6% of subjects in the HT and PTH + HT groups, respectively; serious AEs occurred in 6 (6.7%) and 3 (3.3%) subjects, respectively. The most common AEs were of gastrointestinal origin, with similar proportions of each group reporting these events. At least 1 serum total calcium elevation (>10.7 mg/dL) occurred in 13.3% of the PTH/HT group and 1.1% of the HT group; hypercalciuria (>360 mg/day) occurred at least once in 50% and 34% of subjects, respectively. One subject (PTH + HT) discontinued the study for hypercalcemia and 6 for hypercalciuria (3 in each group).

In conclusion, 12 months of combined PTH/HT therapy was associated with significant increases in LS BMD compared to HT alone. The increases in LS BMD were similar to those previously reported in subjects treated with PTH alone (Hodsman et al., 2003) suggesting that HT does not blunt the anabolic effects of PTH. When used in combination with HT, full-length PTH was generally safe and well tolerated.

P684-Tu

First Experience with Teriparatide as Treatment of Severe Osteoporosis in Daily Routine in Switzerland

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Teriparatide, a recombinant form of human PTH 1–34, has recently been approved for the treatment of severe osteoporosis in Switzerland. Compliance by the patient in the use of a daily subcutaneous injection of teriparatide is important to achieve therapeutic benefits. Although biochemical markers are normally used in clinical trials, they are not yet part of daily routine in Switzerland. Therefore, monthly medical consultations with return of disposable pens used for injection were scheduled. The early assessment of the therapeutic effect of teriparatide on bone mineral density (BMD) with the widely used dual energy X-ray (DXA) technology may be used to maintain patients' future compliance.

Fourteen patients (10 postmenopausal women, 4 men; mean age 68 years) with severe osteoporosis received teriparatide 20 μg once daily and supplementation with calcium and vitamin D. One woman stopped after 3 weeks because of side effects (dizziness and somatic pain). For the other 13 patients, no side effects have been reported. Ten of 13 patients received other osteoporotic treatments (fluoride, calcitonin or bisphosphonates up to 7 years) prior to teriparatide. BMD was measured using DXA (Hologic Discovery) at lumbar spine (LS) and proximal femur [femoral neck (FN) and total hip (TH)] at baseline and after 6 months of therapy.

Mean *T* score at baseline was −3.4 at LS, −2.5 at FN and −2.1 at TH. After 6 months, we observed an increase in LS BMD in all 13 patients, between 2.6% and 13.9% (7.4% mean BMD increase). In contrast, on average, no change of hip BMD could be observed after 6 months as expected. Furthermore, no clinical fracture occurred during this 6-month period.

In conclusion, the regular monthly medical consultations in association with a planned early DXA control led to an excellent compliance, as reflected by the monthly return of empty injectors. All patients responded to teriparatide in terms of BMD increase at LS. The observed increases at LS were similar to those observed in the Fracture Prevention Trial (Neer et al., NEJM 2001). We were not able to determine a negative impact of pretreatment with other antiosteoporotic drugs on BMD response in this small number of patients.

P685-Su

Self-assessment of Health in Relation to Previous Falls among General Population and Osteoporosis Patients

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Objectives: The experience of falling can lead to a reduction in confidence and activity and an increase in the probability of subsequent falls and fractures. In this study, we analyzed the effect of recent falls on self-assessment of health by women in general and by patients with osteoporosis.

Methods: Subject Group 1 consisted of 127 ambulant women with an average age of 60 (29–93) years, 103 of whom were living in the community and 23 were nursing

home residents. Subject Group 2 comprised 40 ambulant female patients at the Osteoporosis Clinic of Kyoto University Hospital who were diagnosed with osteoporosis. The average age of Group 2 was 67 (39–85) years. The SF-36 Questionnaire (a profile measurement of health-related quality of life, HRQOL, with 8 subscales) was used for self-assessment of health. The subjects were also asked whether they had fallen within the last 1 year. Multivariable regression analysis was performed to assess the effect of subject group, age and previous falls on each of the SF-36 dimensions.

Results: Group 2 showed significant negative effects on two SF-36 dimensions, general health ($P = 0.0035$) and vitality ($P = 0.0337$), indicating that osteoporosis patients tend to evaluate personal health as poor and to feel tired in comparison with the general population. Among subjects in Group 1, three SF-36 subscales, role-physical ($P = 0.0085$), role-emotional ($P = 0.0046$) and mental health ($P = 0.0322$) were significantly and negatively affected by previous falls. This implies that women with previous falls tend to have difficulties with daily activities as a result of physical or emotional problems and to feel nervous and depressed in comparison with their counterparts without the experience of falling. When women under 65 were excluded, previous falls were found to have a negative effect on general health ($P = 0.0108$) and vitality ($P = 0.0471$). In Group 2, previous falls showed no significant effect on any of the SF-36 dimensions.

Conclusions: Previous falls are related to negative self-assessment of health in women of advanced age. Osteoporosis patients show lower scores of HRQOL than women in general, regardless of previous falls. From the results reported here and in previous reports, showing that a decline in activity can be a risk factor for falling, it can be concluded that interventions to maintain proper activity to prevent fractures can be beneficial for both elderly persons in general and osteoporosis patients.

P686-Mo

What Degree of Height Loss Affects Health-Related QOL in the Elderly?

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To study the degree of height loss experienced in elderly men and women compared with their youth and what degree of height loss affects health-related QOL in the elderly, a cross-sectional survey with health examinations, including spinal radiographs and questionnaire surveys, was conducted among 1941 participants (657 men and 1284 women) aged 55–99 years in a population-based study (Adult Heath Study (AHS)) in Hiroshima during the period from 2002 to 2004 who responded to a questionnaire survey including EQ-5D. The AHS recruited a cohort of about 20,000 people in Hiroshima and Nagasaki based on the 1950 Japanese national census, and this cohort has received biennial health examinations since 1958. EQ-5D is a questionnaire that comprises items regarding mobility, self-care, usual daily activities, pain/discomfort, and anxiety/depression. Based on body height measurements recorded in medical charts for over 50 years, we obtained the difference between subject height at its peak and at the present.

Average height loss was 1.2 cm for men and 1.3 cm for women in their fifties; 1.5 cm for men and 2.1 cm for women in their sixties; 2.4 cm for men and 3.9 cm for women in their seventies; and 4.1 cm for men and 6.8 cm for women in their eighties. Degrees of height loss most frequently observed were 1–2 cm and 2–3 cm among men and women, respectively, and in about one-third of the women, height loss of 5 cm or more was observed. Height loss was significantly related to prevalent spine fracture, but neither osteoarthritis of spine nor arthritis of knee joints.

The average EQ-5D score in the AHS population was 0.79. EQ-5D score decreased with increasing age and was lower in women than in men. Elderly men and women whose body height decreased more than 2 cm had significantly lower EQ-5D scores compared with those whose body-height change was less than 2 cm, after adjusting for age and sex. Subjects were categorized into four groups of height loss (<2 cm, 2–3, 3–4, 4+ cm), and the larger the degree of height loss, the more EQ-5D decreased in both men and women.

In conclusion, average height loss was more than 2 cm for men and 4 cm for women in Japanese elderly older than 70 years of age. Even among the elderly who had height loss of 2–3 cm, decreased health-related QOL was observed. Important for maintaining QOL among the elderly will be to prevent development of spinal fracture.

P687-Tu

The Effect of 1 year of Alendronate following 1 year of PTH 1–84: Second Year Results from the PTH and Alendronate (path) Trial

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We have previously reported the results of a trial comparing 1 year of therapy with either PTH(1–84) alone, alendronate alone or the two in combination (Black et al., NEJM, 2003). This randomized trial was designed also to evaluate the results of a second year in which PTH(1–84) was discontinued and either followed by no therapy or with the antiresorptive alendronat, ALN. We tested the hypothesis that antiresorptive therapy is required to

maintain the densitometric gains in bone mineral density after 1 year of PTH(1–84).The 119 women who received PTH(1–84) (100 mcg) monotherapy in the first year were randomized to 1 additional year with either placebo (n = 60) or alendronate (n = 59) (10 mg/day) in a protocol that continued to be double-blinded. PTH was stopped in all subjects after the first year. The main endpoints were spine and hip BMD by DXA and by QCT, the latter in a subset (approximately 75%) of participants. During the first year of study among those treated with PTH(1–84) alone, there was a 6.2% mean increase in DXA spine BMD and a 29% increase in trabecular spine BMD (QCT). Comparable increases at the (total) hip were 0.3% by DXA and 8.6% for trabecular hip BMD. The second year, post-PTH changes, in the ALN and placebo groups are described in the table below:

Mean percent changes: 1 year of PTH(1–84) followed by 1 year of ALN or PBO.

Changes within 2nd year Changes over 2 years.

Treatment in year 2 Treatment in year.

Endpoint ALN PBO Difference ALN PBO Difference.

Spine BMD (DXA) +4.9%* −1.7%* 6.6%*** +12%^ +4.1%^ 8.0%***.

Trabec. spine BMD (QCT) +2.7% −9.9%* 12.6%*** +34%^ +13%^ 21%***.

Total. Hip BMD (DXA) +3.6%* 0.03% 3.6%*** +4.5%^ −0.1% 4.6%***.

Trabec hip BMD (QCT) +6.4%* −3.7% 10.1%*** +12%^ +5.4% 6.2%.

*P < 0.01 vs. year 1 value; ***P < 0.01 between groups; ^P < 0.01 vs. baseline value.

Following PTH, there were significant gains in the group treated with ALN, while there were losses in those on PBO. There were striking differences following PTH in those on alendronate compared to those on placebo, particularly for trabecular bone. These data suggest that BMD gains after 1 year of PTH(1–84) are quickly lost in the next year if PTH is not followed by an antiresorptive agent.

P688-Su

Measurement of Quality of Life (QOL) in Patients with Severe Osteoporosis: The ICARO Study (Incidence and Characterization of "Inadequate Treatment Responder patients" in Osteoporosis), Final Cross-sectional Report

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ICARO is a multicenter observational project that aims to identify, among patients with severe osteoporosis, those with "inadequate response to antiresorptive treatment", defined as patients prescribed with antiresorptive drugs (alendronate, risedronate and raloxifene, as reported by Note 79) for at least 1 year, presenting a new fragility fracture (vertebral or non-vertebral) or discontinuing antiresorptive therapy for lack of compliance and/or side effects. The study consists of two phases: a cross-sectional and 12 months longitudinal one. In the cross-sectional phase, postmenopausal women with established osteoporosis, prescribed for at least 1 year with antiresorptive treatment, were enrolled in the study. Compliance to previous treatments and QoL (QUALEFFO-41) were measured.

One thousand four hundred twenty one women with severe osteoporosis, mean age 67.6 (±7.7) years, were studied. Patient with "inadequate clinical response" was defined as a subject presenting a new clinical fragility fracture (with following radiological documentation) after at least 6 months of antiresorptive therapy prescription. 75.3% of "inadequate clinical responders" showed a compliance level >75%, suggesting that the inadequate response is rather due to antiresorptive drug resistance than to drug intolerance. The results on QoL showed a consistent increase throughout the different domains of QUALEFFO-41 in "inadequate clinical responders" compared with "adequate clinical responders" (the higher score indicates a worse quality of life perception). In "inadequate clinical responders," there was a tight correlation between the decrease in QoL and the disease severity (average percentage of multiple clinical vertebral fractures 53.5%).

These preliminary data underline that an "inadequate clinical response" to antiresorptive treatments is associated with a worsening in quality of life parameters.

Table
QUALEFFO-41 domains score*

	Responders	Inadequate responders
Global	38.12 ± 16.7	45.84 ± 18.3
Pain	41.44 ± 24.3	50.58 ± 23.7
Daily life activity	20.21 ± 19.1	25.46 ± 21.8
Housework	31.53 ± 23.2	40.58 ± 25.0
Mobility	31.30 ± 21.0	39.03 ± 23.5
Social activities	48.77 ± 24.6	58.27 ± 26.6
Health condition perception	59.14 ± 20.5	68.45 ± 20.0
Mood	39.81 ± 17.8	45.62 ± 19.6

*P < 0.001 between responders and non-responders in total.

P689-Mo

Secondary Osteoporosis Therapy by Generating Magnetic Field

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To evaluate magnetic therapy (MT) on bone mineral density (BMD), magnetic field therapeutic system "OSTEOPORO-

SIS" ("Direx", Israel) was used. The main principle concludes in generation pulse electromagnetic fields, stimulating the secondary electric field inside bone tissue that determines medical effect: bone mass elevation in whole body, fractures joining acceleration and new ones prevention. Observation group consisted of 32 patients with low forearm BMD in combination with thyroid dysfunction, such as Graves disease (GD)—7 persons; and primary hypothyroidism (PHT)—25 persons. The average age was 44.6 ± 14.1 years. All patients were in medical euthyrosis (PTH median was 0.8 MU/ml in GD and 1.4 MU/ml in PHT). In results of dual-energy X-ray absorptiometry (DEXA) according to WHO recommendations (1994), all patients had a low BMD corresponding to osteoporosis (OP) or severe osteopenia, expressive pain syndrome. Bone fractures were registered in 42.9% (GD) and 24% (PHT). Calcium-phosphate metabolism markers stayed within normal range. All patients had MT course of 20–30 seances (average 23.8 ± 2.65) in combination with calcium and vitamin D preparation ("Calcium-D3 Nycomed" 2 tablets once a day) during MT. All MT parameters were chosen automatically by PC according to individual patient's DEXA indexes. Control investigation was fulfilled in 12 months. All results were processed statistically with "Biostatistica" program.

Results: Pain intensity decreased in both MT groups (GD from 1.4 ± 0.02 to 0.6 ± 0.04 points, $P = 0.000$ and PHT from 1.2 ± 0.04 to 0.6 ± 0.05 points, $P < 0.05$). New fractures were not registered. OP frequency changed from 42.9% to 21.4% in GD; in PHT, 26% and 18% correspondingly. Initially, in GD patients, T criteria average mean was −2.34 ± 0.53 SD, in PHT, −1.95 ± 0.2 SD; in 12 months BMD increased in 2.35% (W = −0.28; $P = 0.01$) and 2.3% (W = −103; $P = 0.03$) correspondingly.

Conclusion: MT had positive effect on BMD and pain syndrome. So, this method may be used for OP therapy in patients with thyroid dysfunction.

P690-Tu

Low Rate of Use of Vitamin D Supplements in Osteoporotic Women

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Objective: To estimate the rate of vitamin D use and evaluate the factors related to its use among osteoporotic women.

Materials and methods: A sample of 1357 women aged 50 and older who reported having osteoporosis was analyzed using combined data from the 2002 and 2003 National Health and Wellness Surveys [NHWS] in France, Germany and the UK. NHWS is a cross-sectional study of consumer attitudes, behaviors and treatment choices as they relate to healthcare. It is based on a nationally representative stratified random sample from the Ipsos panel. In 2002 and 2003, data were collected by mail survey fielded by Ipsos. Response rates were around 50%. The use of vitamin D was defined as either a prescription [Rx] medication or an over the counter product containing vitamin D with or without calcium that had been used in the past month. A logistic regression was performed to determine factors related to vitamin D use.

Results: Of 1357 osteoporotic women, only 17.6% (n = 239) reported use of vitamin D. Among those who were receiving an osteoporosis Rx (n = 806, 61.4%), the rate of vitamin D supplementation was 25.9% (n = 209). Even among women with a history of fracture after the age of 50, the rate of vitamin D use was very low: 20.1% (n = 92). The likelihood of using vitamin D was significantly higher for women with a family history of osteoporosis (OR = 1.47; 95% CI = 1.07–2.01) and significantly lower for those with a history of having had a bone mineral density [BMD] test (OR = 0.48, 95% CI = 0.32–0.71) and for those taking an Rx drug to treat osteoporosis (OR = 0.21; 95% CI = 0.14–0.32).

Conclusions: Although vitamin D is essential for calcium absorption to ensure strong, healthy bones, fewer than one in five women with osteoporosis are taking a vitamin D supplement. Even among high risk patients with a fracture history, only 1 out of 5 patients used vitamin D supplementation. More attention needs to be given to educating osteoporosis patients about the importance of getting enough vitamin D.

P691-Su

Superiority of Alfacalcidol Compared to Vitamin D Plus Calcium Carbonate in Lumbar BMD in Post Menopausal Osteoporosis

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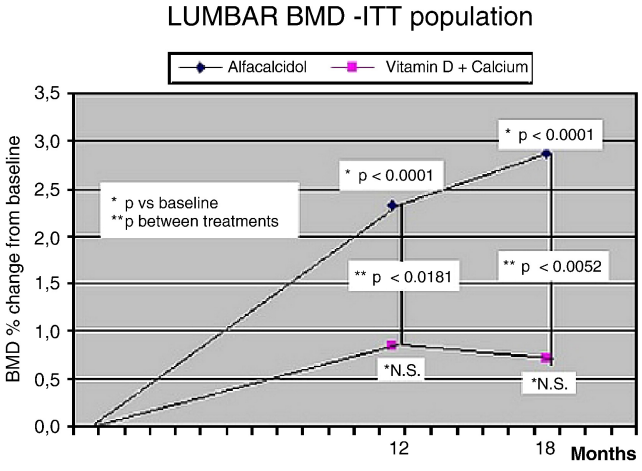
In a randomized multi-center double blind, double dummy parallel group study, a comparison of the efficacy and safety of 1 μg alfacalcidol to 880 IU vitamin D plus calcium

carbonate (1 g calcium) once daily per os was performed on 148 post menopausal osteoporotic patients for 18 months. BMD was measured at baseline, 12 and 18 months. Safety parameters were followed during the entire study.
Lumbar BMD in the alfacalcidol group increased by 2.33 and 2.87% from baseline ($P < 0.0001$), whereas in the vitamin D plus calcium group, the increase was 0.85 and 0.70% from baseline (N.S.) at 12 and 18 months respectively. The higher % changes from baseline in the alfacalcidol group, as compared to the % changes in the vitamin D plus calcium group at both 12 and 18 months, were found to be significant (figure below). No significant differences were noted between the groups in plasma calcium or any other safety or vital parameters.
In conclusion, alfacalcidol was found to be superior in significantly increasing lumbar BMD as compared to vitamin D plus calcium, while safety characteristics in both treatments were similar.
The study was funded by Teva.



P692-Mo
Changes in gGene Expression in Pagetic Osteoblasts and Stromal Cells
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Paget's disease of bone is a common disorder characterized by focal areas of increased bone resorption coupled to increased and disorganized bone formation. The osteoclasts in the pagetic lesion are driving the high bone turnover, however, due to the integral cross-talk between osteoclasts and osteoblasts, we propose that pagetic osteoblasts may also play a key role in the pathology of Paget's disease. To determine the differences in gene expression between pagetic and non-pagetic osteoblasts and their precursors, the mRNA levels of RANK, RANKL, OPG, M-CSF, IL-1beta, IL-6, IL-11, MIP-1alpha, TNFalpha, BCL-2, SHIP, COX-2 and VEGF were investigated in primary cell cultures of human osteoblasts and the mixed-cell population of bone marrow stromal cells. Trabecular bone explants from 10 Paget's patients and 21 control subjects were used to grow osteoblast cultures. Bone pieces washed free of marrow and cellular debris were placed in flasks to culture outgrowth of osteoblast-like cells. In addition, bone marrow samples were collected from the same number of Paget's patients and control subjects. Mononuclear stromal cells from marrow were isolated and grown in culture flasks. RNA was collected from these cells at confluency and quantitative Taqman Real-Time PCR was used to determine gene expression levels. Gene expression was normalized using 18S ribosomal RNA as an endogenous control. The average levels of gene expression in pagetic cells were compared to the average expression levels in non-pagetic cells using one-sample *t* tests. In pagetic osteoblasts, RANKL and BCL-2 displayed significantly lower expression while IL-1beta, IL-6 and IL-11 expression levels were significantly higher. In pagetic stromal cells, RANKL, COX-2 and VEGF displayed significantly lower expression, while levels of RANK, OPG, IL-6 and SHIP gene expression were significantly higher. In both osteoblasts and stromal cells, RANKL/OPG ratio was significantly decreased. Increased levels of IL-1beta, IL-6 and IL-11 are consistent with the over-active pagetic bone microenvironment, while the decreased RANKL/OPG ratio and changes in BCL-2, COX-2 and SHIP would favor suppression of osteoclast formation and activity. This suggests that the RANKL/OPG system is compensatory to other mechanisms that increase osteoclast number and activity within the active pagetic lesion.

P693-Tu
Bisphosphonate Treatment Partially Restores Trabecular Architecture in Juvenile Paget's Disease
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Juvenile Paget's disease (JPD), also known as Idiopathic Hyperphosphatasia (IH), is a serious childhood genetic disorder resulting from deletions of or inactivating mutations in the gene for osteoprotegerin (OPG). JPD is characterized by elevated bone turnover rate. The trabecular architecture in iliac biopsies and tibial autopsy samples is highly unusual, characterized by arrays of parallel trabecular plates aligned with nearby endocortical surfaces. OPG negatively regulates the RANK–RANKL mediated coupling or feedback between osteoblast formation and osteoclast resorption. Thus, the removal of OPG action causes remodeling feedback, resulting in an excessive "run-away" remodeling rate. Another consequence of remodeling feedback is the suppression of the spontaneous nonlinear pattern formation that establishes normal trabecular architecture and the imposition

of simpler but mechanically inferior parallel plate architecture. Therapy for JPD patients is aimed primarily at reducing the excessive turnover rate. Intensive bisphosphonate treatment given to an 11-year-old girl suffering from JPD appeared to arrest the progress of the disease: she remained mobile instead of becoming wheelchair-bound like her affected siblings and had much enhanced bone density with bone turnover rate restored to within a normal range. Figure 1 shows iliac crest biopsies taken from this girl before and after the bisphosphonate treatment, compared to a normal individual. The bisphosphonate treatment caused trabecular thickening by 32% and a partial amelioration of the abnormal parallel plate phenotype. Micro-CT measurements of the degree of anisotropy (MIL method) in trabecular regions from the three biopsies were 9.68, 5.96 and 1.45 (Figs. 1a, b and c, respectively).

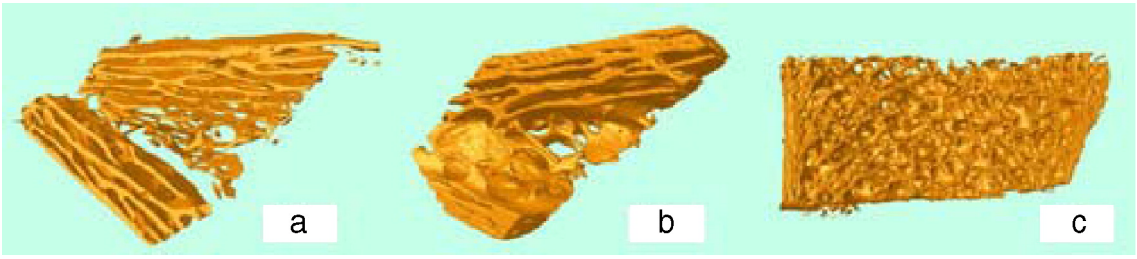

Fig. 1. Micro-CT generated 3d models of iliac crest trabecular biopsies from a JPD patient before (a) and after (b) bisphosphonate treatment, and a control normal individual (c).

P694-Su

Paget's Disease: Clinical Profile of 19 Patients from India

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Paget's disease has traditionally been regarded as very rare or nonexistent in India. Clinic records of nineteen patients with Paget's disease seen over a period of 10 years from various tertiary care centers of India were analyzed, as a part of the initiative of the Indian Society for Bone and Mineral Research. Paget's disease was diagnosed on the basis of classical radiology, elevated serum alkaline phosphatase and bone scan and/or biopsy. Out of 19 patients, 13 were males and 6 were females. Age of presentation of these patients ranged from 10–74 years, with mean (±SD) of 51.6 ± 15.6 years. Out of 19 patients, 11 (57.9%) had polyostotic disease, and the remaining had monostotic disease. Time between onset of symptoms and diagnosis ranged from 0.6 to 20 years with a mean (±SD) of 7.87 ± 6.7 years. Common presenting complaints were headache (42.1%), backache (42.1%), bowing of legs (15.78%), paraparesis (10.5%), facial pain (5.2%), tinnitis (5.2%), exophthalmos (5.2%) and decreased hearing (5.2%). Fracture as a presenting manifestation was observed in 4 patients (21%). Increased head size was noticed in 5 (26.3%) out of 19 patients. Visual impairment and difficult bite were observed in 2 and 1 patient respectively. Interestingly, one patient had recurrent paraparesis. Mean (±SD) serum calcium and phosphate levels were 9.1 ± 5.4 mg/dl and 3.9 ± 0.57 mg/dl respectively. Serum alkaline phosphatase was elevated in all patients, with a mean (±SD) of 1637 ± 1266 and range between 589 to 3422 IU/l. Isotopic bone scan was suggestive of increased uptake in affected areas in all patients. Four patients underwent bone biopsy. Eleven patients were treated with alendronate 40 mg/day for 4 months, while the rest were treated with periodic infusions of intravenous pamidronate. Symptomatic improvement in pain was noticed in most cases. One patient with paraparesis had recurrent neurodeficit secondary to vascular steal phenomenon and improved with alendronate therapy.

This is the first case series of Paget's disease reported from India and confirms the existence of this disease in different regions of India. The common presenting manifestations were bone pains, headache and backache. Polyostotic variety is more common than monostotic. Systematic data compilation involving more centers, including a detailed analysis of response to treatment, which is underway, will help us in managing and understanding this disease better.

P695-Mo

Zoledronic Acid Shows Superior Effect on Paget's Disease Activity that is not Blunted by Previous Treatment Status: A Randomized Controlled Comparison with Risedronate

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Bisphosphonates (BPs) constitute the treatment of choice of Paget's disease of bone (PDB). However, there is increasing evidence that resistance to BPs could have potential clinical implications in patients who have received previous therapy with BPs. Hence, there is still an unmet medical need for an agent that combines a complete response with a faster onset of action, regardless of prior BP treatment.

We assessed the efficacy of zoledronic acid (ZOL, one 5 mg 15-min infusion) versus oral risedronate (RIS, 30 mg/day for 60 days) in two identical randomized, controlled trials. This efficacy assessment focused on therapeutic response over 6 months, defined as a $\geq$75% reduction in serum alkaline phosphatase (ALP) excess or its normalization. At baseline, demographic and background characteristics were comparable between groups. At 6 months, 96% ZOL versus 74% RIS patients ($P < 0.001$) achieved therapeutic response, and 89% ZOL versus 58% RIS patients ($P < 0.001$) reached ALP level normalization. ZOL showed faster onset with shorter median time to first therapeutic response than RIS (64 versus 89 days, $P < 0.001$). The superiority of ZOL was confirmed in subgroup analyses by baseline ALP level (<3 times upper limit of normal ranges [ULN] and 3–6 × ULN). In patients previously treated with oral BPs and in treatment-naive patients, ZOL had a higher response rate compared to RIS (96% versus 55% [$P < 0.001$] and 98% versus 86% [$P = 0.0075$], respectively). Interestingly, while 100% of the patients previously treated with RIS achieved therapeutic response with ZOL, previous treatment with RIS resulted in subsequent blunted response to RIS in 70% of the patients (Table 1). This higher response with ZOL was also seen in subgroups previously treated with other oral BPs. We conclude that ZOL not only is superior to RIS in reducing Paget's disease activity with faster normalization of ALP but also offers the possibility to overcome resistance encountered with oral BPs. These findings provide advantages for ZOL in the clinical management of patients with PDB.

Table 1
Proportion of patients who achieved therapeutic response at 6 months by previous BP usage

Previous BP used	ZOL *n*/*N* (%)	RIS *n*/*N* (%)	*P* value
Oral treatment	53/55 (96%)	33/60 (55%)	<0.001
Risedronate	13/13 (100%)	7/23 (30%)	<0.0001
Alendronate	16/17 (94%)	9/13 (69%)	0.0769
Other orals	24/25 (96%)	17/24 (71%)	0.0216
IV treatment	22/25 (88%)	21/26 (81%)	0.4590
None	80/82 (98%)	65/76 (86%)	0.0075

P696-Tu

Effect of Bisphosphonate Treatment in Pagetic Patients with Skull Involvement

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The aims of this study were to evaluate the response to therapy in disease activity in pagetic patients with and without skull involvement and to compare the usefulness of new bone markers in the evaluation of these patients.

Patients and methods: 40 patients with Paget's disease (12 with skull involvement) treated with tiludronate (400 mg/day × 3 months) and 26 healthy controls were included in the study. Serum total alkaline phosphatase (TAP), bone alkaline phosphatase (BAP), PINP and urinary alpha–alpha CTX, β–β CTX and NTX were measured at baseline and at 1 and 6 months after discontinuation of therapy. Quantitative bone scintigraphy was performed at baseline and at 6 months, and an index of disease activity (SAI) was obtained. Patients were classified into three groups: patients with skull involvement (Sk group), patients without skull involvement and patients without skull involvement but with similar disease activity to those of the Sk group (based on SAI).

Results: All groups of patients showed higher baseline values in all markers compared to controls. At baseline, patients with skull involvement showed significantly higher values in all markers when compared to the rest of the pagetic patients. In addition, there were no significant differences between the group of patients with similar SAI and those with skull involvement except for NTX, which was higher in the latter group. The alpha–alpha CTX was the marker with the highest values in this group of patients (46 times higher than normal values in Sk group vs. 20 times higher in the rest of patients). Six months after discontinuation of therapy, the percentage of patients with markers within the normal range in patients without skull involvement were: 71% for TAP, 71% for BAP, 63% for PINP, 54% for NTX, 48% for alpha–alpha CTX and 52% for β–β CTX; in patients with similar SAI to Sk group, the values were 60% for TAP, 73% for BAP, 50% for PINP, 33% for NTX, 31% for alpha–alpha CTX and 39% for β–β CTX, whereas in patients with skull involvement, they were: 25% for TAP, 18% for BAP, 11% for PINP, 30% for NTX, 17% for alpha–alpha CTX and 17% for β–β CTX. In conclusion, pagetic patients with skull involvement showed a marked increase in bone turnover and a lower response to bisphosphonate therapy. Moreover, alpha–alpha CTX is the marker with the highest increased values in these patients. The results suggest that these patients probably need to be treated with higher doses or more potent bisphosphonates.

P697-Su

C-terminal Parathyroid Hormone-Related Protein (107–139) Exerts Antiapoptotic Effects on Human Osteoblastic Cells In Vitro

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C-terminal parathyroid hormone-related protein (PTHrP) directly inhibits osteoclastic-mediated bone resorption and affects osteoblastic differentiation. This domain and also the N-terminal PTHrP fragment stimulate vascular endothelial growth factor (VEGF) expression in osteoblasts. VEGF is an important cell survival factor, acting through its type 2 receptor (VEGFR2) and the Bcl-2 protein family. We assessed herein the effect of PTHrP (107–139) on osteoblastic cell viability (evaluated by trypan blue and propidium iodide staining), and the possible role of VEGF. Subconfluent MG-63 cells were treated with 100 nM PTHrP (107–139) or 100 nM PTHrP (1–36), for 1–24 h, following by 1 mM dexamethasone or 50 mM etoposide addition for 16 h. Preincubation with each PTHrP peptide significantly inhibited (<50%, $P < 0.05$) cell death induced by dexamethasone or etoposide; but the inhibitory effect of PTHrP (107–139) was 1.6-fold higher and more sustained than that of PTHrP (1–36). In these cells, both PTHrP peptides rapidly stimulated (maximal at 1–3 h, 2–3-fold, $P < 0.05$) the BclxL/Bax ratio and also the activity of Runx2, a transcription factor which appears to modulate the expression of Bcl-2 gene family. Interestingly, while each C- and N-terminal PTHrP peptide (100 nM) similarly stimulated VEGF mRNA, only PTHrP (107–139) enhanced VEGFR2 mRNA (maximal, 2-fold, $P < 0.05$; by real-time PCR) in MG-63 cells. Moreover, the stimulatory effect of this peptide on MG-63 cell viability was abolished by 1 mM SU5416, a VEGFR2 inhibitor. In summary, PTHrP (107–139) appears to exert protective effects on MG-63 cell viability by interacting with the VEGF/VEGFR2 system.

P698-Mo

PTH Activation of ERKs is Mediated Through Both PTH/PTHrP Receptor C-Terminus and Gq Proteins and is Modulated by β-arrestins

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PTH-stimulated cAMP and IP3/Ca signaling is regulated by β-arrestins. PTH also activates ERK1/2, but the PTH/PTHrP receptor (PTH1R) determinants and role of β-arrestins for ERKs activation are unknown. We used Western blot for phospho-ERK1/2 and a serum responsive element-luciferase (SRE-Luc) reporter assay for ERK-mediated transcriptional activation to investigate PTH effects in HEK293 cells transiently expressing wild type (WT) or mutant receptors and arrestins.

With WT PTH1R, PTH(1–34) (100 nM) activated ERKs maximally after 5 min. Phorbol esters (PMA) at low concentrations potentiated PTH stimulation of SRE-Luc, whereas at higher concentrations, they stimulated SRE-Luc in the absence of PTH. In contrast, cAMP stimulation by forskolin did not activate ERKs. In turn, the PKC inhibitor Go6983 (1 mM), but not the PKA inhibitor H89, decreased PTH activation of SRE-Luc by 30%. Mutations in PTH1R intracellular loop 2 that prevent coupling to Gq blunted PTH stimulation of SRE-Luc (−50% vs. WT), while the H223R mutation, which blocks IP3 signaling and constitutively activates cAMP and β-arrestins inhibited both baseline and PTH-stimulated ERK/SRE-Luc activity. PTH1R C-terminus deletions and mutations of proximal serines that prevent β-arrestins recruitment also lowered ERKs/SRE-Luc stimulation by PTH. Moreover, mutating distal polyproline motives reduced by 50% ERK signaling in response to PTH, suggesting that protein–protein interactions with SH2–SH3 containing molecules might be involved. In support of this hypothesis, inhibitors of c-Src (PP2) and PI3K (LY294002) dose-dependently inhibited PTH1R-mediated ERKs/SRE-Luc activation. The role of β-arrestins was further investigated in cells deficient for β-arrestin2 (siRNA or KO) or expressing dominant negative (dn) β-arrestins that prevent receptor internalization. Basal ERK/Sre-Luc activity was suppressed in all these cells, but PTH stimulation of ERKs/SRE-Luc was mostly inhibited by dnβ-arrestins. Moreover, overexpressing constitutively active β-arrestins inhibited SRE-Luc stimulation by PTH, suggesting that arrestins play a dual role on ERKs activation, as both general co-activators (likely through receptor internalization) and specific inhibitors (through uncoupling from Gq) of PTH-stimulated ERKs.

In conclusion, PTH stimulation of ERKs is mediated by receptor coupling to Gq and modulated by β-arrestins and further requires C-terminus-dependent interactions with other cytoplasmic proteins, likely Src and PI3K.

P699-Tu

Essential and Distinct Roles of MAP Kinases in Activation of Osteoblastic Cells by Parathyroid Hormone

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Intermittent administration of parathyroid hormone (PTH) is associated with a positive bone balance and an increase in bone strength. Because of its reducing vertebral fracture risk, this hormone is registered for the treatment of severe osteoporosis. Increased bone formation by PTH is mainly due to activation of osteoblastic cell differentiation (OCD),

an effect largely mediated by the cAMP-Protein Kinase A (PKA) signaling pathway. The molecular mechanism by which PKA and/or the possible implication of other pathways in PTH-induced OCD remains to be investigated. MAP kinases (MAPKs) have recently been shown to play a significant role in OCD, and we investigated their role in mediating OCD by PTH.

In early differentiated MC3T3-E1 cells, 10^{-7} M human PTH(1–34) (hPTH) enhanced alkaline phosphatase (ALP) activity (8–10-fold) and doubled the production of osteocalcin (OC) after 24 h incubation. These effects were fully mimicked by 10 μM forskolin (FSK). They were blunted by H89 (25 μM) but not by Go6983 (10 μM), specific inhibitors of PKA and PKC respectively. The role of MAPKs in PTH-induced OCD was investigated using selective inhibitors. Enhanced activation of ALP induced by either PTH(1–34) or FSK was dose-dependently (5–25 μM) and completely inhibited by SB202190, a specific p38 inhibitor. This compound, however, did not influence PTH-induced activation of either cellular cAMP or OC production. In contrast, inhibitors of ERK and JNK, U0126 and SP600125 (10 μM) respectively, significantly reduced OC production but did not influence PTH effect on ALP.

Associated with these changes, the activity of the three MAPKs, i.e. ERK, p38 and JNK, were transiently (1–3 h) enhanced by 10^{-7} M hPTH, and preliminary data indicate that FSK also mimicked this effect. Finally, 5 weeks hPTH exposure clearly enhanced MC3T3-E1 matrix mineralization assessed by alizarine red staining, an effect completely blunted by 10 μM of the p38 inhibitor.

In conclusion, our results indicate that MAP kinases play a major role in PTH-induced osteoblastic differentiation. Whereas the regulation of ALP and matrix mineralization involve the p38 pathway, ERK and JNK are rather implicated in changes of OC, strongly suggesting that PTH induces osteoblastic differentiation through distinct signaling pathways.

P700-Su

Dose-Dependent Effects of Parathyroid Hormone 1–84 and Teriparatide on Blood Pressure in Rats

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Whitfield et al. (Calcif Tissue Int 60: 302, 1997) showed that a high dose (8 nmol/kg) of parathyroid hormone (PTH) or teriparatide [PTH(1–34)] decreased blood pressure acutely in anesthetized rats when administered by intravenous (iv) or subcutaneous (sc) injection. PTH did not appear to produce as significant a hypotensive effect compared with teriparatide. The present study established the potencies and efficacies of these peptides in lowering blood pressure in rats. To eliminate differences that might result from different rates of peptide entry into the circulation from an sc injection site, or from the effects of anesthesia, we assessed the effects on mean arterial pressure (MAP) of bolus iv injection of PTH and teriparatide in conscious rats with chronic arterial and venous catheters. The peptides were injected at doses of 0.001 to 100 nmol/kg (n = 6–10/dose), and MAP was monitored during the next 20–60 min. MAP decreased rapidly following injection of PTH and teriparatide and usually reached a nadir at about 1 min, before returning towards predose levels at a rate that was dose-dependent. Doses >0.01 nmol/kg significantly decreased MAP; PTH was significantly less hypotensive than teriparatide at doses of 0.1–10 nmol/kg. The ED_{50} for decreasing MAP was 2.3 and 0.8 nmol/kg for PTH and teriparatide, respectively. C-terminal PTH fragments (7–84), (39–84) and (53–84) did not affect MAP at doses up to 10 nmol/kg; future studies will assess whether they influence the hypotensive effects when given in combination with PTH and teriparatide. In conclusion, this study showed that PTH is a markedly less potent hypotensive agent than teriparatide when administered by bolus iv injection in normal conscious rats. In clinical practice, dizziness and syncope are not infrequent adverse events associated with teriparatide. The extent to which these effects relate to the hypotensive actions of the drug observed in rats, and the relative differences with PTH, remain to be determined.

Table
Change (±SE) in MAP (mm Hg)

Dose (nmol/kg)	PTH	Teriparatide	P value
0.1	−4 ± 1	−8 ± 1	0.044
1	−11 ± 1	−22 ± 3	0.003
3	−22 ± 2	−32 ± 2	0.005
10	−25 ± 2	−38 ± 2	<0.001
30	−31 ± 2	−38 ± 3	0.089

P701-Mo

Down-Regulation by Fibroblast Growth Factor 23 of Human 25-hydroxyvitamin D3 1alpha-hydroxylase Promoter Activity

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Tumor-induced osteomalacia (TIO) is characterized by phosphaturia, hypophosphatemia and osteomalacia caused by the secretion of tumor-derived phosphatonins. Subjects with TIO show low serum levels of 1,25(OH)2D3 and inadequate parathyroid hormone levels. The clinical syndrome consists of muscle weakness and osteomalacia. Phosphatonins that have been described are secreted frizzled related protein 4 (sFRP4) and fibroblast growth factor 23 (FGF23). The biological activity of FGF23 is modulated through partial proteolysis, exerted by subtilisin-like proteases and possibly PHEX. Mutations of the FGF23

cleavage site (e.g. FGF23R176Q) result in a gain of function due to proteolysis resistance, the cause of autosomal dominant hypophosphatemic rickets (ADHR). There are controversial data about the responsibility of phosphatonins for decreased concentrations of 1,25(OH)2D3. The enzyme responsible for the conversion of 25(OH)D3 to 1,25(OH)2D3 is 25(OH)D3 1a-hydroxylase. We investigated the responsiveness of human 1a-hydroxylase promoter activity to FGF23 in 293 cells. A 1413 bp fragment of the promoter of human 1a-hydroxylase was amplified from a BAC clone, sequenced and full-length and 5′ deletion constructs were cloned into the luciferase reporter vector pGL3basic. FGF23 cDNA was amplified from a tumor and cloned into pcDNA3.1 expression vector. The gain of function mutant FGF23R176Q was cloned by site-directed mutagenesis. Reporter gene constructs were transfected into 293 cells, and luciferase activity was measured. FGF23 responsiveness was analyzed by cotransfection of FGF23 expression plasmid. FGF23 protein expression was verified by Western blot. Cotransfection of FGF23 and FGF23R176Q resulted in marked reduction of the wild type promoter activity as well as a series of 5′ deletion constructs. A −255 bp fragment still showed FGF23 responsiveness, which implicates a regulatory element in this section. The inhibitory effects of FGF23R176Q were more pronounced compared to the wild type form, indicating proteolytic metabolism of the wild type protein in 293 cells. Our findings are in favor of a negative regulation of the 1a-hydroxylase promoter in HEK293 cells by FGF23. This would be in line with clinical findings in TIO, where low 1,25(OH)2D3 levels are coincident with high serum levels of FGF23. The regulatory element which mediates FGF23 responsiveness located in the −255 bp construct is being characterized.

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P702-Tu

The Effect of Phosphate Ingestion on Serum Fibroblast Growth Factor and Indices of Phosphate Homeostasis

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We measured fibroblast growth factor 23 [FGF-23], phosphate and creatinine in serum and urinary phosphate and creatinine between 20:00 and 06:00 h in 6 subjects [age 45 ± 4 years, M4, F2]. The measurements were made on samples prior to and after ingestion of 750 mg of phosphate [Sandoz] at 22:00 h. Total urinary phosphate excretion [TuPo4], phosphate excretion index [PEI], percentage tubular reabsorbed phosphate [TRP] and the ratio of phosphate clearance to creatinine clearance [Pcl] were calculated in urine collected between 20:00–22:00 h and urine collected from 22:00–06:00 h. The percentage change of the phosphate indices was compared with those from 6 subjects matched for age and sex who did not receive additional phosphate. In the PO4-treated group, mean baseline serum FGF-23 was 109 RU/L [range 56–220] which increased on average 315% [range 71–518] to a maximum of 395 RU/L [104–1038] reached on average 2 h post ingestion; in 4/6 subjects [67%], FGF-23 returned to pre PO4 values after 3.5 h [means 84 v 79 RU/L], while the remaining 2 subjects, with higher baseline concentrations, took longer to return to baseline. All had reached baseline within 7 h from the ingestion of PO4. Serum PO4 increased by 31% from 1.11 mmol/L [1.03–1.22] to a maximum of 1.45 mmol/L [1.25–1.66] 3 h post PO4 ingestion. In the control group during this period of time, FGF-23 increased 25% from a mean of 85 RU/L [60–110] to 106 RU/L [72–130] and serum PO4 by 15% from 1.23 mmol/L to a maximum of 1.42 mmol/L. In the PO4-treated group, TRP decreased from a mean of 78.2% to 76.8% [2%], PEI from +0.10 to +0.06 [40%], whereas Pcl increased 4.5% from 0.22 to 0.23 and TuPO4 340% from 5.9 to 25.5 mmol. For the control group, during the same period, TRP increased from a mean of 78.9 to 85.6 [8.5%], PEI decreased 114% from +0.07 to −0.01 and Pcl from 0.21 to 0.14, a decrease of 33%, TuPO4 increased 82% from a mean of 4.1 mmol to 7.5 mmol. The ingestion of PO4 at 22:00 h induces a rapid increase in circulating FGF-23 that alters the overnight handling of PO4 by decreasing tubular reabsorption and increasing PO4 excretion and PO4 clearance compared with an age-and sex-matched control group.

P703-Su

Prevalence and Causes of Osteopenia in End-Stage Renal Disease Patients Assessed by Quantitative Ultrasonometry

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Various forms of renal osteodystrophy are associated with low bone mineral density (BMD) and increased fracture risk in dialysis population. The aims of our study were: (1) to assess the prevalence and distribution of low bone mass at hand fingers in hemodialysed pts by quantitative ultrasonometry (QUS), (2) to determine what factors contribute to osteopenia in such pts, (3) to demonstrate the validity of QUS to detect bone loss in CRF pts on maintenance hemodialysis (HD).

We studied 70-point-prevalent maintenance HD pts (38 M; 32F/18 postmenopausal) with mean age 45.2 ± 13.5 years and mean duration of HD 22.0 ± 21.9 months. 33 pts (47%) were treated with calcitriol, and 4 pts (5.7%) received HRT. Bone structural densitometric analysis was made at hand fingers (with predominantly cancellous bone) by ultrasound densitometer DBM Sonic 1200, Igea, Italy. Amplitude-dependent speed of ultrasound (Ad-SoS, m/s) and compo-

site ultrasound bone profile score (UBPS) were measured. Results were expressed as *t* score and *Z* score. Serum calcium, phosphate, osteocalcin and total alkaline phosphatase activity and PTH were also determined.

Results: Mean biochemical values of 70 pts were as follows: serum Ca2+ 2.39 ± 0.34 mmol/l, P3+ 1.98 ± 0.48 mmol/l, tAP 56.3 ± 32.9 IU and PTH 35.5 ± 22.2 pmol/l. The mean values of Ad-SoS (1980.1 ± 104.7 m/s). *Z* score (−1.41 ± 1.49 SD) and *t* score (−2.1 ± 1.50 SD) were slightly decreased, revealing moderate decrease of BMD-osteopenia. Ad-SoS and UBPS correlated negatively with the age of pts ($r = -0.44$; $P < 0.001$ and $r = -0.41$; $P < 0.001$ respectively). Age-matched speed of ultrasound (*Z* score) showed inverse correlation with duration of HD treatment ($r = -0.28$; $P < 0.02$) and serum PTH concentration ($r = -0.37$; $P < 0.01$). We also found positive correlation of HD treatment time with serum phosphorus concentration ($r = 0.46$; $P < 0.001$) and with serum PTH concentration ($r = 0.45$; $P < 0.001$).

Conclusions: (1) BMD at hand fingers was decreased (osteopenia or osteoporosis) in 74% of HD patients; (2) prolonged HD treatment was associated with relatively higher PTH values and marked hyperphosphatemia; (3) patients age, duration of HD and higher PTH concentration may contribute to trabecular bone loss in such patients; (4) we were able to show that in secondary hyperparathyroidism bone density was lower not only in cortical bone as described elsewhere, but also in predominantly trabecular bone of hand fingers, (5) phalangeal QUS seems to be a feasible method to investigate BMD in HD patients.

P704-Mo

Quantitative Ultrasound in Adults with End-Stage Renal Failure: A Longitudinal Study

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The aim of the longitudinal study was to assess skeletal status in 29 subjects (18 males and 11 females) with end-stage renal failure (ESRF) being on regular hemodialysis. Control group consisted of 494 healthy subjects (305 males and 189 females). In subjects studied (patients and controls), additional factors known to affect bone metabolism (chronic diseases or prolonged medications) were not present. Skeletal status was assessed by device DBM Sonic 1200 (IGEA, Carpi, Italy) which measures amplitude-dependent speed of sound, Ad-SoS, m/s at hand proximal phalanges II–V. All measurements were performed by the same operator, and CV% was 0.72% in males and 0.43% in females. Skeletal measurements were performed 3 times: at the baseline and 6 and 12 months later. RMS_CV% was 0.72% in males and 0.43% in females. The value of Ad-SoS, *t* score and *Z* score at the baseline were significantly lower than in controls ($P < 0.05$). The mean values of Ad-SoS decreased over a period of observation; in a whole group from 1979 ± 106 m/s to 1928 ± 105 m/s, $P < 0.0001$, in males from 2003 ± 93 m/s to 1949 ± 111 m/s, $P < 0.001$, and in females from 1940 ± 121 m/s to 1894 ± 108 m/s, $P < 0.05$. Ad-SoS *Z* scores dropped significantly over a period of the study in whole group (−1.14 ± 1.64 to −2.08 ± 2.26, $P < 0.01$), in males (−0.63 ± 1.44 to −1.74 ± 2.29, $P < 0.0001$) and in females non-significant decrease was observed. Using the Least Significant Change (LSC) values for skeletal measurement, a decrease in Ad-SoS was noted in 16 subjects (52%). The values of PTH were over a normal limit and did not change during a study. In the whole group, main factors negatively influencing current Ad-SoS values were duration of dialysis, age and PTH.

Concluding, the skeletal status in subjects with ESRF on hemodialysis was seriously affected, and longitudinal measurements showed its aggravation over a time of the study.

P705-Tu

Bone Loss in Kidney Recipients is due to Increased Bone Resorption

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Bone metabolism disturbances occur in the majority of kidney transplant recipients. These disturbances are due to bone metabolism disturbances that have occurred before transplantation and persist with some degree after and to other factors negatively influencing the posttransplant bone metabolism (i.e. immunosuppressive agents). Bone metabolism was evaluated using noninvasive parameters in 44 kidney transplant recipients (20 male, 24 female), aged 21–64 years, with good and stable renal function (creatinine clearance >50 mL/min.). Bone mineral density (BMD) was estimated in the lumbar spine, femoral neck and distal third of the radius using dual energy absorptiometry (DEXA) 0.25–181 months after transplantation. The control DEXA was performed 11–36 months after the first (if first DEXA was made ≤6 months posttransplant, $n = 16$, the control DEXA was made after 11–14 months). The BMD changes (delta BMD, ±%) were calculated per 12 monthss. The role of bone metabolism disturbances in BMD changes was investigated. The following serum parameters were estimated: iPTH, total alkaline phosphatase, crosslaps, Ca, Pi. IPTH sera values were above the upper reference value in 30%, Ca in 50%, alkaline phosphatase in 14% and crosslaps

in 64% of patients. PTH sera values correlated significantly positively with Ca and alkaline phosphatase and negatively with Pi. Alkaline phosphatase correlated significantly positively with crosslaps. Crosslaps values correlated significantly and negatively with delta BMD for the lumbar spine, femoral neck and distal radius. Bone resorption was increased in many kidney transplant recipients. Bone resorption may not have been increased solely due to PTH, other factors also seemed to play a role. Increased bone resorption was not accompanied by bone formation increase and resulted in bone loss in kidney transplant recipients.

P706-Su

Bone Mineral Density and Osteoprotegerin in Hemodialysis Patients

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Osteoprotegerin (OPG) is involved in renal osteodystrophy. We investigated the osteoprotegerin role in uremic bone disease, evaluating possible relationship between serum OPG, and intact parathyroid hormone (iPTH), osteocalcin, bone alkaline phosphatase, vitamin D3, calcium, phosphate, insulin-like growth factor (IGF-1) lumbar spine and hip bone mineral density assessed using dual X-ray absorptiometry and quantitative ultrasound on the calcaneus and on the proximal phalanges of the hand.

36 hemodialysis patients (mean age ± SEM: 69 ± 4 years) on maintenance acetate-free biofiltration and 36 age-, sex- and BMI-matched healthy subjects with no metabolic bone disease were recruited.

Serum calcium, phosphate, bone alkaline phosphatase, osteocalcin, IGF-1, iPTH and OPG were significantly higher in hemodialysis patients compared with controls. The hip bone mineral density and ultrasound parameters of calcaneum and phalanxes were significantly lower in hemodialysis than controls.

Grouping patients according to their iPTH levels, below or above 100 pg/ml, we found significantly higher bone alkaline phosphatase and osteocalcin levels in the group with elevated parathyroid hormone.

Grouping the patients in two groups according to their mean OPG value (14 pmol/l), we observed a significant decrease in lumbar spine, trochanteric and Ward's triangle bone mineral density in patients with high OPG value with respect to the group with low OPG. The reduced bone mineral density in the high OPG group might be due to a significant decrease in serum IGF-1 (110 ± 15 vs. 191 ± 19 ng/ml, $P < 0.005$) and vitamin D3 (33 ± 3.9 vs. 52 ± 3.8 ng/ml, $P < 0.001$) observed in this group with respect to the group with low OPG.

A negative correlation has been shown between OPG and IGF-1 ($r = -0.64$, $P = 0.032$). PTH positively correlated with bone alkaline phosphatase ($r = 0.69$, $P = 0.038$) and osteocalcin ($r = 0.92$, $P < 0.001$). The group with OPG < 14 pmol/l and iPTH < 100 pg/ml showed lower ultrasound bone profiler index with respect to OPG < 14 pmol/l and iPTH >100 pg/ml group (0.16 ± 0.032 vs. 0.27 ± 0.083, $P < 0.05$).

We conclude that OPG increase might, at least in part, represent a compensatory response to elevated bone loss and therefore might be helpful to identify patients with major reduction in trabecular bone density, moreover, OPG in combination with PTH might be useful as a tool for non-invasive diagnosis of renal osteodystrophy at least in the range of parathyroid hormone values where a clinical diagnosis is in doubt.

P707-Mo

Dexamethasone Stimulates Odontoblast-like Cell Differentiation in Human Dental Pulp Cultures

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Regenerative dental pulp strategies require the identification of precursors able to differentiate into odontoblast-like cells that secrete reparative dentin after injury. Pericytes have the ability to give rise to osteoblasts, chondrocytes and adipocytes, and it is currently suggested that odontoblast-like cells could derive from these perivascular cells. In order to gain new insights into this hypothesis, we investigated the effects of dexamethasone (Dex), a synthetic glucocorticoid employed to induce osteogenic or odontoblastic differentiation in vitro in a previously reported model of human dental pulp cultures. This model contains pericytes as identified by their expression of alpha-smooth muscle actin (SMA) and their specific ultrastructural morphology. [3H] thymidine incorporation, cell counting and analysis by flow cytometry indicated that Dex (10−8M) significantly inhibited cell proliferation and markedly reduced the proportion of SMA+ cells. Conversely, Dex strongly stimulated alkaline phosphatase (ALP) activity and induced expression of the transcript encoding the major odontoblastic marker dentin sialophosphoprotein (DSP-PP) as observed by RT-PCR. In contrast, expression of markers of osteoblastic/odontoblastic differentiation such as PTH/PTHrp receptor, osteonectin and Cbfa1/osf2 as well as expression of lipoprotein lipase, an adipocyte-specific marker investigated by RT-PCR, were not modified by Dex treatment. Finally, analysis by flow cytometry evidenced that Dex increased the proportion of cells expressing STRO-1, a marker of multipotential mesenchymal cells. In conclusion, these observations indicate that dexamethasone regulates the commitment of dental pulp cell-derived progenitors into odontoblast-like

cells while reducing the proportion of SMA positive cells. These results provide new perspectives in deciphering the cellular and molecular mechanisms leading to reparative dentinogenesis.
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P708-Tu

Effect of Enamel Matrix Derivative on Osteoblastic Differentiation of Rat Calvaria Cells in Culture

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Objective: Enamel matrix derivative (EMD) is known to stimulate periodontal tissue regeneration. The aim of this study was to evaluate the influence of EMD on osteoblastic differentiation of fetal rat calvaria cells (RC cells) in culture.
Materials and methods: RC cells were cultured in alpha-MEM containing 10% FBS, antibiotics, 50 É g/mÜP ascorbic acids and 2 mM beta-glycerophosphate. After RC cells were treated with EMD, alkaline phosphatase (ALP) activity and bone-nodule (BN) formation were measured. Northern blot analysis was also performed to determine expression of bone-matrix proteins such as osteopontin and osteocalcin. In addition, the participation of TGF-beta1 with EMD's effect was evaluated.
Results: Both ALP activity and BN formation were inhibited by EMD in a dose-dependent manner. Northern blot analysis revealed that treatment with EMD on RC cells increased mRNA expression of osteopontin and decreased that of osteocalcin. On the other hand, activated form of TGF-beta1 protein was found in the conditioned medium, and its origin was confirmed from the treated EMD contents. Addition of anti-TGF-beta1 antibody partially restored the inhibitory effect of EMD on ALP activity.
Conclusion: The present study shows that EMD inhibited osteoblastic differentiation of RC cells through a partial mediation of activated TGF-beta1.

P709-Su

Serum 25-hydroxyvitamin D Level in type 2 Diabetes Mellitus: Association with Microvascular Complications and type of Treatment

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Serum 25-hydroxyvitamin D (25-OHD) concentration is postulated to reflect most accurately vitamin D stores. Vitamin D deficiency causes secondary hyperparathyroidism, which can lead to osteomalacia, irreversible bone loss and increased risk of fracture. The existence of diabetes mellitus is now considered to be a risk factor to induce bone loss and osteoporotic fracture. Moreover, it has also been suggested that hypovitaminosis D may be a significant risk factor for glucose intolerance.
Methods: We conducted the observational study in 581 Japanese with type 2 diabetes and 51 normal subjects and analyzed the relationship between serum 25-hydroxyvitamin D (25-OHD) concentration and the clinical features associated with type 2 diabetes. We examined the incidence of osteoporotic bone deformity and fracture during 2 years after the measurement of serum 25-OHD.
Results: Mean serum 25-OHD concentration in type 2 diabetes patients was 17.0 ± 7.1 ng/ml (Mean ± SD) in winter, not statistically different from normal population (17.5 ± 3.6 ng/ml). The prevalence of hypovitaminosis D (<20 ng/ml) was 70.6%. Serum concentrations of 25-OHD were associated with HbA1c (P = 0.013) and age (P = 0.070) but were not related to body mass index or the duration of diabetes. The levels of 25-OHD were significantly lower in the population with apparent microvascular complications, although serum creatinine levels were below 2.0 mg/dl. Serum 25-OHD levels in the group treated with insulin were the lowest. Simple regression analysis revealed that serum 25-OHD concentration was associated with the future osteoporotic bone deformity or fracture in the patient aged over 50 years (n = 447, P = 0.04). In addition, the highest incidence of the osteoporotic fracture in the patients treated with insulin was observed.
Conclusions: Multiple microvascular complications and insulin treatment in type 2 diabetes patients are the risk factors for hypovitaminosis D and osteoporotic fractures.

P710-Mo

The Levels of 25 Hydroxy Vitamin D and PTH in 834 Women with Osteoporosis Attending an Osteoporosis Center

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Vitamin D is needed to maintain calcium absorption and skeletal integrity as much in older as in younger people. It is required for efficient absorption of dietary calcium and for normal mineralization of bone. Reduction in vitamin D levels is associated with a compensatory increase in the level of parathyroid hormone which, in turn, stimulates bone resorption and bone loss.

The objective is to determine the levels of 25 hydroxy vitamin D (25OH vit. D) and PTH levels in osteoporotic women attending in our osteoporosis center and to establish the threshold level of 25OH vit. D which determine secondary hyperparathyroidism.
Subjects were 834 osteoporotic women, with a range age of 56.1 ± 12 years, excluding other metabolic bone diseases and secondary osteoporosis.
We determined 25 OH vit. D levels in early postmenopausal women and late postmenopausal women, the prevalence of vitamin D deficiency in age decades and seasonal variation of vitamin D. We studied also the correlation between PTH and vitamin D to establish the threshold level of 25 OH vit. D which determines secondary hyperparathyroidism. 25 OH D and PTH intact molecule were measured with ELISA kits. The vitamin D deficiency was define as the levels under 12 ng/ml, and the normal range was 26, 58 ± 10 ng/ml. Data were compared using Student's paired *t* test. Data are expressed as mean ± standard deviation, and a $P < 0.05$ was considered statistically significant.
32.2% of the total number of osteoporotic women had deficient 25 OH vit. D levels, and 42.3% had 25OH vit. D levels under normal mean value. Comparable results were obtained also in age decades, with an increased especially in the 7th and 8th decade of age. We obtained a significant correlation ($P < 0.05$) between seasonal variation of vitamin D levels. There was a significant negative correlation between PTH and 25OH vit. D levels ($P < 0.001$) in the total group of study, and we observe that PTH level increased when the level of 25OH vit. D decreased under 20 ng/ml.
Our data confirm that PTH levels increases significantly with age and is negatively correlated with vitamin D levels. There was a high prevalence of vitamin D deficiency in our osteoporotic patients and also significant seasonal variation in the level of vitamin D.

P711-Tu

Global Prevalence of Vitamin D Inadequacy among Community Dwelling Women with Osteoporosis

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Objective: To describe the distribution of serum 25-hydroxyvitamin D [25(OH)D] levels among post-menopausal women with osteoporosis in various regions.

Materials and methods: Cross-sectional study of 1244 community-dwelling osteoporotic women from 18 countries, recruited from May through September, 2004. Serum 25(OH)D and PTH were measured; information about general health, medication, supplement use, sun exposure, skin tone and diet was collected. Descriptive statistics were used to estimate mean serum 25(OH)D and PTH levels by region and the frequency of vitamin D inadequacy at different cutpoints.
Results: Mean age (SD) was 67.7 (8) years, range 42 to 92, with 36% >70 years. 52% reported taking a vitamin D supplement; 63% were receiving pharmacologic therapy for the treatment of osteoporosis. Overall mean 25(OH)D was 28.0 ng/ml (SD = 12.4, range 7–120, median = 26); mean serum PTH was 29.0 pg/ml (SD = 15.9). Approximately 59% of women had 25(OH)D <30 ng/ml. PTH values began to rise at 25(OH)D levels <30 ng/ml, supporting 30 ng/ml as the appropriate cutpoint to define vitamin D inadequacy. Distribution of vitamin D inadequacy varied by region (see Table).
Conclusion: Vitamin D inadequacy is widespread among postmenopausal women with osteoporosis, even in countries with ample sunlight. In this study, conducted in 18 countries from Europe, Middle East, Asia-Pacific and Latin America, 59% of postmenopausal women with osteoporosis had vitamin D inadequacy. These results underscore the importance of increasing awareness of the need for adequate vitamin D supplementation in women with osteoporosis.

Table
Distribution of Vitamin D Inadequacy by region

Region (N)	<30 ng/ml (%)	Mean 25(OH)D (range in region)
Europe (502)	52	30.6 (27.4–35.1)
Middle East(165)	82	19.9 (19.5–21.3)
Asia (296)	57	27.6 (20.4–32.3)
Latin America(182)	53	30.4 (27.1–36.7)
Pacific Rim (99)	59	27.9 (Australia)

P712-Su

A Precise and Sensitive Method for the Determination of 25-hydroxyvitamin D in Human Plasma using High-performance Liquid Chromatography-tandem Mass Spectrometry

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Vitamin D is an important regulating factor of calcium metabolism. Vitamin D deficiency or insufficiency is a risk factor for bone metabolic diseases such as rickets, osteomalacia and osteoporosis. Recently, it has become evident from epidemiological studies that serum 25-OH-D levels correlate negatively with serum PTH levels, thus much effort has been focused on defining a reference value for the serum concentration of 25-OH-D needed for the diagnosis of vitamin D insufficiency/deficiency. For this purpose, we developed a precise and sensitive method to determine 25-

hydroxyvitamin D (25-OH-D_2/-D_3) in human plasma using HPLC-tandem mass-mass spectrometry with atmospheric-pressure chemical ionization (LC-APCI-MS/MS). The method involves the use of deuterated 25-OH-D_3 as an internal standard compound for 25-OH-D_2/D_3, which was synthesized in our laboratory, and the selection of a precursor and product ion with a MS/MS multiple reaction monitoring (MRM) method. The method for sample preparation was as follows. After addition of [$^2H_6$]-25-OH-D_3 as an internal standard into the 0.1 mL of serum or plasma sample, 0.2 mL of methanol was added for protein removal. The mixture was shaken and centrifuged at 3000 rpm for 15 min. The supernatant was applied to a Bond Elute C_{18} silica column (Waters, USA) and washed with 15 mL of methanol:water (30:70, v/v). 25-OH-D_2, 25-OH-D_3 and 24,25(OH) $_2D_3$ were eluted into the same fraction with 6 mL of methanol:acetonitlile (20:80, v/v). The elute was evaporated, and the resulting residue was dissolved with 100 μL of methanol. The 50 μL aliquots were applied to the LC-APCI/MS/MS system. The average intra-assay and inter-assay validation values (RSD) for 25-OH-D_3 and 25-OH-D_2 were 5.7% and 4.5%, respectively and 2.5% and 5.1%, respectively. The average spiked recoveries from authentic compounds added to normal human plasma samples for 25-OH-D_3 and 25-OH-D_2 were 103.8% and 98.8%, respectively. Mean plasma concentrations of 25-OH-D_3 and 25-OH-D_2 in healthy subjects were 20.5 ng/mL and 0.4 ng/mL, respectively. We conclude that this novel LC-APCI-MS/MS method would be useful for the evaluation of vitamin D status in postmenopausal women and elderly subjects and provides useful information on the diagnosis, treatment and prevention of osteoporosis with vitamin D.

P713-Mo

Vitamin D Status, Parathyroid Function and Bone Mineral Density in Japanese Adolescent Males and Females

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Aims: Data required for setting the reference values of serum concentrations of 25-hydroxyvitamin D (25-D) and parathyroid hormone (PTH) and bone mineral density (BMD) for healthy adolescent males and females are limited in Japan as well as other nations. Since a nutrient, vitamin D and an endocrine hormone, PTH are equally essential for bone mass accrual during adolescence, their serum reference values are important for the diagnosis of vitamin D status and prevention of low BMD. The aim of this study is to measure serum levels of 25-D and PTH and BMD in healthy adolescents and to examine the association of vitamin D status and PTH function with BMD in adolescents.
Methods: A total of 632 adolescents (Group A: 99 males and 95 females aged 12–13 years, Group B: 104 males and 146 females aged 15–16 yrs, Group C: 84 males and 104 females aged 17–18 years) from urban Tokyo had enrolled in this study, and they were defined as normal based on health history and questionnaire. Plasma concentrations of 25-D and intact PTH were measured. Calcaneal stiffness was measured by a Lunar Achilles A-1000 ultrasonometer. Subjects were also measured for height, weight and BMI and completed a questionnaire on exercise history, diet and lifestyle factors. Statistical analyses were performed using JMP 5.0.1 J.
Results: Mean plasma 25-D levels in Groups A, B and C were 49.9, 46.3 and 43.5 nmol/L, respectively, and a significant age-dependent decrease ($P < 0.0001$) was observed. Serum 25-D levels of males were consistently higher than those of females. Mean serum PTH levels in Groups A, B and C were 45.3, 37.2 and 32.2 pg/mL, respectively, and a significant age-dependent decrease ($P < 0.0001$) was observed. Unlike 25-D, no gender difference in serum PTH levels was observed in each age group. Plasma 25-D levels correlated negatively with PTH levels in Groups B and C, but not in Group A. *Z* score of calcaneal stiffness correlated positively with plasma 25-D levels in Groups B ($P = 0.0131$) and C ($P = 0.0038$), but not in Group A. Calcaneal stiffness correlated positively with plasma 25-D levels in adolescent females ($P = 0.0055$) and tended to correlate with plasma 25-D levels in adolescent males ($P = 0.0536$).
Conclusion: These results suggest that plasma concentrations of 25-D and PTH are strictly controlled by the bone-related and age-related unknown mechanisms during adolescence, and vitamin D status would be more important for females to optimize bone formation.

P714-Tu

A New, Commercial, Non-isotopic Method for the Measurement of 1,25-dihydroxy Vitamin D in Human Serum and Plasma

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The hormone 1,25-dihydroxyvitamin D (1,25D) is produced by hydroxylation of 25-hydroxyvitamin D (25D) in the kidney. It is one of the major regulators of calcium metabolism and acts on a variety of target tissues including the intestine and parathyroid gland.
Using the simple IDS immunoextraction method currently used in the IDS Gamma-B 1,25-Dihydroxy Vitamin D radio immunoassay (RIA), we have developed an enzyme immunoassay (EIA) for the measurement of 1,25 vitamin D in human serum and plasma.
Delipidated samples are incubated with a highly specific solid phase bound anti 1,25D monoclonal antibody. The solid phase is washed and the purified 1,25D eluted and dried. The dried elute is reconstituted with sheep anti-1,25D

polyclonal antibody. After an incubation step, the solution is added to a microtiter plate coated with an anti-sheep antibody. Following a further incubation, biotin labeled 1,25D is added, and incubation continues. The microtiter plate is then washed, and the amount of bound biotinylated 1,25D is determined by an Avidin labeled HRP/TMB colorimetric detection system. The endpoint color developed for samples is inversely proportional to the amount of 1,25D present.

The following performance for the IDS OCTEIA 1,25-Dihydroxy Vitamin D EIA was recorded. The assay has a range of 0 to 500 pM. Sensitivity was 6.3 pM. Correlation performed using linear regression with the IDS Gamma-B 1,25-Dihydroxy Vitamin D RIA was EIA = 0.996 RIA – 0.65 pM with a correlation coefficient (r value) of 0.960 (n = 167). Intra-assay precision on 20 replicates of 3 samples was 17.8% (17.6pM), 10.8% (47.4pM) and 9.0% (140.6pM). Inter-assay precision (n = 25) from the same samples was 14.0% (18.1pM), 15.6% (50.8pM) and 13.8% (133.6pM). The mean linearity of 5 samples diluted in assay buffer was 92%. Mean recovery again based on 5 samples was 94%.

The IDS OCTEIA 1,25-Dihydroxy Vitamin D EIA demonstrates excellent correlation with the existing IDS Gamma-B 1,25-Dihydroxy Vitamin D RIA, and being non-isotopic has numerous advantages over existing isotopic methods.

P715-Su

A Simple Method for Measurement of Vitamin D Binding Protein Capacity (DBPC) in Serum

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Vitamin D binding protein or Gc Globulin (DBP) is the major carrier of 25 hydroxyvitamin D (25D) and 1,25 dihydroxyvitamin D (1,25D) in blood. Methods that measure DBP in terms of mass do not necessarily indicate the functionality of this protein for carrying vitamin D compounds. We have developed reagents that enable the functional binding capacity of DBP to be measured in serum.

Saturation of DBP was achieved by adding excess 25D (500 μL of 1200 nM) to 20 μL sample at 37°C for 30min. After 10 min chilling on crushed ice, cold charcoal reagent (500 μL) was added to the mixture and incubated for 1 h on the ice to remove unbound or low affinity albumin bound 25D. Following centrifugation of the charcoal, high affinity DBP bound 25D remains in the supernatant and was measured using the IDS Gamma B 25hydroxyvitamin D RIA kit.

The assay has a range of 1–10 μM DBPc. Sensitivity measured by 20 replicates of BSA Buffer plus 2SD was 0.87 μM. Intra-assay precision on 20 replicates of 3 samples was 6.9% (1.5 μM), 8.2% (5.2 μM) and 6.3% (4.9 μM). Inter-assay precision (n = 32) from the same samples was 14.3% (1.5 μM), 10.2% (4.7 μM) and 7.8% (4.5 μM). The mean linearity of 5 samples diluted in BSA Buffer was 106% (1/2 dilution 108%, 1/4 dilution 105%). The mean value of 28 normal serum samples was 5.4 μM (range 2.4–8.6 μM).

The overall assay is completed in less than 5 h and can be used to obtain 25D and DBPc levels from the same assay. DBPc measures could also be used with a total 1,25D measure to calculate the relative amounts of 'free' 1,25D.

P716-Mo

Hypervitaminosis D as a Cause of Calcinosis Cutis

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Objective: To present two cases of calcinosis cutis with hypercalcemia.

Methods: Two cases of calcinosis cutis with hypercalcemia are presented, and a mechanism relating them is postulated.

Results: The first case is a 78-year-old female who presented with severe hypercalcemia and hyperphosphatemia. She was found to have widespread subcutaneous calcification sparing the internal organs, which was confirmed on biopsy to be calcinosis cutis. This was thought to be due to liquid silicone injections done previously. PTH and 25-OH-VitD levels were normal. The second case is a 19-year-old female with B precursor acute lymphoblastic leukemia who presented with extensive waxy, verrucous, tender plaques over the flexures of her arms and legs. Skin biopsy was consistent with calcinosis cutis, and there was no evidence of metastatic calcification in other organs. She was found to have severe hypercalcemia and hyperphosphatemia, with a normal parathyroid hormone (PTH) but low 25-hydroxy-vitamin D (25-OH-VitD) level.

Conclusion: Hypercalcemia and hyperphosphatemia in both cases are likely associated with hypervitaminosis D. This is due to dysregulation of the vitamin D 1-alpha-hydroxylase enzyme in granuloma macrophages and lymphoma cells respectively. The distribution of calcium deposits suggests that local diffusion of active vitamin D from stratum basale keratinocytes combined with elevated systemic levels may trigger calcium influx into dermal cells. High intracellular calcium levels cause precipitation of calcium deposits which then aggregate.

P717-Tu

Vitamin D Inadequacy: Global Prevalence and Skeletal Implications

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Objective: Osteoporosis is a chronic, progressive disease characterized by reduced bone mass and microarchitectural

deterioration of bone. Vitamin D is an essential component of osteoporosis management strategies. This study examines the role of vitamin D in osteoporosis and reviews current knowledge about the prevalence of vitamin D inadequacy and recommendations for supplementation.

Methods: A non-systematic review of recent publications that reported the prevalence and consequences of low serum 25-hydroxyvitamin D [25(OH)D].

Results: Inadequate serum vitamin D is an important risk factor for osteoporosis and fractures. It is associated with impaired calcium absorption and increased parathyroid hormone levels, leading to increased bone resorption and bone loss. Recent data also suggest that vitamin D inadequacy is associated with age-related muscle weakness, musculoskeletal pain and increased body sway, risk of falls and falls-related fractures. Nevertheless, many people have inadequate vitamin D levels, especially postmenopausal women with osteoporosis. Several studies demonstrate that more than 50% of patients with osteoporosis have inadequate vitamin D, irrespective of latitude. For example, in a study of community-dwelling postmenopausal osteoporotic women living in southern California, a highly sunny climate, 53% had serum vitamin D <30 ng/mL. In another study, 97% of patients with a history of fractures or falls had serum 25(OH)D <30 ng/mL. The positive effects of supplementation – the most effective means of correcting low vitamin D – include increased bone mineral density, decreased bone turnover and reduced fracture risk. Moreover, vitamin D may increase muscle strength and reduce the risk of falls. However, patients do not consistently take daily vitamin D supplements. For example, patients with hip fractures demonstrated poor adherence to vitamin D and calcium, despite having received detailed information about the importance of supplementation.

Conclusions: The prevalence of vitamin D inadequacy is high in many populations that are geographically and culturally diverse and tends to be especially high in postmenopausal women with osteoporosis. Although supplementation is an effective means of improving vitamin D status, adherence to supplementation recommendations is low. Greater awareness of the importance of vitamin D for skeletal health and more aggressive supplementation are needed, especially in populations at high risk for inadequacy.

P718-Su

1,25(OH)2D3 Suppression of Sp1/NF-Y Transactivation of the Human PTH Promoter Requires an Intact AF-2 Domain

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We previously identified a highly conserved Sp1 DNA element in mammalian PTH promoters that acted as an enhancer of gene transcription and bound Sp1 and Sp3 proteins present in parathyroid gland nuclear extracts. More recently, an NF-Y element (NF-Yprox) was also described by our group, which was located ca. 30 bp downstream from the Sp1 site in the human PTH (hPTH) promoter and acted as a weak enhancer of gene transcription. We now report that Sp-proteins, either Sp1 or Sp3, binding to the Sp1 element together with NF–Y interactions at the NF–Yprox element can synergistically enhance transcription of a minimal hPTH promoter construct far greater than either factor alone. Positioning of the Sp1 DNA element appears to be critical for this synergism as deviations of one-half of a helical turn caused a ca. 60% decrease in transactivation. The NF–Yprox element also overlaps with the repressor DNA binding site for the vitamin D receptor (VDR) heterodimer complex and the two factors compete for DNA-binding in vitro. Co-expression of the VDR heterodimer complex results in strong 1,25-dihydroxyvitamin D3 (1,25(OH)2D3)-dependent suppression (>80%) of Sp1/NF-Y synergistic transactivation of the hPTH promoter. Other vitamin D analogs can also suppress transcription, although none exceeded the potential of the natural hormone. Finally, suppression of the hPTH promoter construct by 1,25(OH)2D3 is lost or impaired when transient expression of the wild-type VDR in the heterodimer complex is replaced by VDR AF-2 domain mutants L417S or E420Q. In summary, synergistic transactivation of the hPTH promoter was observed with the combined expression of Sp1 and NF-Y transcription factors. Sp1/NF-Y transactivation of the hPTH promoter can be suppressed by 1,25(OH)2D3 and other analogs but requires ligand interactions with an intact VDR AF-2 domain. Data suggest that this model system will be useful to study hormone-dependent gene repression and screen the repressive potential of vitamin D analogs.

P719-Mo

Fulvestrant, an Estrogen Receptor Antagonist, Blocks Proliferation and Differentiation of Cultured Primary Human Osteoblasts

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Osteoblasts have been cultured from cancellous bone biopsies of patients undergoing orthopedic surgery after bone fractures of the lower limb in elderly patients ($n = 21$; age: 74–97, media $n = 87$) and younger patients ($n = 13$; age: 15–68, media $n = 28$). Primary osteoblast cultures were successfully established in 24% (5/16) of elderly and 92% (12/13) of young patients. The cells were tested for osteoblast markers using light and immunofluorescence microscopy and real-time reverse transcription polymerase chain reaction (rtRT-PCR). All cultures formed bone-like nodules of calcified extracellular matrix. The cells stained positive for

vimentin (all cells positive) and STRO-1 (stromal stem cell marker; 1–5% positive cells) and expressed mRNA for Cbfa1, BMP-2, BMP-4, BMP-6, BMP-7, AP, collagen type 1, RANK-L, estrogen receptor alpha and beta.
The cells were cultured in the presence or absence of fulvestrant (1nM, M) for up to 1 week before tested for changes to the cells 100 nM, 10 using light and electron microscopy, rtRT-PCR and proliferation assays. Fulvestrant decreased proliferation of the cells in concentration-dependent way and induced differentiation of cells towards lipocytes. These results allow the following conclusion:

1. The efficiency of osteoblast culture from cancellous bone derived from young individuals is much better than from elderly individuals.
2. However, there is no difference between the cultured cells of either group.
3. Blocking of estrogen receptors impairs proliferation of osteoblasts at an early stage of differentiation.
4. Blocking of estrogen receptors induces differentiation of lipocytes, indicating that deficiency diverts differentiation.

P720-Tu

The Protective Effect of Body Mass Index on Osteoporosis

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Objective: In a large number of samples, we assessed the possible effect of body mass index (BMI) on the development of osteoporosis.
Methods: We studied 2878 patients that were admitted to Istanbul Medical Faculty, Department of Physical Medicine and Rehabilitation Osteoporosis Unit between 1994 and 2004.
Bone mineral density (BMD) was measured in the femoral neck and spine by using dual-energy X-ray absorptiometry (DEXA). BMD scores at he femoral neck, lomber spine and total BMD scores are noted. Body weight and height were recorded simultaneously, patients were weighted with underwear, and height was recorded without shoes. BMI was calculated as weight (kg) divided by height (m^2).
Results: There were 2878 patients (144 men and 2734 women) in the study. The patient who had secondary osteoporosis was not included in this study. The mean age of the patient was 63.40 ± 10.55, and the mean BMI was 27.41 ± 4.59. The mean calcium was 9.47 ± 0.48 mg phospfor 3.69 ± 0. 54 mg alkaline phosphatase 157.33 ± 74 mg. BMI was well correlated with total BMD. Especially the patients who had high BMI have higher BMD. The patients who had better neck BMD were the ones that had the highest BMI.
Conclusion: This study confirms the protective effects of a high BMI on femoral and lomber bone mineral density among subjects.

Table 1
Correlations between BMD and BMI

	Values	BMI	L3 BMD	L1.4B MD	Neck BM	Total BMD
BMI	r	(–)	0.103	0.118	0.182	0.309
	p	–	(0.000)	(0.000)	(0.000)	(0.000)
L3 BMD	r	0.103	(–)	0.905	0.380	0.439
	p	(0.000)	–	(0.000)	(0.000)	(0.000)
L1.4 BMD	r	0.118	0.905	(–)	0.413	0.460
	p	(0.000)	(0.000)	–	(0.000)	(0.000)
Neck BM	r	0.182	0.380	0.413	(–)	0.664
	p	(0.000)	(0.000)	(0.000)	–	(0.000)
Total BMD	r	0.309	0.439	0.460	0.664	(–)
	p	(0.000)	(0.000)	(0.000)	(0.000)	–

** Correlation is significant at the 0.01 level.
BMD: Body Mineral Density; BMI: Body Mass Index.

P721-Su

The Osteogenic Potential of Sulfated Glycosaminoglycans

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Background: During osteogenesis, osteoblast progenitors are recruited and progressively differentiate into osteoblasts that produce a mineralized extracellular matrix (ECM). Although most of the organic components of ECM are comprised of collagen, growing evidence suggests that the most bioactive element of the developing ECM is its heparan sulfate (HS) glycosaminoglycan complement. These linear, unbranched sugars contain protein-binding domains that regulate the flow of adhesive, mitogenic and differentiative influences that coordinate osteoblast development. Among the HS-binding factors known to be important to this process are FGF-2 and BMP-2. The aim of this study was to compare the osteogenic effects of HS with heparin, rhFGF-2 and rhBMP-2 and heparin together with rhFGF-2 and rhBMP-2.
Method: Varying doses of HS, heparin, rhFGF-2 and rhBMP-2 were applied to rat calvarial osteoblast progenitor cells (RC) and the effects on cell cycle, proliferation and differentiation analyzed. Cell cycle was determined by propidium iodide incorporation, proliferation by cell number and differentiation by a combination of alkaline phosphatase, alizarin red, sirius red and von-Kossa staining and real-time PCR for the following mRNA transcripts: cbfa-1, alkaline phosphatase, osteopontin and osteocalcin.
Results: We show that HS and heparin have significant effects on osteoblast growth and development, albeit at varying levels. Cultures exposed to high dose HS showed significant inhibition of both proliferation and differentiation, while low dose HS had no effect. In contrast, cultures were not responsive to varying doses of heparin, however, when combined with FGF-2 or BMP-2, varying effects were observed. Notably, heparin/FGF-2 significantly increased

proliferation compared to FGF-2 alone. Furthermore, FGF-2 and heparin/FGF-2 were shown to reduce the expression of alkaline phosphatase and collagen and completely block mineralization. When combined with BMP-2, heparin increased the differentiative effects of BMP-2.
Conclusion: Sulfated glycosaminoglycans are important regulators of osteogenesis whose effects are determined by growth factor interactions.

P722-Mo
Intra Articular Mal Union of Distal Radius Treated by Chondro Costal Graft
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Introduction: 3 cases of intra articular mal union of distal radius treated by chondro costal graft are reported with a minimum follow up of 2 years.
Materials and methods: One 22-year-old patient with a dislocated radiocarpal joint 6 months after an injury and two patients (48 and 53 years old) respectively 1,5 and 2 years after a intraarticular fracture of distal radius reported two mains complaints: pain and stiffness. Location of the loss of cartilage was central in two cases and palmar in the last case. A dorsal approach in two cases, a palmar approach in the last case allowed reduction and reconstruction of the destroyed radial part of joint. A chondro costal graft harvested on the eight's rib was inserted and fixed by plate in place of the articular impaction. Plaster cast of 3 months in the first case and 1 month in the two last cases followed the articular reconstruction.
Results: Aucune complication n'a été observée. Union was achieved in all 3 cases. Integration of the graft was evaluated with RMI. At the highest follow up, functional result is excellent in the first case. Motion and grasp are similar than the contralateral side. For the two other patients, motion in flexion–extension reached respectively 74% and 69% of controlateral side, and grasp reached respectively 62% and 73% of controlateral side.
Conclusion: Reconstruction of a partially destroyed articular surface by a costal graft is reliable and allows filling and resurfacing an articular cartilage void. If chondro costal graft is currently used in maxillo facial surgery, it is the first report in articular distal radius non union.

P723-Tu
Distal Radius Fracture and Injectable Cement: Useful or Not?
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Aim: The purpose of the study was to evaluate the feasibility of Norian SRS bone cement injected into a distal radius following reduction and stable fixation in preventing shortening and loss of pronation–supination.
Materials and methods: Between 1998 and 2000, 48 patients with a mean age of 65 (54–82) sustained distal radius fracture (AO classification stage A in 26 cases, B in 15 cases, C in 7 cases) with metaphyseal comminution. Functional and radiological outcomes of the wrist (O' Brien scorring, Gartland and Werley scoring, DASH) were evaluated with a mean follow up of 46 months (36–56) by a surgeon not involved in treatment. Fixation was performed in 34 cases by pins, in 14 cases by dorsal plate, in 2 cases by external fixator.
Results: 4 patients lost of follow up and 5 mal union were excluded of final evaluation. 3 RSD were pointed on the 39 evaluated patients. O'Brien scoring reached 84/100 (54–100), Gartland and Werley scoring reached 4.6 (0–11) with 89% excellent and good results, DASH reached 23.6 (5.8–62.7). Ulnar variance changed less than 2 mm between postoperative time and maximal follow up in 88%. There were no clinically adverse effects, but one case of volar extrusion of injected Norian was pointed with resolutive evolution. Bone substitute was always in place at the longest follow up.
Discussion: Adams, Pogue, Mc Queen pointed the biomechanical and clinical advantage to fill the void secondary to the comminution to avoid the shortening of the radius. First cases reported by Kopylov and Jupiter and prospective series of Kopylov, Sanchez Sotello and Cassidy proved the interest of an adaptative injectable cement in case of comminution. Injectable bone substitute allows to maintain the ulnar variance in competition with bone graft or bio ceramic.
Conclusion: Norian is able to fill a metaphyseal void, but fixation of the fracture remains necessary.

P724-Su
Septic non union of tibia solved by free flap and induced membrane
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One case of septic non union of tibia secondary to open fracture is reported in a 43-year-old male. Treatment consisted in resection of septic diaphyseal bone (11cm) and inflammatory tissue, stable fixation by external fixator and free latissimus dorsi flap. The loss of bone was solved by Masquelet technique which consists in inducing a foreign body membrane with a cement spacer. This spacer allows to maintain to remove all the bone it needs, to maintain the length (with the external fixator) and to induce the membrane. In the original technique described by Masquelet, the spacer is removed at 2 months and the fill is voided by autograft. As reported by Pelissier, the induced membrane secretes some growth factor (TGF, BMP) and

avoids the destruction of the autograft by surrounded muscle.
In the reported case, empty iliac crest did not allow to obtain autograft, and after removing cement, the fill was voided with granular of interconnected biphasic ceramic. Immediate weight bearing occurred with the external fixator maintained 2 years without complications. The X-ray assessment allowed to demonstrate that months after months bone cortex appeared secondary of conjunction of stimulating bone cell by weight bearing under external fixation and growth factors secreted by the induced membrane. Clinical and radiological results are reported with 1 year of follow up. This case of induced membrane for bone loss and only bone substitute is the first to be reported.

P725-Tu
Physician and Patient Satisfaction, Tolerability and Safety during Raloxifene Treatment in PM Women
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Raloxifene is indicated for the treatment of osteoporosis in postmenopausal women. The decision to continue or terminate treatment based on patient and physician treatment satisfaction and overall safety is often made within the first 4 months. This project was designed to obtain data of these variables after 3 to 4 months of Raloxifene therapy. Patient satisfaction of 1,250 postmenopausal women (66.8 ± 10.3 years) with osteoporosis or osteopenia as diagnosed by DEXA was assessed by asking if patients would continue with Raloxifene after receiving more details about its properties and by rating treatment tolerability on a 5-point rating scale. Physician satisfaction of 187 physicians (GPs, rheumatologists, gynecologists) was assessed based on overall satisfaction, willingness to continue therapy and overall positive assessment compared with other treatment options after 4 months. Predefined symptoms (gastrointestinal symptoms, breast tenderness/pain, vaginal bleeding/spotting, skin/mucous membrane symptoms, and mental problems) were assessed at baseline and after 3 and 4 months of therapy. Overall safety was assessed by recording the patients' spontaneously reported symptoms within the first 4 months. After 4 months, 84.9% of the patients wished to continue Raloxifene treatment; 82.6% assessed its tolerability to be very good or good, respectively. 89.4% of the physicians confirmed general satisfaction with treatment and 86.4% wished to continue therapy. 70.6% of the physicians considered Raloxifene as advantageous compared with other treatments. The percentage of patients reporting a severe or moderate degree of all predefined symptoms had decreased statistically significantly ($P < 0.0001$, McNemar test) after 4 months compared with baseline (improvement from 6.6% to 10.1%). 10.4% of the patients spontaneously reported any type of symptoms. Hot flushes were most frequently reported (16.9%), followed by oedema (13.8%), abdominal symptoms (7.7%), nausea, and headache (5.4%).
The evaluation showed an excellent patient and physician satisfaction with Raloxifene in a routine clinical setting over 4 months. Its good safety profile as revealed by the decreasing frequency of predefined symptoms and low incidence of spontaneously reported symptoms makes it ideally suited in the long-term treatment of postmenopausal women with osteoporosis.

SATELLITE SESSIONS

Comparative Endocrinology of Calcium Regulation

CE01
Calcium Deposition and Resorption in the Sternal $CaCO_3$ Deposits of *Porcellio Scaber* (Isopoda), Ultrastructure and Molecular Mechanisms
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Terrestrial isopods are interesting models to study biomineralization processes including epithelial calcium and proton transport. Before molting, *Porcellio scaber* forms large calcium reservoirs at the first four sternites consisting of amorphous $CaCO_3$ and an elaborate organic matrix. The structural organization of the deposit facilitates the quick mobilization of Ca^{2+} ions that are used to mineralize the new cuticle after each molt. During formation and degradation of the deposits, Ca^{2+}, HCO_3^- and protons are transported across the anterior sternal epithelium (ASE). The ASE differentiates for epithelial ion transport by increasing the surface area of the basolateral and apical plasma membranes and the size and number of mitochondria. These differentiations are virtually absent in the posterior sternal epithelium (PSE), which therefore can be used as a control tissue. Expression analysis of a plasma membrane Ca^{2+} transport ATPase, a Na^+/Ca^{2+} exchanger and a V-type H^+-ATPase suggests that these mechanisms contribute to deposit formation and degradation. Immunofluorescence and ultrastructural detection of portasomes in the basolateral and apical plasma membranes indicate a polarity reversal of the V-type H^+-ATPase between deposit formation and degradation, respectively. X-ray microprobe analysis of freeze-dried cryosections of shock frozen sternal epithelia suggests the contribution of a subcellular compartment to intracellular calcium transport. In situ hybridization and changes in the activity of the smooth endoplasmic reticulum Ca^{2+}-ATPase (SERCA), as measured by the in situ Ca-oxalate method, suggest that this compartment may be the smooth endoplasmic reticulum. A model derived from these results suggests a mechanism by which the epithelium can switch between its function in mineral deposition and degradation.
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CE02

Left Out in the Cold: Low Temperature Induced CA Dyshomeostasis Alters Expression of Calcium Transporting Proteins in Crayfish Tissues

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Historically, our laboratory has used vectorial movement of Ca as a model to study expression of Ca pumps, exchangers and channels as epithelial tissues respond to increased Ca flux associated with cyclical biomineralization events. The molting cycle however is episodic and under hormonal control. In order to better understand the regulation of genes encoding for Ca transporting proteins, we have searched for other environmental stressors that can reliably perturb cellular Ca homeostasis. Acclimation to low temperature in a range of plants and ectothermic animals has been shown to elicit an influx of Ca into the cytosol through altering the composition of membrane lipids and leakage of Ca into the cell through Ca channels. When the temperate crayfish experiences cold acclimation (from 20°C to 4°C for 4 weeks), expression of the epithelial Ca channel (ECaC) was upregulated within 24 h. Within days, the cell compensated for this Ca influx and the potentiality for cytotoxicity by increasing expression of the Ca exporting proteins (Na^+/Ca^{2+} exchanger, NCX and plasma membrane Ca^{2+} ATPase, PMCA). Real time PCR has shown that relative quantitation of these genes increased from 2–9-fold; protein levels corresponding increased. In all tissues studied, NCX, the workhorse for basolateral Ca efflux showed greater increase than PMCA, the pump that fine-tunes cytosolic Ca level. Cold acclimation will be used in further studies to explore the spatial and temporal regulation of these Ca import/export proteins and the genes that encode them (supported by NSF grant IBN 0076035 to MW).

CE03

Functional Analysis of Bone Regulatory Genes in the Fish Models Medaka and Zebrafish

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Small laboratory fish like the Medaka (*Oryzias latipes*) and zebrafish (*Danio rerio*) offer many experimental advantages as in vivo models to study disease-related processes. They produce large numbers of completely transparent embryos, have relatively compact genomes and are easy to keep under laboratory conditions. The transparency of fish embryos allows for example real-time analysis of skeletal development in living specimens. Using the calcium-binding fluorochrome calcein and confocal laser scanning microscopy in Medaka, we followed the formation of calcified bone from day 6 of embryonic development until day 20 post hatching. We are using this model to characterize the genetic networks regulating bone formation and remodeling. We showed that despite the large evolutionary distance many known factors regulating bone formation are conserved between fish and humans. This includes osteoprotegerin (OPG), a secreted glycoprotein belonging to the tumor necrosis factor receptor (TNFR) superfamily and key regulator of bone resorption in mammals. In Medaka embryos, OPG expression starts at stages when first skeletal elements are already detectable. Putative consensus binding sites for transcription factors were identified in the promoter region of the Medaka OPG gene indicating possible evolutionary conservation of gene regulatory mechanisms between fish and mammals. Clinorotation experiments using Medaka larvae showed that OPG is regulated by simulated microgravity at the transcriptional level similar to the situation in human cell cultures (Kanematsu et al., Bone 30, 2002). In mammals, tuberoinfundibular peptide of 39 residues (TIP39) is a potent activator of the PTH-2 receptor but acts as an antagonist on the PTH-1 receptor. Despite the known interaction with the PTH receptor family, the function of TIP39 is largely unknown. We identified and isolated the TIP39 genes in both Medaka and zebrafish. Compared to the processed TIP39 of humans, both fish peptides show a high degree of sequence conservation (with 59% aa identity). In fish, TIP39 is expressed in a restricted domain of the embryonic forebrain and in the adult brain. To elucidate the function of TIP39, we presently perform Morpholino-based antisense approaches. Taken together, fish models offer excellent experimental approaches to characterize skeletal factors in a whole-animal model system, with relevance to human diseases. This work is supported by the European Space Agency.

CE04

Physiological Role of Calcitonin in Cartilaginous Fish and Cyclostomes

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Calcitonin (CT) is a 32-amino-acid hypocalcemic hormone that can mineralize bones by suppressing the activities of osteoclasts in mammals. This hormone is secreted from the C-cells of the thyroid gland in mammals and from the ultimobranchial gland (UBG) in non-mammalian vertebrates.

We indicated that stingray, *Dasyatis akajei*, has one pair of large UBGs which contain abundant CT. Therefore, we purified a CT from the stingray UBG and determined the

amino acid sequence of it. Stingray CT has a hypocalcemic activity in rat and approximately 2.4–6.2 times more potency than mammalian CTs, but 2.3–2.5 times less active compared with chicken, salmon and eel CT. Using ultimobranchialectomized stingray, we demonstrated that CT functions to calcium excretion via bile. In addition, estrogen receptor has been detected in the stingray UBG by a binding assay, Western blotting and Northern blotting. Therefore, estrogen directly acts on the UBG and may stimulate CT secretion in reproductive period.

From morphological observation and a hypocalcemic rat bioassay of the branchial region, which possibly contains UBG, it has been concluded that Cyclostomata, such as lamprey and hagfish, have no CT-producing cells. However, a CT-like substance has been detected in the plasma of lamprey (*Lampetra japonica*), and the molecular weight of this substance was estimated to be 3.5 kDa, which is equal to that of salmon CT, using Western blotting methods. In other species of Cyclostomata (hagfish, *Eptatretus burgeri*), the immunoreactive substance (3.5 kDa) was also present and shown to have hypocalcemic and hypophosphatemic activities in rat. In lamprey, we indicated that CT-like substance has a co-relationship with gonadal maturation. This suggests that this substance has some roles in reproductive physiology. In hagfish, CT-like substance was correlated with plasma calcium levels. Therefore, the CT-like substance in hagfish may participate in calcium homeostasis, as does calcium excretion via bile, because the hagfish has the highest calcium concentration in the bile determined to date among vertebrates, with a level corresponding to 12-fold that in the plasma.

Considering these facts, it is concluded that CT in cartilaginous fish and CT-like substance in cyclostomes function to regulate calcium homeostasis and reproductive physiology.

CE05

Duplicate Zebrafish *PTH* Genes are Expressed along the Lateral Line and in the Central Nervous System during Embryogenesis

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Parathyroid hormone (PTH) plays a critical role in calcium metabolism in tetrapods. The primary site of PTH expression is the parathyroid glands, although it is also detected in the thymus and hypothalamus. Fish lack anatomically distinct parathyroid glands, and the first animals to evolve parathyroid glands were the amphibians. However, fish do have PTH family ligands (Danks et al., 2003, JBMR 18: 1326–1331) and receptors (Rubin et al., 1999a, J Biol Chem 274:28185–28190; Rubin et al., 1999b, J Biol Chem 274:23035–23042) which are functionally similar to their mammalian counterparts. We report the expression patterns of duplicate zebrafish *pth* genes during embryogenesis. Both zebrafish *pth1* and *pth2* transcripts are expressed along the lateral line before the migration of the lateral line primordium and later in development Pth protein is detected in lateral line neuromasts by immunohistochemistry. Furthermore, *pth1* transcripts are detected in the central nervous system in the ventral neural tube and in the developing jaw. These temporally and anatomically restricted expression patterns imply a novel role for PTH family hormones during embryonic development of the zebrafish and allow for the genetic dissection of PTH function in this model organism.

CE06

Further Investigation of the Desensitization of Relaxant Responses to PTH and PTHRP in Pregnant Mouse Myometrium in Vitro

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It was previously shown that N-terminal parathyroid hormone (PTH) and parathyroid hormone related peptides (PTHrP) relax spontaneous contractility of 4 days pregnant mouse uterine and avian oviducal tissues in vitro in a concentration-dependent fashion[1]. These responses are reduced by nitric oxide synthase inhibitors and furthermore, the murine responses appear to be desensitized following 3–4 h repeated exposure to the peptides. In the present study, we continued these investigations. Tissues from 2, 4, 7 and 9-day pregnant mice were incubated in De Jalon's solution containing 1.2 mM Ca^{++} and gassed with 5% CO_2/95% O_2 until regular spontaneous contractions were established. Peptides were added to according to a 5-min cycle followed by washout and recovery of contractions before further additions of peptide. hPTH(1–34) and hPTHrP(1–34) caused similar concentration related inhibition of contractions over a concentration range of 10^{-9} to 10^{-7} M. These responses were rather inconsistent in 2-day pregnant tissues, were marked by 4 days, but were much less apparent in 7 and 9-day tissues. The desensitization in 4-day pregnant tissues following 4 h repeated exposure to a sub-maximal concentration of peptide appeared to be specific for each peptide, so that relaxant responses were re-established when the alternative peptide was used. Furthermore, hPTH(1–34) or hPTHrP(1–34) desensitized tissues showed a concentration-dependent relaxant response to the selective β_2-adrenergic receptor agonist, salbutamol. Continuous exposure to a single dose of peptide (10^{-7} M or 3×10^{-8} M) for 4 h produced a relaxant response which lasted for approximately 30 min, after which contractions started to

reappear. Following washout at 4 h, a further single dose of the homologous or heterologous peptide gave a variable but partial relaxant response. It is concluded that N-terminal PTH peptides may play a regulatory role in controlling endometrial quiescence during early pregnancy and that the in vitro responses to these peptides become specifically desensitized following repeated acute exposure and also chronically as the pregnancy progresses.

1. Francis M. et al. Gen. Comp. Endocr. 2003;133:243–251.

CE07

Parathyroid Hormone (PTH) and PTH-related Protein Co-regulate Murine Fetal–Placental Calcium Metabolism

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Calcium metabolism in the fetus is regulated differently from the adult, reflecting unique needs of the developing fetus and the role of the placenta. Both PTH and PTHrP are present in the fetal circulation and are capable of activating the PTH/PTHrP receptor, unlike the adult in which normally only PTH circulates. We have been examining the relative roles of PTH and PTHrP in regulating fetal calcium metabolism by systematically studying the physiological consequences of partial or complete absence of PTH and PTHrP in intact fetal mice.

In this paper, we will review data from several relevant knockout models. The *Pthrp* null lacks PTHrP; the *Pth* null lacks PTH; the *Gcm2* null lacks parathyroids but retains thymus-derived PTH; the *Hoxa3*-null lacks parathyroids, PTH and parathyroid-derived PTHrP; the *Pthrp/Hoxa3* double mutant lacks PTH and PTHrP; and the *Pth/PthrpReceptor* null lacks effective N-terminal actions of PTH and PTHrP but retains actions of PTH and PTHrP mediated by other receptors.

Normally, the fetal blood calcium is raised above the maternal calcium level in the presence of low fetal serum PTH levels. Loss of both PTH and PTHrP reduces the blood calcium to well below the maternal level (*Hoxa3, Pthrp/Hoxa3* double mutant, *Pth/PthrpReceptor* null) whereas loss of either PTH or PTHrP reduces the blood calcium to the maternal level. All models shared a similar degree of marked hyperphosphatemia and modest hypomagnesemia.

Calcium is actively transferred across the placenta. All models have relative hypocalcemia and might be expected to upregulate placental calcium transfer in response to this stimulus. Only the *Pth/PthrpReceptor* null and *Gcm2* null fetuses upregulated placental calcium transfer to 150% and 119% of the control value, respectively. Placental calcium transfer was not significantly different from control in *Pth* null or *Hoxa3* null but was reduced in the *Pthrp* null to 80% of control.

The results demonstrate that PTH and PTHrP both play roles in regulating fetal blood calcium and placental calcium transfer. Absence of either will reduce the blood calcium and raise phosphorus. Absence of PTHrP reduces the rate of placental calcium transfer, but absence of PTH may be what distinguishes *Pth* null from *Gcm2* null fetuses in their inability to increase placental calcium transfer.

In summary, PTH and PTHrP are both present in the fetal circulation and play interlocking roles in regulating calcium homeostasis and placental calcium transfer.

CE08

Expression and Localization of Calcium Binding Protein in the Chicken Small and Large Intestine with Aging

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Calcium metabolism in egg-laying hens is extraordinary when compared with all other classes of vertebrate, because they lay an egg with hard eggshell that consists of 5.7 g calcium carbonate containing about 2.3 g of net calcium. It is generally accepted that dietary calcium is actively absorbed in small intestine, such as duodenum and jejunum. At the enterocytes of small intestine, a 28 kD-sized calcium binding protein (CaBP-D28k) is highly accumulated and plays an important role as a calcium transporter across enterocytic cytoplasm. The levels of intestinal CaBP-D28k are closely correlated with active calcium absorption. However, the expression of CaBP-D28k in chicken large intestine has not been fully clarified. In the present study, we observed changes of CaBP-D28k expression and localization in the chicken small and large intestine with aging.

Female White Leghorn chickens were sacrificed at 40 (immature), 260 (high egg production) and 640 days (low egg production) of age, and duodenum, jejunum, ileum, cecum and colon were excised for this experiment. Western blot analysis represented that the expression degrees of CaBP-D28k in the intestines of 260-day hens were following; duodenum > jejunum > cecum > ileum > colon. Furthermore, immunohistochemical analysis of 260-day hens showed that CaBP-D28k was localized in enterocyte through tip-crypt axis of villi of the small intestine but only in villus tip enterocytes of the cecum and the colon. On the other hand, 40-day chicks showed CaBP-D28k expression in duodenum, jejunum and ileum but not in cecum and colon, and the expression degrees of CaBP-D28k were lower in 40-day chicks compared with 260-day hens. Furthermore, the localization and expression of CaBP-D28k in each intestinal segment of 640-day hens were similar to that of 260-day hens.

From these results, it is concluded that egg-laying hens apply to extend active calcium absorption into large intestines from small intestines in compensation for the high demand of calcium for eggshell formation.

CE09

Valproate has Strain-specific Effects on Bone Mineral Content in Mice

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Long-term therapy with anti-epileptic drugs (AED) is associated strongly with increased fracture risk, but the mechanism by which AED use is associated with decreased bone mineral density and increased bone fragility is poorly understood. There are currently no established animal models of AED-induced bone disease.

Our aim was to develop a mouse model to investigate the effects of AEDs on total bone mineral content (BMC) and to better characterize the metabolic and structural bone changes associated with AED treatment. The ultimate aim of this project is to identify the mechanisms including any genetic factors underlying this effect.

Seven different inbred strains (n = 40 per strain, n = 10 per diet) of 8–9-week-old mice were placed on a diet mixed with 0, 2, 4 or 6 g/kg valproate (VPA) for 8 weeks. Then, total BMC, fat mass and lean mass were assessed using dual energy X-ray absorptiometry (DXA). BMC was corrected for total body weight and total lean mass to account for differences in animal size.

Statistical analysis using ANOVA identified BALB/C as being sensitive to VPA-induced bone disease showing significant differences (95% CI) of 10.4% (2.7%–18.2%) and 8.4% (0.1%–16.2%), respectively, in weight-adjusted BMC compared with control mice while on the 2 and 6 g/kg VPA diets ($P < 0.05$). 129T2 was identified as a strain resistant to the effects of VPA on BMC at all doses. Other VPA-sensitive and resistant strains also have been identified. Further investigation of these mouse strains and the identification of metabolic and genetic factors involved may help to elucidate the mechanisms underlying the effects of AED treatment on bone health. This approach will facilitate the design of therapeutic strategies for the prevention and treatment of AED-associated bone disease and the pre-clinical testing of potential interventions.

CE10

Expression of Matrix Metalloproteinases 2 and 9 and Reck During Amelogenesis

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Proteinases play important roles during dental enamel formation, namely, processing and degradation of enamel proteins. Matrix metalloproteinases (MMP), which are zinc-dependent endopeptidases, play a pivotal role in extracellular matrix (ECM) turnover. Reck gene is widely expressed in normal human tissues and is a membrane-anchored glycoprotein that regulates MMP activity. During embryogenesis and organogenesis, MMP-2 and -9 are involved in physiological tissue remodeling events. Our goal is to evaluate the expression pattern of MMP-2, -9 and Reck during the secretory and maturation phases of amelogenesis in continuously growing incisor of rats. Hemi-mandibles (n = 5) were collected from 50-day-old rats. Samples were fixed in 10% buffered formalin (pH 7.2), demineralized and embedded in paraffin wax. Sections (5 μm thickness) were stained with Masson's Trichrome. Immunohistochemistry, using polyclonal antibodies against MMP-2, -9 and Reck, was performed using the StreptABComplex/HRP duet kit, with biotinylated secondary antibody and a chromogenic substrate mixture. The sections were counterstained with Harris's hematoxylin. Immunohistochemistry of demineralized material provided evidence for stronger MMP-2 staining in enamel organ than in the cytoplasm of secretory ameloblasts but diffuse immunostaining on ameloblasts at maturation stage. MMP-9 was detected in secretory ameloblasts (Tomes Process), in the cytoplasm of ameloblasts at maturation stage, and enamel organ. Reck immunostaining was observed in the cytoplasm of odontoblasts, probably at Golgi complex, in maturation and secretory ameloblasts, odontoblasts and enamel organ. Our results support the first evidence that MMP-2, -9 and Reck are differentially expressed during the phases of amelogenesis, playing an active role during ECM remodeling and suggesting a regulation mechanism of Reck upon metalloproteinases activity.

Keywords: Reck, matrix metalloproteinases, extracellular matrix, amelogenesis.
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CE11

Effects of Endocrine Disruptors on Avian Medullary Bone Formation

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In recent years, a number of man-made chemicals, such as organochlorine pesticides (DDT and its metabolites), bisphenol-A (a chemical used in the manufacture of polycarbonate-derived products and epoxy resins), dioxin-

like chemicals and others, have shown to be able to mimic endogenous hormones. It has been hypothesized that alternations in normal pattern of endocrine systems seen in some populations of wildlife are linked with exposure to these chemicals. Especially in avian, it has been reported that DDT, its metabolites and others cause the eggshell thinning. Medullary bone is a specific tissue of female birds and plays an important role as a calcium reservoir for eggshell formation. This bone is induced by increasing amounts of estrogen during the onset of sexual maturation and observed in marrow cavities of long bones. Therefore, it is easy to induce medullary bone formation by the treatment of male birds with estrogen. In the present study, we observed effects of endocrine disruptors on medullary formation in male Japanese quails and estimated the estrogenic activity of bisphenol A and DDT metabolite.

Matured male Japanese quails were injected i.m. daily with bisphenol A (10 mg or 1 mg), p,p′-DDE (DDT metabolite; 10 mg, 5 mg, 1 mg or 0.2 mg) or 0.1 ml vehicle (corn oil) for 7 days, respectively. After that, femurs were excised, fixed and sectioned according to general protocols, and the sections were histochemically observed with a light microscope. As a result, p,p′-DDE-treated quails represented that medullary bone was developed in marrow cavities of femurs. On the surface of medullary bone, active osteoblasts were also observed with ALP histochemistry. On the other hand, in bisphenol A-treated quails, some differentiation of bone lining cells into osteoblasts was observed on endosteal surface of cortical bone, but medullary bone was rarely developed in the marrow cavities, differing from p,p′-DDE-treated quails.

In conclusion, these results suggested that p,p′-DDE stimulates the medullary bone formation as estrogen-like endocrine disruptors. This means p,p′-DDE disrupts normal endocrine system of calcium metabolism including medullary bone formation. Additionally, it is possible that medullary bone is a useful biomarker of endocrine disruptor pollution.

CE12

Cortical Bone Porosity and Osteoblast Gene Expression during Growth of the Immature Avian Skeleton

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Bones increase their diameter by incorporation and infilling of primary osteons. Comparing chickens with fast (F) and slow (S) growth potential, we have previously reported that the tibia of fast growing birds had increased cortical porosity, which may explain their lowered mechanical properties. The aims of this study were to directly determine the infilling rate of the primary osteons and to compare osteoblastic characteristics of both strains. To quantify the rate of osteon infilling, tibiae were removed from 21-day-old chicks, which had been double labeled with calcein. The mineral apposition rate was F = 11.51 μm/day; S = 28.16 μm/day, $P < 0.001$. Osteoblasts from 4 birds/strain were grown and expanded in culture. Osteoblast proliferation was determined by tritiated-thymidine uptake and differentiation by alkaline phosphatase (ALP) activity. At pre-confluency, cell proliferation was higher in the slow growing birds (F = 7439 dpm; S = 11732 dpm, $P < 0.001$), but this pattern was reversed at confluency (F = 10,491 dpm; S = 1979 dpm, $P < 0.001$) and post confluency (F = 4564 dpm; S = 1702 dpm, $P < 0.001$) and is a likely consequence of the earlier impairment of proliferation by contact inhibition in the slow growing strain. ALP activity (pNPP hydrol/30 min/mg protein) was only detected at post-confluency and was higher in the fast growing strain (F = 1188; S = 216, $P < 0.001$). Osteoblastic gene expression was determined by RT-PCR and quantified by densitometry (cnt/mm^2). A higher level of osteopontin (F = 382; S = 1205, $P < 0.01$) and BSP (F = 33; S = 262, $P < 0.05$) expression was observed in the slow growing birds. The serotonin receptor, considered to have a role in mechanoregulation, was more highly expressed in the fast chicks (F = 565; S = 241, $P < 0.05$) as was Runx2 (F = 312:S = 72, $P < 0.001$). In conclusion, porosity was shown to be due to a lack of infilling within the primary osteons. Osteoblast proliferation was faster in the slow growing birds whereas differentiation was slower. This is in accord with our previous hypothesis that the fast growing birds are characterized by an increase in transit time through the osteoblast lineage, which may be driven by the high levels of Runx2 expression. Osteopontin and BSP are associated with mechanical loading, but the significance of the lower expression levels in the fast growing birds requires further study. However, the upregulation of serotonin expression may reflect the greater loads experienced in the fast growing birds in vivo.

CE13

Expression Profiles of *Xenopus Banded Hedgehog*, a Homolog of Mouse Indian Hedgheog, Are Related to the Late Development of Endochondral Ossification in *Xenopus Laevis*

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Late development of endochondral ossification occurs at the boundary between the growth cartilage and bone marrow during the formation of long bones in *Xenopus laevis*. Since the Indian hedgehog (Ihh) is involved in endochondral ossification in mouse, we investigated the expression of *Xenopus banded hedgehog* (*X-bhh*), which is

a homolog of mouse *Ihh*. RT-PCR analysis demonstrated that the *X-bhh* mRNA was detected from an early stage of limb formation to formation of femurs in mature frogs, and it was associated with the expression of *Xenopus-ptc1* (*X-ptc1*), *Xenopus-gli1* (*X-gli1*), *Xenopus-type II collagen* (*X-col II*), *Xenopus-runx2* (*X-runx2*) and *Xenopus-osteocalcin* (*X-ocn*) mRNAs. In situ hybridization revealed that chondrogenic cells observed at early limb development expressed *X-bhh* and *X-gli1*. At later stages of limb development, chondrocytes, located slightly away from the boundary between the cartilage and bone marrow, expressed the *X-bhh*, *X-ptc1* and *X-gli1* mRNAs; however, the mesenchymal cells at the boundary failed to express these mRNAs. The *X-bhh*, *X-ptc1* and *X-gli1* mRNAs as well as those of *X-runx2* and *X-ocn* were expressed by the mesenchymal cells in the periosteal region at the tip of the cortical bone, indicating an intimate relationship between *X-bhh* expression and bone formation in this region. Considered collectively, the present study suggests that X-bhh evolutionally acquired the function to induce osteogenesis; however, the expression profile of X-bhh in epiphysis is closely related to the late development of endochondral ossification in *X. laevis*.

CE14

Changes in Serum Parathyroid Hormone and Electrolyte Concentrations and Urinary Excretion of Electrolytes in Horses with Experimental Endotoxemia

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We have documented that hypocalcemia and hypomagnesemia are frequent findings in horses with sepsis and endotoxemia. We hypothesized that, in horses, endotoxemia triggers a systemic inflammatory response that results in hypocalcemia and hypomagnesemia. The goal of this study was to determine the effect of endotoxin (LPS) administration to healthy horses on serum parathyroid hormone (PTH), ionized (Ca^{2+}) and total calcium (tCa), ionized (Mg^{2+}) and total magnesium (tMg), phosphate (Pi), potassium (K^+), sodium (Na^+), chloride (Cl^-) and insulin concentrations, and on the urinary excretion of these electrolytes.

Twelve mares were infused with *E. coli* LPS (30 ng/kg/h/IV) for 1 h. Six mares were infused with saline (controls). In LPS-infused horses, heart rate increased from 40.0 ± 1.3 to 70.0 ± 9.0 beats/min, respiratory rate from 12.7 ± 1.0 to 21.1 ± 3.0 breaths/min, body temperature from 37.4 ± 0.03 to 38.9 ± 0.6°C, and TNF-α concentrations from 6.6 ± 3.5 to 507 ± 260 pg/mL ($P < 0.05$). White blood cell count decreased from 7570 ± 600 to 1960 ± 560 cells/μL. Serum concentrations of Ca^{2+} decreased from 6.5 ± 0.3 to 6.0 ± 0.3 mg/dL, Mg^{2+} from 0.53 ± 0.06 to 0.43 ± 0.04 mmol/L, tMg from 0.78 ± 0.05 to 0.62 ± 0.08 mmol/L, K^+ from 4.3 ± 0.4 to 3.0 ± 0.5 mEq/L, and Pi from 3.4 ± 0.5 to 1.7 ± 0.5 mg/dL ($P < 0.05$). Insulin increased from 9.4 ± 3.6 to 50.5 ± 9.6 μIU/mL and PTH from 1.3 ± 0.4 to 6.0 ± 5.2 pmol/L. In some horses ($n = 2$), PTH did not increase despite hypocalcemia. Urinary fractional excretion of Ca^{2+} (FCa) decreased from 4.7 ± 1.4 to 1.7 ± 1.2%, of Mg^{2+} (FMg) from 36.6 ± 6.5 to 11.7 ± 7.3% and K^+ (FK) from 37.9 ± 11.3 to 17.7 ± 6.2% ($P < 0.05$). Fractional excretion of Pi (FP) increased from 0.02 ± 0.02 to 0.14 ± 0.07% and Na^+ (FNa) from 0.26 ± 0.13 to 1.2 ± 0.5% ($P < 0.05$).

Based on our findings, we believe that endotoxemic horses developed hypomagnesemia and hypokalemia from shifting Mg^{2+} and K^+ to the intracellular compartment and hypocalcemia from movement of Ca^{2+} to the interstitial compartment and intracellular subcompartments. The decrease in FCa, FMg and FK makes urinary loses of these electrolytes an unlikely cause of their serum disturbances, but rather a compensatory mechanism. Some of our findings can be explained by the increased serum PTH and insulin concentrations and activity of the Ca^{2+}-sensing system. In conclusion, endotoxemia in horses resulted in electrolyte abnormalities that included hypocalcemia, hypomagnesemia, hypokalemia and hypophosphatemia.

CE15

Prevention with DCAD of Hypocalcemic Postparturient Paresis of Dairy Cows

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Hypocalcemic postparturient paresis is a disorder of dairy cows caused by the sudden increase of calcium secretion into the colostrum. In high-yielding high-parity cows, hypocalcemia develops after calving, and the cows begin measures to increase serum Ca concentration by enhancing its reabsorption from the renal tubules, its mobilization from the bone reserves and its absorption from the intestinal tract. During the dry period, only Ca lost due to the fetal and endogenous fecal drain (2 to 7 and 5 to 7 g/Ca per day, respectively) has to be replaced and the mechanisms for replenishing plasma Ca are thus relatively inactive. Because the cow's milk contains 1.2 g Ca/L, more than 25 g of Ca is needed in the Ca pool during lactation. The colostrum contains approximately 2.3 g Ca/L. After calving, the Ca that is absorbed from the ingested food is momentarily insufficient to replace this loss. A cow producing 10 L of colostrum loses about 23 g of Ca in the first single milking. This amount of Ca is about nine times higher than that present in the entire plasma Ca pool (2.5–3 g) and more than two times that present in the extracellular pool (9 to 11 g). The manipulation of the dietary cation–anion difference [DCAD = $(Na^+ + K^+) - (Cl^- + SO_4^{2-})$] makes it possible to maintain the cows in metabolic acidosis during the critical period that precedes calving,

presumably via a mechanism that involves the strong ion difference in the extracellular fluid. As a consequence, the mobilization of calcium is enhanced and the incidence of the disorder is decreased. Conversely, a dietary induced metabolic alkalosis leads to a more severe degree of hypocalcemia and the incidence of the disease is increased. The underlying mechanisms of the prevention of the disease are only partially understood. Nevertheless, this preventive method is already widely applied with success in clinical practice.

CE16

Regulation of Skeletal Demineralization During Lactation and its Recovery after Weaning

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It is well appreciated that peak bone mass is achieved in women in their early 20s and that this level is normally maintained until menopause. If there is a significant insult to bone during this time (prolonged illness or immobility, high-dose corticosteroid therapy, etc), the skeleton will lose mineral content and will not fully regain it afterward.
Conversely, it is not well appreciated that the female skeleton undergoes a significant change during pregnancy and lactation, from which it recovers fully. On average, women lose 5–10% of skeletal mineral content during 6 months of exclusive lactation and, most importantly, fully regain that mineral content within a few months of weaning. These daily losses appear to be met by a temporary partial demineralization of the skeleton which is mediated by PTH-related protein secreted from the breast, in combination with low estrogen levels. The restoration of calcium to the skeleton occurs rapidly but by means that are not understood.
Our laboratory is systematically examining how the skeleton recovers after weaning. The mechanism by which the skeleton recovers might be adapted into new approaches to stimulating the skeleton to increase its mineral content in clinical conditions such as osteoporosis.
In this paper, we will review studies that we have undertaken in normal mice and in mice that lack PTH, the vitamin D receptor or calcitonin. Studies have included serial measurements of bone mineral density and content, ash weight, hormones, serum and urine minerals, μCT and mechanical strength of bone.
Normal mice increase skeletal mineral content by 12–20% during pregnancy, lose about 30% during 3 weeks of lactation and fully recover after 14 days. These changes are more marked in trabecular bone. Mice lacking PTH achieve the normal increase in bone mineral content during pregnancy, lose during lactation and recover fully after weaning. Mice lacking calcitonin achieve the normal increase in bone mineral content during pregnancy but lose twice the normal amount of mineral during lactation, dropping to about 45% of pre-pregnancy bone mineral content. Despite these severe losses, the skeletons of mice lacking calcitonin recover fully after weaning.
In summary, mice experience rapid and severe losses of skeletal mineral content that are fully reversed after weaning. None of the classic calcitropic hormones appear to be required to achieve full recovery of the skeleton.

CE17

Bone Metabolism of Milk Goat and Sheep During Second Pregnancy and Lactation

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Substantial losses of skeletal tissue occur during pregnancy and lactation. The goal of the present study was to follow these changes in pregnant and lactating goat and sheep and to compare these two species during their second lactation.
Blood samples were collected from 12 dairy goat and sheep monthly during gestation, 2 to 3 days postpartum (pp), 2 weeks pp, 4 weeks pp and then monthly during lactation until 7 months after parturition. Total bone mineral content (BMC) and total bone mineral density (BMD) were quantified using peripheral quantitative computed tomography at the same intervals as the blood was taken. Bone resorption was assessed in serum using two different domains of the carboxyterminal telopeptide of type I collagen (ICTP and crosslaps). Bone formation was quantified in serum with osteocalcin (OC) and bone-specific alkaline phosphatase (bAP).
Mean ICTP and crosslaps concentrations of the two animal groups showed an increase in the last month of gestation. In contrast, mean OC concentrations decreased slowly from the 2nd month of pregnancy until the first week pp. Furthermore, mean bAP activities showed a similar time course. Total BMC and BMD decreased also from the 4th month of pregnancy until the first week pp in both groups. Afterwards, BMC increased again during lactation. BMD levels of sheep and goat returned to prepartum levels during lactation.
In conclusion, in goats and sheep at parturition and in early lactation, the process of bone remodeling is enhanced. The bone resorptive phase of bone remodeling is accelerated but is uncoupled from the process of bone formation. This allows the animal to achieve calcium homeostasis at the expense of bone. Increased bone remodeling during lactation may represent physiological mechanisms to help the maternal skeleton adapt to greatly increased requirements due to enormous calcium losses in milk. Later in lactation presumably the animals come back into positive calcium balance and bone remodeling now has bone resorption and formation phases tightly coupled. Interest-

ingly, in these species, the bone loss in the second pregnancy and lactation measured with BMC and BMD is not as prominent as in the first lactation but shows almost the same course, although the animals gave more milk in the second lactation. It seems that the organism adapts to the circumstances more easily in the second lactation compared to the first lactation in these two species.

CE18

Immunohistochemical Localization of Endothelial Nitric Oxide Synthase and its Response to N-terminal PTH Peptides in Pregnant Mouse Uterine Tissues

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Parathyroid hormone (PTH) and PTH-related peptide (PTHrP) act via a common receptor (PTH_1R) involving several second messenger systems and have previously been shown to modulate the contractile state of numerous types of smooth muscle.[1,2] We previously suggested that relaxant effects of N-terminal PTH and PTHrP in pregnant mouse endometrial tissues are mediated via nitric oxide (NO) signaling, although it is not yet clear if this is endothelial or neuronally derived. Furthermore, we reported that relaxant responses to these peptides become desensitized following repeated exposure in organ bath preparations. A role for PTHrP[3] and NO[4] has been suggested in maintaining mammalian pregnancy. This study aims to examine differences in endothelial (e)NOS localization in untreated, bPTH(1–34)) and hPTHrP(1–40)-treated uterine tissue excised from 4-day pregnant mice. Uterine horns from freshly killed 4-day pregnant mice were removed and placed in an organ bath in De Jalon's ringer solution at 37°C gassed with 95% O_2/5% CO_2 and incubated with either PTH(1–34) or PTHrP(1–34) (3×10^{-8} M) for 1 min, or repeatedly exposed to the peptides over 4 h. Tissues were then removed from the bath, rapidly frozen and mounted. Immunohistochemical localization of eNOS in 10-μm-thick cryostat sections was conducted using a polyclonal anti-eNOS primary antibody raised against the C-terminal of the peptide and a fluorescein-conjugated secondary antibody. Sections visualized by confocal laser scanning microscopy were compared qualitatively. eNOS was located primarily in the myometrial layers. Single exposure to PTH(1–34) or PTHrP(1–34) caused no change in fluorescence compared to that in non treated tissues, whereas repeated exposure to the peptides substantially decreased fluorescence. We conclude that desensitization of relaxant responses to N-terminal PTH peptides is associated with a reduction in tissue eNOS content.

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Satellite Symposium: Osteoporosis and Fragility Fractures: A New Approach

Supported by Eli Lilly

SS01

Following Up on Patients with Osteoporosis

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Therapies for the treatment of osteoporosis are effective in reducing fracture incidence after 2-4 years of treatment. However, it would be useful to have a method to determine whether or not a patient is responding to therapy in the short-term, soon after initiation. Currently, measurements of bone mineral density and biochemical markers of bone turnover, both surrogates for fracture risk, have been used to monitor patients on therapy. Bone mineral density changes of a magnitude to be detected in a clinical setting may need perhaps a year of treatment, whereas changes in markers of bone turnover in response to treatment are identifiable within 1-3 months with a much greater magnitude of change, and reach full response within 12 months.

Clowes et al (J Clin Endocrinol Metab 2004) found that patient adherence to treatment was increased when patients were provided with information on their response to therapy using bone turnover markers. Biochemical markers of bone turnover, therefore, are useful tools in monitoring the response to therapy. These bone turnover markers include PINP, BSAP, CTx/Cr, NTx/Cr, and osteocalcin. The sensitivity or responsiveness of these markers is dependent, however, on the specific therapy. Bjarnson et al (Osteoporos Int 2001), in a study of raloxifene, was the first to describe a relationship between change in markers, specifically osteocalcin and bone specific alkaline phosphatase (BSAP), and future fracture risk. Using a new marker of bone formation, type I procollagen N-terminal propeptide (PINP) in the Multiple Outcomes of Raloxifene (MORE) trial, we not only confirmed the utility of osteocalcin and BSAP in predicting vertebral fracture risk, but found that change in PINP at 1 year accounted for 28% of the reduction in fracture risk, more than that of change in BMD measurements (Reginster et al., Bone 2004). PINP, which also has the highest or best signal to noise ratio, has been used to identify responders to teriparatide therapy, a bone-forming agent, based on a threshold response of 15 μg/L at 3 months.

Measurements of biochemical markers of bone turnover, particularly PINP are of value in monitoring patients' responses to osteoporosis therapies.

SS02

Are All Fragility Fractures Equal?

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Over the past few years epidemiologic studies and national registries have provided information about the incidence of nonvertebral fractures in several regions of Europe.
These studies have increased our understanding of the societal impact of hip, as well as other nonvertebral fractures. For example, nonvertebral non-hip fractures have been shown to account for 90% of total fractures up to age 80 and for 59% of total fractures after age 80 (Kanis 2000).
Studies have also provided information about risk factors for fracture, as well as the cost of caring for fractures patients and the change in quality of life resulting from a fracture.
Large randomized placebo-controlled trials of several osteoporosis treatments have been published that provide information about the effect of treatment on the risk of spine, as well as hip and other non-spine fractures. Available data suggest that the reduction of hip fractures and of other nonvertebral non-hip fractures is in the same order of magnitude. Currently there is a requirement in Europe for the conduct of specific trials both for spine and hip fractures. Given the current state-of-the-art it may no longer be ethical or practical to conduct studies to establish the effect of a treatment specifically on hip fractures. Therefore, the effect of treatment on the incidence of overall nonvertebral fractures appears to be a justified primary endpoint in the evaluation of new agents for treatment of osteoporosis. This new strategy does not preclude the specific effect of a new agent on hip fracture as a secondary endpoint.

SS03

Use of Antiresorptives and Bone-Forming Agents in the Management of Osteoporosis

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Anti-resorptive therapies increase bone mineral density (BMD) and reduce fracture risk. They do not, however, stimulate formation of new bone. In fact, they reduce bone formation. Thus, they cannot cure osteoporosis. Recently parathyroid hormone became available to treat both men and women with osteoporosis. Unlike anti-resorptive agents, once daily parathyroid hormone administration actually increases bone formation and it can cure osteoporosis in animals. PTH is the only true anabolic therapy that is available for people with osteoporosis. The ability of once-daily PTH to increase bone mass was first described in the 1920 s yet the observation was largely ignored because PTH was known to cause bone loss in people with hyperparathyroidism. Despite a series of small uncontrolled studies starting in the 1980 s suggesting that once-daily PTH also increases bone mass in humans, skepticism continued regarding its anabolic potential. Eventually, numerous animal studies demonstrated the powerful anabolic effect of once-daily PTH and similar results were obtained from randomized controlled trials in humans. In 1994 we first reported that once-daily hPTH-(1-34) prevented bone loss and actually increased BMD in women with endometriosis with acute estrogen deficiency due to GnRH analog therapy. Three years later, Lindsay et al. reported that adding hPTH-(1-34) to long-standing HRT increased BMD markedly in postmenopausal women. In 2001, Neer et al. published the landmark study demonstrating that once-daily hPTH-(1-34) therapy markedly reduces the risk of spine and non-spine fractures in women with postmenopausal osteoporosis. That study led to the approval of PTH as a therapy by regulatory agencies. Similar data were subsequently reported in men. When compared directly with alendronate, PTH clearly increases BMD much more. When combined with alendronate, however, the anabolic effect of PTH on spine and hip BMD is reduced compared with PTH monotherapy. When PTH therapy is stopped, BMD appears to fall though this decline can be prevented with an anti-resorptive agent. PTH therapy is a major advance in our ability to treat people with osteoporosis.

Satellite Symposium: The Evolution of Bisphosphonate Dosing: Improving Therapeutic Outcomes in Osteoporosis

Supported by Roche/GSK

SS04

Adherence, Patient Preference and Dosing Frequency: Understanding the Relationship

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Adherence to medications used in chronic diseases is suboptimal, averaging only 50%.[1] Poor adherence is a particular concern in asymptomatic conditions and is further complicated in osteoporosis as patients are unable to monitor their response to therapy. Inadequate long-term adherence to osteoporosis therapy negatively impacts on therapeutic outcomes, resulting in smaller decreases in bone turnover, lower BMD gains and a greater risk of fractures.[2]
Evidence from other chronic diseases suggests that reducing dosing frequency can improve therapeutic adherence. A recent study of oral bisphosphonates, the first-line treatment of choice for osteoporosis, has demonstrated an improve-

ment in adherence with weekly over daily dosing, although persistence and compliance with weekly regimens remain suboptimal. Prescription claims data for 288 women starting a new prescription for weekly or daily alendronate were obtained over a 12-month period from a German general practitioner database.[3] Persistence rates at 6 months were only 56% and 41%, falling to 47% and 28% at 12 months for the weekly and daily cohorts, respectively. The mean 12-month medication possession ratio (the number of days drug was supplied divided by the number of follow-up days), which is a measure of compliance, was 52% and 38%, for the weekly and daily groups, respectively. Similar studies conducted in the USA confirm these findings.[4,5]

Extending the bisphosphonate dosing interval beyond weekly is predicted to provide additional adherence benefits to postmenopausal patients. This is supported by a recent survey of current weekly oral bisphosphonate users ($n = 393$).[6] The majority (67%) expressed a preference for once-monthly dosing, with 'less frequent administration' and 'better fit with lifestyle' being the most common reasons. Ongoing prospective clinical studies are further evaluating patient preferences for once-monthly versus weekly oral regimens.

The strong patient preference for extended dosing intervals is expected to be reflected in improved therapeutic adherence and thus treatment outcomes.

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SS05

Beyond Weekly Dosing: Preclinical and Clinical Experience

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Beyond weekly oral bisphosphonate dosing likely requires high antiresorptive potency and favorable tolerability, enabling practical administration of the increased doses required for efficacy over the dosing interval. To date, the potent, nitrogen-containing bisphosphonate, ibandronate, is the only oral bisphosphonate to be extensively evaluated for its suitability with extended intervals.

Preclinical studies have evaluated the feasibility of intermittent ibandronate regimens in various animal models of osteoporosis.[1] Appropriately dosed, intermittent regimens were shown to produce similar therapeutic benefits to continuous regimens. Increases in bone mass and maintenance or improvements in bone strength and architecture were observed, with no ill effects on bone mineralization.

Following these positive preclinical observations and successful phase II clinical evaluation,[2] a randomized, double-blind, placebo-controlled, phase III study (BONE) was initiated to establish the antifracture efficacy of a 2.5 mg daily oral ibandronate regimen and provide 'proof of concept' for antifracture efficacy with an intermittent (dosing interval >2 months) ibandronate regimen.[3] A total of 2946 women (55–80 years, ≥5 years postmenopausal, lumbar spine BMD t score < −2.0 in ≥1 vertebra [L1–L4] and 1–4 prevalent vertebral fractures) received 3 years' treatment with oral ibandronate, either intermittently (20 mg every other day for 12 doses every 3 months) or continuously (daily; 2.5 mg), or placebo. All participants received daily calcium (500 mg) and vitamin D (400IU).

At 3 years, intermittent ibandronate provided a substantial reduction in the risk of new vertebral fractures versus placebo (50%). This is the first time that an intermittently administered bisphosphonate has prospectively demonstrated significant antifracture efficacy. Importantly, this risk reduction was comparable to that observed with the daily regimen (62%). Similar, sizeable increases in BMD and decreases in biochemical marker of bone turnover were also reported with both schedules. Independent of the dosing regimen, ibandronate therapy was well tolerated, with safety similar to placebo.

The comprehensive oral ibandronate development program, including the positive BONE study outcomes, provides important insights into the development of simplified, beyond weekly bisphosphonate regimens in PMO.

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SS06

Once-Monthly Dosing: An Effective Step Forward

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In postmenopausal osteoporosis (PMO), reducing the frequency of bisphosphonate dosing may improve patient adherence. In a phase I, dose-ranging study (MOPS),[1] the safety and tolerability of once-monthly oral ibandronate regimens were similar to placebo. Dose-related decreases in biochemical markers of bone resorption were observed, highlighting a potential for significant clinical efficacy.

In the subsequent MOBILE study, 1609 women with PMO (aged 55–80 years, ≥5 years since menopause, lumbar spine [L2–L4] bone mineral density [BMD] t score < −2.5 and ≥−5) were randomized to 2 years of double-blind treatment with oral ibandronate, given either once monthly (50 + 50 mg [single doses, consecutive days], 100 mg [single day] or 150 mg [single day]) or daily (2.5 mg). All participants received daily calcium (500 mg) and vitamin D (400 IU).

At 12 months, lumbar spine BMD (primary efficacy endpoint) increased in all groups (4.3%, 50 + 50 mg; 4.1%, 100 mg; 4.9%, 150 mg and 3.9%, 2.5 mg); the increases in the once-monthly groups were statistically non-inferior, and the increase in the 150 mg group statistically superior (P = 0.002) to those in the daily group. Proximal femur BMD (total hip, femoral neck and hip trochanter) also increased markedly, and to a similar extent, in all groups. Increases in BMD at the lumbar spine and total hip, both from baseline and above predefined thresholds of 6% (lumbar spine) and 3% (total hip), were obtained in larger proportions of women in the 100 mg and 150 mg groups than in the daily group. Serum CTX (sCTX) levels decreased markedly from baseline in all groups (62.8–75.8%); significantly more patients achieved falls in sCTX of >30%, >50% and >70% from baseline in the 150 mg group than in the daily group ($P < 0.05$). Ibandronate was well tolerated by patients in all four groups. The overall incidence of adverse events (including upper gastrointestinal events) in the once-monthly groups was similar to that in the daily group. In women with PMO, oral ibandronate given once-monthly is at least as effective and similarly well tolerated as when given daily.

Reference

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Satellite Symposium: Fighting Osteoporosis on Two Forms

Supported by Servier

SS07

Uncoupling Bone Formation and Bone Resorption: A Novel Therapeutic Approach

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Bone remodeling plays a major role in the maintenance of the mechanical integrity of the skeleton. This involves a well-coordinated balance between bone formation (by osteoblasts) and bone resorption (by osteoclasts). The activities of osteoblasts and osteoclasts are regulated by a large variety of systemic and local hormones and factors. The main systemic regulators of bone remodeling are parathyroid hormone (PTH), calcitriol, and calcitonin, but many other substances may affect bone remodeling, such as prostaglandins, thyroid hormones, glucocorticoids, sex hormones, growth factors, and interleukins. Bone formation is promoted via the stimulation of osteoblast proliferation and/or differentiation by, for example, insulin-like growth factor (IGF) and transforming growth factor β (TGF-β). Recent advances indicate that the central nervous system also modulates bone formation through the sympathetic system. There are many cytokines that can stimulate bone resorption, including interleukin 1, 6, 11, and 17, tumor necrosis factor α (TNF-α), and the receptor activator of nuclear factor κB ligand (RANK-L), which binds to the RANK receptor, leading to increased formation of osteoclasts and to their activation. Another player is osteoprotegerin, which prevents binding of RANK-L to RANK, thereby inhibiting bone resorption. Because bone formation and resorption are coupled, an antiosteoporotic agent that influences one may alter the other as well. Hence, antiresorptive agents also decrease bone formation, and bone-forming agents also increase bone resorption. There is great interest, therefore, in agents that may positively uncouple bone turnover, like strontium ranelate, which is associated with increased bone formation and decreased bone resorption.

SS08

Pharmacological Approach for Optimizing Bone Formation and Bone Resorption

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Strontium ranelate is a newly developed drug that has been shown to be effective in decreasing the risk of fractures in postmenopausal women. In contrast to other available treatments for osteoporosis, strontium ranelate induces opposite effects on bone resorption and formation. This original dual mode of action was demonstrated both in pharmacological studies in animals and in experimental studies on bone cells in vitro. In the model of osteopenia induced by ovariectomy in adult rats, strontium ranelate (12-week and 52-week studies) decreased bone resorption but maintained bone formation, as documented by biochemical markers and histomorphometric parameters. This dual effect resulted in prevention of bone loss induced by estrogen deficiency assessed by bone mineral content and trabecular bone volume. In the model of osteopenia induced by hind limb immobilization in rats, strontium ranelate reduced histomorphometric and biochemical parameters of bone resorption and reduced long bone loss, as assessed by bone mineral content and bone volume. In normal mice, strontium ranelate increased bone formation and reduced bone resorption, resulting in increased vertebral bone mass without any alteration of bone mineralization. This dual mode of action of strontium ranelate on bone formation and resorption was confirmed at the mandibular bone level in intact monkeys. In vitro, strontium ranelate was shown to enhance preosteoblastic cell replication and collagen synthesis in rat calvaria and osteoblastic cell cultures as well as in mouse osteoblastic cell cultures. At the same time in this mouse model, strontium ranelate increased alkaline phosphatase activity without affecting bone mineralization. On the other hand, strontium ranelate decreased bone resorption in rat calvaria or mouse long bones in organ culture systems and decreased the resorbing activity of isolated rat or mouse

osteoclastic cells. Additionally, recent data indicate that strontium ranelate can increase apoptosis in isolated rabbit osteoclasts. In clinical trials (Spinal Osteoporosis Therapeutic Intervention [SOTI]), the evaluation of bone markers showed that after 3 months, bone alkaline phosphatase levels were significantly higher in the strontium ranelate group than in the placebo group (treatment-related increase of +8.1%, $P < 0.001$) whereas C-telopeptide of type I collagen (CTX) levels were lower in the strontium ranelate group than in the placebo group (treatment-related difference of −12.2%, $P < 0.001$) and at all time points. These pharmacological and clinical studies show, therefore, that strontium ranelate acts by increasing bone formation and decreasing bone resorption, an effect that results in improved bone mass in vivo.

SS09

Implications for Bone Quality and Strength

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Various bone resorption inhibitors have been shown to decrease the risk of osteoporotic fractures. However, there is still a need for agents promoting bone formation by inducing positive uncoupling between bone formation and bone resorption. In vitro studies have suggested that strontium ranelate enhances osteoblastic cell replication leading to an increase in bone-forming activity. Simultaneously, strontium ranelate dose-dependently decreases osteoclastic activity. In vivo studies indicate that strontium ranelate stimulates bone formation and decreases bone resorption, and prevents bone loss and/or promotes bone gain. This positive uncoupling between bone formation and bone resorption results in bone gain and improvement in bone geometry and microarchitecture.

In intact female rats, a 2-year period of exposure to strontium ranelate significantly increased bone mechanical properties of vertebrae and midshaft femur. All the determinants of bone strength were positively influenced by the treatment like bone mass, dimension, microarchitecture, and intrinsic bone tissue quality. The increment in bone mechanical properties was characterized by an increase in ultimate strength but also by a dramatic improvement in energy to failure, which was essentially due to an increment in plastic energy. Such modifications observed with strontium ranelate treatment are in good agreement with the improvement in intrinsic bone quality and also in trabecular bone mass leading to greater bone resistance. These results strongly suggest that new bone formed following strontium ranelate treatment is able to withstand greater deformation before fracture while possessing similar elastic properties to normal bone. Furthermore, treatment with strontium ranelate fully prevents the deleterious effect of ovariectomy on bone strength. In this model, a 1-year period of exposure to strontium ranelate significantly prevents alteration of bone mechanical properties of vertebrae and proximal tibia in association with a partial preservation of the trabecular microarchitecture: a dose-dependent effect on the bone volume/trabecular volume ratio and trabecular number and thickness.

In conclusion, strontium ranelate, a new treatment of postmenopausal osteoporosis, acts through an innovative mode of action, both stimulating bone formation and inhibiting bone resorption, resulting in a rebalance of bone turnover in favor of bone formation. Strontium ranelate increases bone mass while preserving the bone mineralization process and improving intrinsic bone tissue quality, resulting in an improvement in bone strength and bone quality.

SS10

Vertebral and Hip Antifracture Efficacy of Strontium Ranelate

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Strontium ranelate is a new treatment for patients with postmenopausal osteoporosis registered in the European Union for the prevention of vertebral and hip fractures. Strontium ranelate has a dual mode of action, both increasing bone formation and decreasing bone resorption, which rebalances bone turnover in favor of bone formation and increases bone strength.

The efficacy of strontium ranelate, 2 g per day orally, in the treatment of postmenopausal osteoporosis has been investigated in an international (12 countries), large-scale, multicenter (75 centers), phase 3 program. The prevention of vertebral and peripheral fractures has been assessed, as main criteria, in SOTI (Spinal Osteoporosis Therapeutic Intervention) and in TROPOS (TReatment Of Peripheral OSteoporosis), respectively, both these studies being randomized, double-blind, and placebo-controlled, with a main statistical analysis over 3 years of treatment. A preplanned pooled analysis of SOTI and TROPOS was also performed over 3 years.

A total of 1649 (mean age ± SD: 69.7 ± 7.3 years) and 5091 (mean age ± SD: 76.8 ± 5.0 years) postmenopausal women were included in SOTI and TROPOS, respectively, with patients receiving calcium and vitamin D supplementation throughout the studies. At baseline, the mean (SD) lumbar spine bone mineral density (BMD) T-score was −3.6 (±1.2) in SOTI and the mean (SD) femoral neck BMD T-score was -3.1 (±0.6) in TROPOS.

Significant early (after 1 year) and sustained (over 3 years) vertebral antifracture efficacy of strontium ranelate was demonstrated in patients with prevalent vertebral fracture with reductions in the relative risk of 49% ($P < 0.001$) and 41% ($P < 0.001$) in the strontium ranelate group compared with placebo. In addition, the relative risk of clinical vertebral fracture was significantly reduced by 52% ($P = 0.003$) after 1 year and by 38% ($P < 0.001$) over 3 years in

the strontium ranelate group compared with placebo. Strontium ranelate was also demonstrated to significantly decrease the relative risk of vertebral fractures by 48% ($P < 0.001$) in patients without prevalent vertebral fracture over 3 years, versus placebo. Strontium ranelate was proven to significantly reduce the relative risk of peripheral fracture by 16% compared with placebo ($P = 0.04$); in osteoporotic patients aged 74 years or over, strontium ranelate achieved a significant decrease in the relative risk of hip fracture of 36% ($P = 0.046$).

The change in BMD was measured at 3 years in both studies: compared with placebo, in SOTI, lumbar BMD was significantly increased by 14.4% in the strontium ranelate group, while in TROPOS, femoral neck BMD was significantly increased by 8.2% (relative changes from baseline between groups, $P < 0.001$ in both studies).

Thus, strontium ranelate, 2 g per day orally, is a new, effective treatment for patients with postmenopausal osteoporosis for the prevention of vertebral and hip fractures.

SS11

Recent Advances in the Management of Postmenopausal Osteoporosis

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Bone loss in osteoporosis is related to the imbalance between bone resorption and bone formation. Bisphosphonates and selective oestrogen receptor modulators decrease bone loss by inhibiting the rate of osteoclastic bone resorption and have been shown to decrease the incidence of fragility fractures. The 1−34 fragment of human parathyroid hormone has been shown to increase bone formation more than resorption by increasing osteoblastic activity, and also to decrease the risk of fragility fractures. An ideal approach to osteoporosis would be a treatment that is capable of combining these effects of increasing bone formation and reducing bone resorption.

Strontium ranelate, a new antiosteoporotic treatment, is the first to both increase bone formation and decrease bone resorption, rebalancing bone turnover in favor of formation. It has been demonstrated to prevent vertebral and hip fractures in a wide range of patients.

Following the preplanned pooled analysis of the two pivotal phase 3 studies, SOTI (Spinal Osteoporosis Therapeutic Intervention) and TROPOS (TReatment Of Peripheral OSteoporosis), strontium ranelate was also shown to be effective even in elderly patients (age $\geq$80 years), who were demonstrated to have a significant reduction in both vertebral (relative risk reduction [RRR], −32%; $P = 0.013$) and peripheral (RRR, −31%; $P = 0.011$) fractures versus placebo, over 3 years. In addition, strontium ranelate was proven to be effective in postmenopausal patients at risk of osteoporosis, as it significantly reduced the vertebral fracture risk by 72% in patients with lumbar and/or femoral neck osteopenia (both scores being nonosteoporotic) and no prior fracture, over 3 years, versus placebo ($P = 0.045$).

Strontium ranelate has a good general (especially at the upper gastrointestinal level) and bone tolerability profile and has been shown to significantly improve the quality of life of patients, measured with the QUALIOST questionnaire.

Thus, strontium ranelate, 2 g per day orally, is a new, effective, and safe agent for the first-line treatment of postmenopausal patients with osteoporosis, whatever the severity of the disease, even in elderly patients, thereby improving their quality of life.

Update

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Corrigendum

Corrigendum to "IBMS-ECTS 2005 Abstracts" [Bone 36 (2005) S466]

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The authors of abstract P725-Tu wish to make a correction to the author order. For the readers' convenience, the entire abstract appears below.

P725-Tu

Physician and Patient Satisfaction, Tolerability and Safety during Raloxifene Treatment in PM Women

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Raloxifene is indicated for the treatment of osteoporosis in postmenopausal women. The decision to continue or terminate treatment based on patient and physician treatment satisfaction and overall safety is often made within the first 4 months. This project was designed to obtain data of these variables after 3 to 4 months of Raloxifene therapy. Patient satisfaction of 1250 postmenopausal women (66.8 ± 10.3 years) with osteoporosis or osteopenia as diagnosed by DEXA was assessed by asking if patients would continue with Raloxifene after receiving more details about its properties and by rating treatment tolerability on a 5-point rating scale. Physician satisfaction of 187 physicians (GPs, rheumatologists, gynecologists) was assessed based on overall satisfaction, willingness to continue therapy and overall positive assessment compared with other treatment options after 4 months. Predefined symptoms (gastrointestinal symptoms, breast tenderness/pain, vaginal bleeding/spotting, skin/mucous membrane symptoms, and mental problems) were assessed at baseline and after 3 and 4 months of therapy. Overall safety was assessed by recording the patients' spontaneously reported symptoms within the first 4 months. After 4 months, 84.9% of the patients wished to continue Raloxifene treatment; 82.6% assessed its tolerability to be very good or good, respectively. 89.4% of the physicians confirmed general satisfaction with treatment, and 86.4% wished to continue therapy. 70.6% of the physicians considered Raloxifene as advantageous compared with other treatments. The percentage of patients reporting a severe or moderate degree of all predefined symptoms had decreased statistically significantly ($P<0.0001$, McNemar test) after 4 months compared with baseline (improvement from 6.6% to 10.1%). 10.4% of the patients spontaneously reported any type of symptoms. Hot flushes were most frequently reported (16.9%) followed by edema (13.8%), abdominal symptoms (7.7%), nausea, and headache (5.4%).

The evaluation showed an excellent patient and physician satisfaction with Raloxifene in a routine clinical setting over 4 months. Its good safety profile as revealed by the decreasing frequency of predefined symptoms and low incidence of spontaneously reported symptoms makes it ideally suited in the long-term treatment of postmenopausal women with osteoporosis.

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