Invited speakers

I1 – Transgenic models in osteoporosis and skeletal aging

M Amling, M Priemel, AF Schilling, P Pogoda, JM Rueger

Department of Trauma Surgery, Hamburg University School of Medicine, Hamburg, Germany

While our understanding of the developmental biology of the skeleton, like that of virtually every other subject in biology, has been transformed by recent advances in human and mouse genetics, we still know very little, in molecular and genetic terms, about skeletal physiology. Thus, among the many questions that are largely unexplained are the following: why is osteoporosis mainly a women's disease? How is bone mass maintained nearly constant between the end of puberty and the arrest of gonadal functions? Molecular genetics has emerged as a powerful tool to study previously unexplored aspects of the physiology of the skeleton. Among mammals, mice are the most promising animals for this experimental work. This has been previously demonstrated e.g. trough the tremendous impact of different osteopetrotic models on our molecular understanding of osteoclastic bone resorption. Until recently the only way to study bone loss situations and osteoporosis in mice was by using ovariectomy with all its limitations. Today, however, we have access to more sophisticated osteoporotic mouse-models from four different origins: Transgenic mice (HSV-TK), knock-out mice (OPG; TTD-XPA), inbred-strains (SamP6), and trough physiological modulation (icv application). These new models have already taught us several important lessons. The first is, that bone remodeling is more than just an autocrine/paracrine process. Multiple experimental evidence has demonstrated that the latter regulation exists, but genetics proves that there is no functional cross-control between resorption and formation. The second lesson is that remodeling is, at least in part, subject to central regulation. Thus, osteoporosis is partly a central or hypothalamic disease. The most dramatic change and the most important advantage we feel is, that today we have models to test a new hypothesis regarding the etiology of osteoporosis before it turns to dogma. Taken together, mouse-studies may lead to a shift in our physiological understanding of skeleton biology and to the emergence of novel paradigms. These, in turn, should help us to devise new treatments for degenerative diseases of the skeleton such as osteoporosis and its associated clinical problems.

I2 – The physiological role of liver-derived IGF-I

C Ohlsson

RCEM, Dept of Internal Medicine, Sahlgrenska University Hospital, Göteborg, Sweden

Insulin like growth factor-I (IGF-I) is important for postnatal body growth and is expressed in most tissues with the highest expression found in the liver. The relative importance of liver-derived IGF-I versus locally produced IGF-I is unclear. We have abolished IGF-I production in the livers of mice by using the Cre/loxP recombination system. These mice demonstrated complete inactivation of the IGF-I gene in the hepatocytes. This lead to a decrease in serum IGF-I by 85% confirming that the liver is the principal source of IGF-I in the blood. We have earlier shown that the reduction in serum IGF-I concentration had no discernible effect on longitudinal bone growth in the long bones. However, recent studies demonstrate that the crown-rump length and the periosteal cortical bone growth are partially reduced in the liver-inducible-IGF-I knockout (LI-IGF-I-/-) mice. Long-term metabolic studies on these mice showed that they have a decreased fat mass (-25%) at 12.5 months of age. Both females and males had significantly reduced peritoneal and parametrial/epididymal fat pads. LI-IGF-I-/- mice also demonstrated elevated insulin and cholesterol levels. Furthermore, we have recently demonstrated that liver-derived IGF-I is an important regulator of cardio vascular functions. In conclusion, liver-derived IGF-I regulates periosteal cortical bone growth, carbohydrate- and fat- metabolism and cardio vascular functions, while it is not required for longitudinal bone growth.

I3 – Bone-cartilage interface: a common interest of bone and cartilage researchers or no man's land

K Väänänen

Institute of Biomedicine, Department of Anatomy, University of Turku, Turku, Finland

During the development of human skeleton cartilage anlage gives birth to bone and skeletal growth is ensured by continuous "transformation" of growth plate cartilage to primary spongiosa. At this interface calcified cartilage is under continuous vascular invasion and resorption and somehow induces formation of primary spongiosa. Several cellular processes are thus in operation simultaneously in a very restricted tissue area; namely vas-

cular invasion, cartilage mineralization and subsequent cartilage resorption, bone formation and mineralization as well as bone resorption. A number of recent studies suggests that in addition of endocrine regulation several paracrine and autocrine regulatory processes are in function. Some of these processes will be discussed in further details.

In the adult skeleton zone of calcified cartilage provides a critical interface between articular cartilage and bone. The physiological role of this structure is still poorly understood. It serves as a structural barrier between cartilage and subchondral bone and obviously forms also an important diffusion barrier for most, if not all biological substances. The role of calcified cartilage e.g. in osteoarthrosis remains an enigma. It has been proposed to have some function in the remodellation of articular cartilage. Possible direct regulatory role(s) of bone to cartilage metabolism and vice versa are still obscure and remains to be clarified. Identification and revealing of new paracrine mechanisms involved in cellular activities at cartilage-bone interface are of outmost importance in order to understand better the pathophysiology of joint and bone diseases and to develop new therapeutic strategies for them.

I5 – Use of biochemical markers in the early detection of RA and OA

J Risteli

Dept. of Clinical Chemistry, University of Oulu, Oulu, Finland

Rheumatoid arthritis (RA) and osteoarthritis (OA) lead to destruction of bone and cartilage. In RA there is a chronic inflammation, which results in local degradation of connective tissue, synovial alterations, periarticular osteoporosis and bone erosions. OA is a non-inflammatory disease with intermittent inflammatory episodes. The reason for joint failure in OA is not known, but there are changes in the metabolism of the surface of the cartilage. The progression of cartilage lesions probably requires stiffened subchondral bone.

One of the degradation products of bone type I collagen, ICTP, which mainly reflects matrix metalloproteinase activities, is often increased in the serum of patients with RA. Elevated ICTP is a predictor of a more erosive disease course. Other serum markers of type I collagen degradation, CrossLaps or NTx, reflect the activity of cathepsin K, these assays being sensitive to physiological bone turnover. Thus ICTP reflects pathological degradation, whereas CrossLaps is increased when normal bone turnover is locally or generally increased. Also urinary Glc-Gal-PYD and CTX-II (degradation marker of type II collagen) and serum MMP-3 (stromelysin-1) may be associated with the progression of joint damage in RA. The synthesis rate of type I collagen, as evidenced by PINP and PICP in serum, is usually not increased, whereas the circulating concentration of the aminoterminal propeptide of type III collagen (PIIINP) is often increased in early RA, reflecting synovial activity. Cartilage-derived protein fragments (e.g. from aggrecan or COMP) may be increased in synovial fluid, but seldom in blood. Serum hyaluronan and YKL-40 also reflect synovial disease activity.

In osteoarthritis, the above markers are usually normal, although occasionally elevated levels can been found. Production of an alfa1-homotrimer of type I collagen has been reported. This variant of bone type I collagen shows local reduction of mechanical stability and decreased mineralisation. We have developed an immunoassay (hotPINP) to measure its rate of synthesis, but more studies are needed to evaluate its significance.

I6 – Use of magnetic resonance techniques in the early detection of RA and OA

M Ostergaard[1,2]

[1] Depts of Rheumatology and MRI, Copenhagen University Hospital at Hvidovre, Denmark
[2] Depts of Rheumatology, Copenhagen University Hospital at Herlev, Denmark

The cornerstones of diagnosis and monitoring of rheumatological diseases are the history and physical, laboratory and radiographic examinations. However, the soft tissues, which are the site of primary involvement in most rheumatological disorders, are not or only very poorly visualised by radiography. The cartilage can only be evaluated indirectly, through assessment of joint space narrowing. In inflammatory joint diseases, the conventional clinical and laboratory methods are neither very sensitive nor specific, particularly not in the early phases of disease.

MRI allows multiplanar imaging with unprecedented soft tissue contrast and high spatial resolution. Bone, cartilage, synovium, tendons, tendon sheaths, ligaments, entheses and muscles can all be visualised. Thus, MRI allows ìwhole organ evaluationî.

Numerous MRI techniques for assessment of cartilage have been reported, including conventional imaging techniques focusing at signal intensity changes reflecting inner structure changes and/or visualization of surface lesions, but also quantitative techniques as thickness mapping and volume measurements. More sophisticated techniques for tissue characterization (e.g. glycosaminoglycan content and collagen structure) exist. Albeit promising, the use of these techniques in OA clinical trials and, particularly, clinical practice is still very limited. Improved validation and feasibility combined with more effective treatments would be expected to change this.

There is accumulating evidence that MRI in early RA and other inflammatory joint diseases identifies both inflammatory and destructive joint changes, in periph-

eral as well as axial joints, with a higher sensitivity than conventional methods. MRI appears useful in detection and monitoring as well as prognostication of early RA, but larger long-term follow-up studies are needed.

The superior sensitivity of MRI may be of major significance in both clinical trials and practice, e.g. lead to an earlier diagnosis and more sensitive monitoring of treatment. In trials, using MRI may allow reductions in the trial size and length due to more sensitive separation of responders from non-responders.

I7 – ODF, RANK and osteoprotegerin regulation of osteoclastic activity

Ö Ljunggren

Dept of Medical Sciences, Uppsala University, Sweden

Osteoclastic activity is regulated by signals from the bone forming osteoblasts. This has been known for two decades. However, the exact mechanism by which the osteoblasts transmit a signal to the osteoclasts has only recently been discovered. The initial finding was the cloning of osteoprotegerin (OPG), a secreted member of the tumor necrosis factor receptor family. Transgene mice overexpressing OPG developed severe osteopetrosis. OPG was found to be secreted from several cell types, eg the bone marrow stromal cells, and is regulated by several of the known bone resorbing factors. The exact role of OPG was however not known until the further discovery of two cell surface bound proteins called osteoclast differentiating factor (ODF; also called RANK ligand) and RANK (Receptor activtor of NF kappa beta). ODF is expressed on the surfaces of the osteoblasts / bone marrow stromal cells, and RANK is expressed on the surfaces of the osteoclast progenitor cells, and on mature osteoclasts. ODF binding to RANK is the signal that causes the osteoclast progenitor cells to differentiate along the osteoclast pathway, and is also essential for the maintenance of osteoclastic activity. OPG, finally, is a decoy receptor interrupting this signalling by binding to ODF. Hormones and cytokines that stimulate bone resorption, such as PTH, PGE2, and various cytokines, act via the osteoblasts. All these agents have receptors on the osteoblast and not on the osteoclasts. Stimulation of osteoblasts by bone resorbing substances upregulate the expression of ODF on the cell surfaces, and down regulate the amount of secreted OPG. Thus, it is the relative proportions of OPG and ODF in the bone marrow microenvironment that determine the osteoclastic activity.

Conclusion: OPG, ODF and RANK are the essential signalling molecules in the regulation of osteoclastic activity. Bone resorbing agents affect osteoblasts, causing them to upregulate the cell surface protein ODF, and to down regulate OPG. Binding of ODF to RANK on osteoclast surfaces is the signal that cause enhanced bone resorption.

I8 – Role of receptor activator of nuclear factor-kappaB ligand and osteoprotegerin in bone biology

LC Hofbauer

Department of Medicine, Philipps University, Marburg, Germany

Receptor activator of NF-kappaB ligand (RANKL), its receptor, receptor activator of NF-kappaB (RANK), and the decoy receptor, osteoprotegerin (OPG) constitute a novel cytokine system. RANKL produced by osteoblastic lineage cells is the essential factor for osteoclast formation, fusion, activation, and survival, thus resulting in bone resorption and bone loss. RANKL activates its specific receptor, RANK located on osteoclasts. The effects of RANKL are counteracted by OPG which acts as a neutralizing receptor. RANKL and OPG are regulated by various hormones (glucocorticoids, estrogen) and cytokines. Transgenic and knock-out mice with excessive or defective production of RANKL, RANK, and OPG display the extremes of skeletal phenotypes, osteoporosis and osteopetrosis. Abnormalities of the RANKL/OPG system have been implicated in the pathogenesis of postmenopausal osteoporosis, rheumatoid arthritis, Paget's disease, periodontal disease, benign and malignant bone tumors, bone metastases, and hypercalcemia of malignancy, while administration of OPG has been demonstrated to prevent or mitigate these disorders in animal models. The discovery and characterization of RANKL, RANK, and OPG and subsequent studies has changed the concepts of bone and calcium metabolism, has led to a detailed understanding of the pathogenesis of metabolic bone diseases, and may form the basis of innovative therapeutic strategies.

I10 – Skeletal and articular biomechanics

E Czerwinski

Dept of Orthopaedics, Medical College of Jagiellonian University, and Krakow Centre of Osteoporosis. Krakow, Poland

Skeleton provides mechanical integrity, protection of vital organs and locomotion for human body (also: mineral storage and hemopoetic function). Bones building the skeleton are unique connective tissue, extremely mineralized (trabecular bone 30%; cortical 70%). Minerals in form of hydroxyapatite crystals are bound to collagen fibers. That combination of two opposed materials, brittle and rubbery, results in a very high mechanical quality. Cortical bone of femur shows resistance on: compression 159 Mn/m^2, tension 107 Mn/m^2, torsion 53 Mn/m^2. Bone presents perfect micro- and macro architecture, which is permanently remodeled in answer to current mechanical requirements. Bone cells reacts to mechanical, electrical and biochemical stimuli. If the response of bone cells is

non adequate, microcrack may appear. Those are possible to be repaired, but in osteoporotic conduction this process may be too slow, and further microcracks will follow with the treats of fracture.

Limb motion takes place in an articular joint. Bone, as a passive force lever is moved by the muscle force, under the control of neurological system. Motion is controlled by unconditional and conditional reflexes, based on information from sensors in tendons, muscles and sense organs. Upper limb joint are only under the tension of muscle power, while in lower extremities body weight is an additional force. This force is increased five times on running. Motion between two bodies under the pressure inevitably includes friction and material wear. Biological response of the articular cartilage to this phenomenon is the capacity of cushion schlock abortions and lowest possible friction coefficient.

On the contrary to bone, cartilage is non-mineralized, but contains up to 75% of water. Fibres of collagen constructs fine network of architecture keeping inside clusters of chondral matrix. This consists of proteoglycan agrecan build of gluglycosaminoglycan bound to hyaluronian. Those have hydrostatic tensions trying to expand as much as a collagen fiber allows them. Specific orientation of collagen fibers, in five different layers of cartilage, constitutes the best possible mechanical construction for stress absorption and distribution. Chondrocytes maintain matrix network by balancing synthesis and degradation of matrix products. Cyclic loads increases while static decrease chodrocytes activity.

Friction in an articular joint is the lowest achievable in normal condition on our planet (friction coefficient 0.001–0.005.). This is achieved by synovial fluid lubrication. Specific micro- and macro unevenness of the cartilage surface submits, a unique, bio-hydro-dynamic lubrication of the joint, what is out of range in technical world.

Through remodeling process, cartilage can adapt or optimize its properties to meet changes in function demand. If this process is not efficient, deterioration of mechanical properties will follow. Primary fibrillation in the superficial layer of cartilage will occur and acceleration material wear might proceed. Catabolic processes will supervene in cartilage metabolism and osteoarthritis will be unavoidable.

The role of biomechanics in bone and join pathology is still underestimated.

I11 – The relevance of structural integrity for bone strength

C-C Glüer

Medizinische Physik, Klinik für Diagnostische Radiologie, Universitätskrankenhaus Kiel, Germany

According to the consensus definition of osteoporosis this disorder is not only characterized by low bone mass but also microarchitectural deterioration. When this definition was developed in 1993 only very limited tools for assessing bone microstructure in vivo were available. Only in the past few years detailed knowledge about the 3-dimensional structure of trabecular bone and the role of cortical porosity have become available. Methods like high resolution computed tomography, high resolution magnetic resonance imaging and micro computed tomography have been developed and they allow to depict bone microstructure with about 100–200 micrometer in vivo and down to about 1 micrometer in vitro. There is still no consensus on how to describe microstructure comprehensively. On the one hand there are parametric methods: relevant features of bone are characterized by specific structural variables (e.g. trabecular spacing, thickness, shape, connectivity etc.). The complexity of the resulting variable sets may limit the utility of such approaches and therefore alternative methods have been developed. Most notably, finite element analysis allows to model the structural integrity of whole bones provided that sufficient computing power is at hand. Methods of texture analysis, on the other hand, may allow to define families of bone with individually different but similar structural patterns (e.g. Markov point processes). Which of these methods will work best to improve fracture risk assessment remains to be determined. However with the new technologies we are for the first time in the position to assess the clinical significance of poor microarchtiecture.

I12 – Genetics of osteoporosis

B Langdahl

Aarhus Bone and Mineral Research Group, Dept. of Endocrinology, Aarhus Amtssygehus, Denmark

Osteoporosis is a common disease with a strong genetic component. Family and twin studies have shown that several of the features that are known predictors of fracture risk: bone mineral density, ultrasound properties of bone, bone turnover and hip geometry as well as fracture risk per se are influenced by genetic factors. It has also been demonstrated that the genes involved are likely to be many each with a modest effect.

Linkage studies in humans have defined several loci on chromosomes 1, 5 and 6 that show linkage to bone mineral density. The causative genes have not yet been identified. Linkage studies have also been performed in experimental animals and linkage has been found between several loci and bone mineral density or bone structure. The genes that will be discovered from these animal studies will most likely be in two categories: genes that influence bone mineral density in both animals and humans and genes that are only relevant in the animals examined, but may provide information about basic mechanisms or pathways.

So far most work in the field of genetics of osteopo-

rosis in humans has been done as candidate gene studies in populations or in cases and controls. The most widely studied genes are the vitamin D receptor (VDR), the collagen type Ia1 (COLIA1) and the oestrogen receptor (ER). An Sp1 binding site polymorphism has also been identified in the COLIA1 gene, which predicts osteoporotic fractures independently of bone mass, and there is functional evidence to suggest that this polymorphism influences gene transcription and subsequently bone quality. Polymorphisms of the VDR have been associated with bone mass in several studies and there is evidence to suggest that these effects may be modified by dietary calcium and vitamin D intake. The exact effects of these polymorphisms are still to be unravelled. Polymorphisms in the ER gene have been associated with BMD and increased fracture risk, but the underlying mechanisms are presently unknown. Unfortunately, many of these candidate studies are underpowered and this has led to conflicting results in different populations.

Despite the fact that osteoporosis generally is a polygenetic disease, abnormalities in bone mass can be caused by mutations in a single gene. Recently, mutations in the LRP-5 gene were demonstrated to cause either osteoporosis-pseudoglioma or a high bone mass phenotype. These findings lead to the discovery of the importance of the Wnt-signalling pathway in bone metabolism.

Knowledge about the genes involved in the pathogenesis of low bone mass and osteoporotic fractures are important since it can lead to improved fracture risk assessment, discovery of pathways and factors that are involved in bone metabolism and design of new treatments to prevent or treat osteoporosis.

I13 - Assessment of fracture risk

O Johnell

Department of Orthopaedics, Malmoe University Hospital, Malmö, Sweden

The diagnosis of osteoporosis is based on a BMD measurement and expressed as T-scores. The golden standard for BMD measurement is at present the proximal femur by DXA. However, the clinical significance of osteoporosis rests with the fractures that arise as a consequence of osteoporosis. Low bone mass is an important component of the fracture risk but BMD measurement does not capture all abnormalities in the skeleton that contribute to skeletal fragility. The use of T-score is according to the WHO definition based on DXA measurement of the hip, spine or wrist and the T-scores for different sites have different absolute risks of fractures. At present the T-score cannot be used for other measurements than those in the WHO definition. To incorporate these techniques absolute risk is probably the best.

Furthermore, other risk factors such as propensity to fall is important. Several other risk factors for osteoporosis also contribute to the fracture assessment, e.g. low BMI, previous fragility fracture, use of glucocorticoids, heredity etc.

Thus there is a distinction to be made between the diagnosis of osteoporosis and assessment of fracture risk which implies a distinction between diagnostic and intervention thresholds.

Intervention thresholds should be based on fracture risk probability. The best is to use a 10-year fracture risk and incorporate several risk factors with and without BMD. This also implies a case-finding approach since there are no studies to support a screening strategy at present.

The risk assessment can be made in several steps. One step is if there are strong risk factors such as age and previous fractures, then an intervention based on the risk factors with a very high absolute 10-year risk, if there is an intervention trial showing that treatment is beneficial in this risk group. For weaker risk factors BMD measurement has to be performed to identify high risk patients.

The treatment thresholds can be based on cost effectiveness analysis, both for men and women based on the moribidity of all osteoporotic fractures.

Assessment of fracture risk will improve the detection of patients for intervention and select the right patients.

I14 – Designing transgenic mouse models for cartilage disorders

E Vuorio

Department of Medical Biochemistry and Molecular Biology, University of Turku, Turku, Finland

The extracellular matrix of hyaline cartilage forms a complex, multicomponent system of collagen fibrils containing several different collagen types, and of proteoglycan molecules which are entrapped or bound to the fibrils. This structure and physiologic exposure of cartilage to alternating pressure, makes it is essentially impossible to study structure-function relationships of different cartilage components in vitro. Cartilage thus forms a good example of a complex biological system where genetically engineered mice provide distinct advantages over other experimental models as they allow the effects of abnormal gene function to be studied in their natural environment.

Several different approaches are currently in routine use for production of genetically engineered mice depending on the type of question to be asked. As the techniques and subsequent characterization of resultant phenotypes are costly and time consuming, careful planning of the experiments is a must. The aim of the workshop is to introduce to the audience the different approaches available for production of transgenic mouse models for cartilage diseases.

For reproduction of dominant negative mutations, for overexpression of a normal gene, and for aberrant expression of a gene, production of traditional transgenic

mice by microinjection is still a feasible approach. However, for reproduction of recessesive phenotypes inactivation or targetting engineering of the endogenous allele must be employed. Finally, technical developments have made it possible to construct conditional knock-out mice, where the desired genetic change will take place at a later stage of development using cre recombinase to modify target DNA at loxP sites.

Phenotypic characterization of the resultant mice is another challenging part of transgenics research. Such phenotypes may range from severe derangement of skeletal development (and intraembryonic lethality) to very mild phenotypes occurring only upon ageing or as an increased trend to develop a degenerative disease 8such as osteoarthrosis). These phenotypes illustrate the dual role of cartilage as a transient model for bone formation, and as a permanent tissue providing structural support e.g. in articular, aural and nasal cartilage. Finally, some examples of transgenic mouse models for cartilage disorders, both chondrodysplasias and osteoarthritis, will be presented.

I15 – Transgenic Del1 mice as model of cartilage disorders with special emphasis to knee osteoarthritis

A-M Säämänen

Department of Medical Biochemistry and Molecular Biology, University of Turku, Turku, Finland

The working hypothesis was, that mutations in type II collagen reduce structural integrity of collagen network in cartilage, and predispose to osteoarthritis (OA). This presentation aims at describing the authors practical experience in characterizing the OA phenotype in Del1 mice.

Methods: Del1 mice were generated by oocyte microinjection of mouse Col2a1 transgene with a 150 bp deletion of exon7 - intron7 into C57Bl/6 x DBA founder. Changes in the knee joint morphology and molecular biology were studied by radiological, histological, immunohistochemical and mRNA expression methods between 2 to 22 months of age in male Del1 mice heterozygous for the transgene locus and in nontransgenic littermates that served as controls. Every 20th section of paraffin embedded hind limbs was hematoxylin-eosin stained, and evaluated for changes in histology. Cartilage erosion was scored with respect to depth of penetration (grades 0-4). Life-long voluntary running was used to characterize the cartilage resiliency.

Results: Cartilage erosion initiated by superficial fibrillation at 3 months of age. It progressed to tide mark by 6 months and to subchondral bone by 9 to 15 months, and was accompanied by bony sclerosis, degeneration of the menisci, mineralization of various joint structures and subchondral cysts. Similar, but less severe and later appearing changes were also observed in nontransgenic littermates. Running increased the severity of OA in Del1 mice, suggesting reduced mechanical properties of articular cartilage.

Onset of OA was accompanied by up-regulation of COMP mRNA synthesis, altered tissue distribution and increased secretion into serum. Simultaneously, MMP-13 mRNA expression increased in deep calcified cartilage and adjacent subchondral bone, suggesting bone remodeling. A possible secondary response to cartilage erosion was observed in synovial tissue, which was hyperplastic and rich of TIMP-1 and MMP-13.

Conclusion: Knee joint degeneration in Del1 mice greatly resembles human OA. Del1 mice provide a well-characterized model to study molecular changes in OA development with possible applications in testing various therapeutic approaches for the disease.

I16 – New treatment developments: SERMS and PTH

EF Eriksen

Eli Lilly & Co., Indianapolis, USA

Selective estrogen receptor modulators (SERMS), of which raloxifene (Evista) is the only one on the market, act as antiresorptive drugs. They inhibit bone turnover and increase BMD. Spinal fractures are reduced by 30–50%. Traditionally, the reduction of bone turnover is associated with preservation of bone structure and reduced bone loss. However, recent data have demonstrated that this may not be the only mechanism, whereby raloxifene exerts its positive action on bone. Over a wide range of BMD changes (-8 to +8% over 3 years) raloxifene shows a persistent reduction in fracture incidence. This indicates BMD independent positive effects on bone quality, most of which are still poorly defined. Moreover, the presence of preserved antifracture efficacy despite loss of bone mineral, calls in question the use of BMD for monitoring effects of raloxifene in the clinical setting. Bone markers may be a better choice in this situation. With the current controversy around the cardiovascular effects and the breast cancer issues related to HRT, Raloxifene emerges a drug well suited for long term prophylaxis. This SERM reduces the risk of breast cancer, lowers cholesterol and has recently shown additional protective effects on cardiovascular events in high-risk women.

The 1-34 fragment of PTH (PTH(1-34), teriparatide) has shown pronounced anabolic effects in a large controlled trial. Recent analyses of bone biopsies have demonstrated that the drug, not only improves cancellous bone architecture, but also has pronounced positive impact on cortical bone. Moreover, the drug changes geometry of several bones of the appendicular skeleton, thereby further improving their biomechanical properties. Follow up of patients using teriparatide for a median of 21 months have shown persistence of antifracture effi-

cacy for an additional 26 months.

Teriparatide elicits significant improvements of bone quality over a period of 18-24 months. After termination of treatment the doctors using this drug are faced with a new dilemma, – how to maintain the improvements achieved. In this context a whole new area of maintenance therapies opens up. The use of bisphosphonates, SERMS and HRT in this context will be discussed.

I17 – New treatment developments (II)

MJ Seibel

Dept of Endocrinology & Metabolism, University of Sydney at Concord, Australia

The prevention and treatment of osteoporosis aims at reducing bone loss, normalising bone turnover and avoiding new or further fractures. Over the past decades, a multitude of drugs or components with either antiresorptive (ie. osteoclast inhibiting) or anabolic (i.e. osteoblast stimulating) or even combined activities has been identified. Some of these have been tested in clinical phase II and III trials, but the majority of the newer substances is still on its way from the bench to the bed.

Amongst anti-resorptive agents, most progress has been made with the development of potent, third generation Bisphosphonates, such as Alendronate, Ibandronate, Risedronate, and, most recently, Zolendronate. While promising clinical results have been presented for the selective estrogen-receptor modulators (SERMs, e.g. Raloxifene, Lasofoxifene), the skeletal effect of other components such as statins, calcium receptor modulators, osteoprotegerin, anti-cytokines, proton pump inhibitors, nitric oxide modulators and certain enzyme inhibitors is still under investigation.

As far as anabolic treatments are concerned, the use of parathyroid hormone (PTH), PTH-related peptide and their analogues in osteoporosis have yielded exciting new results and justifiably stimulated high expectations. Other less well-evaluated anabolic strategies may include the application of high dose oestrogens, statins, strontium salts, growth factors, prostaglandins, endothelins, amylin, mechanoreceptor modulators and cellular signalling targets such as SMADs.

In the light of secular demographic trends in most countries and the anticipated worldwide epidemic of osteoporotic fractures, the development of effective, safe and cost-efficient drugs for the treatment and prevention of osteoporotic bone disease seems absolutely paramount.

I18 – Statins and bone: evidence of bone anabolic and antiresorptive in-vitro effects of statins

L Mosekilde

Aarhus Bone and Mineral Research Group, Aarhus University Hospital, Aarhus, Denmark

Recent studies have suggested that statins in addition to their lipid lowering effect may be useful in the treatment of skeletal disorders, as statins may exert antiresorptive as well as bone anabolic effects. The intracellular effects of statins may be similar to the mechanism of action of nitrogen-containing bisphosphonates (i.e. aminobisphosphonates). Both drugs act on the intracellular mevalonate pathway by which cholesterol is synthesed from 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA). Statins reduce the cholesterol synthesis by inhibiting the HMG COA reductase, whereas aminobisphosphonates inhibit another enzyme further downstream the mevalonate pathway. Thereby, the synthesis of small lipid chains is reduced. Normally, these small lipid chains exerts important intracellular functions, as they are coupled to glutamyl transpeptidases (GTPases) enabling these GTPases to be anchored into the cell membrane. By blocking this process, amionbisphosphonates cause dysfunction and apoptosis of osteoclasts. Thus, by blocking the mevalonate pathway, statins may (like aminobisphosphonates) exert antiresorptive effects. However, although in vitro studies have demonstrated that statins decrease the formation of osteoclast there is a lack of experimental data on the potential antiresorptive effects of statins. In addition to a potential antiresorptive effect, statins may exert bone anabolic effects by increasing the synthesis of bone-morphogenetic protein 2 (BMP-2). BMP-2 is a growth factor that stimulates osteoblast differentiation and causes new bone formation. Statins have been shown to stimulate osteoblast proliferation and differentiation as well as new bone formation in organ cultures of neonatal murine calvaria. Furthermore, 4 weeks of treatment with simvastatin or lovastatin have been shown to cause a 35% increase in trabecular bone volume in ovariectomized as well as in intact rats. Similarly, in rats treated with either simvastatin or saline by a gastric tube for 3 months, the femoral periosteal middiaphysial bone formation rate increased significantly in the simvastatin treated animals compared with the saline group. Consequently, experimental studies on the intracellular effects of statins on bone are promising. Treatment with statins may like aminobisphosphonates reduce bone resorption and in addition exert bone anabolic effects. Further experimental studies on the mechanism of action on bone should focus on the bioavailability of statins. The statins that are commercially available at present are designed for treating hypercholesterolaemia. They are extensively metabolised in the liver, and only a small part of a peroral statin dose reaches the skeletal tissue. Thus, it may be necessary to administrate statins in a manner by which they reaches the systemic circulation before they are metabolised in the liver (e.g. trans-

dermal application) in order to obtain clinical relevant concentrations in bone tissue.

I19 – Statins and bone: evidence from clinical studies

L Rejnmark

Aarhus Bone and Mineral Research Group, Aarhus University Hospital, Aarhus, Denmark

Statins have been suggested as potential agents in the management of osteoporosis. In several epidemiological studies, treatment with statins has been associated with a decreased fracture rate, whereas no effect on fracture rates has been found in subjects with hyperlipidaemia treated with non-statin lipid-lowering drugs. According to these studies, statins may reduce the risk of fractures by as much as 60%. However, other epidemiological studies as well as reanalysis of adverse event records from large drugs trials in hyperlipidaemic subjects have failed to demonstrate an association between statin treatment and fracture risk. Moreover, most epidemiological studies have failed to demonstrate an association between dose of statin, length of treatment, and risk of fracture. So fare, there have been no randomised-controlled trials on statins and fracture rates. Similarly, there is a lack on studies on the potential effect of statins on bone tissue. Although animal experimental studies in rodent have shown promising results with increased bone formation and increased bone strengths in response to statin treatment, conflicting results have emerged from human studies. An increased bone mineral density (BMD) has been found in one study, whereas other studies have failed to demonstrate an effect of statins on BMD. Similarly, conflicting results on levels of biochemical bone markers have been reported, as increased, decreased, or unchanged levels have been found in statin treated subjects. Explanations for the conflicting results could be that most of the studies have been either uncontrolled or of a very short duration (few weeks).

In a recent cross-sectional study from our group, 140 postmenopausal women, who had been treated with a statin for more than two years were compared with 140 age- and sex-matched postmenopausal controls. BMD did not differ between the two groups. However, plasma levels of markers of bone-formation and -resorption were significantly (10–15%) lower in the statin treated women, indicating a week antiresorptive effect of statins on bone. Thus, at present no clinical studies have been able to demonstrate a biological effect of statins on bone that may account for the marked reduced fracture risk shown in some epidemiological studies. Long term randomised controlled studies are needed to resolve whether statins affects bone and fracture risk in a clinically relevant manner.

I20 – Densitometry in children

R Lorenc, H Matusik, P Pludowski, M Jaworski

Dept of Biochemistry and Experimental Medicine, The Children's Memorial Health Institute, Warsaw, Poland

Bone mass of the whole skeleton as well as its selected parts depends directly upon volume or size analyzed segments and the density of the contained within its periosteal envelope mineralized tissue. The techniques of single and dual energy absorptiometry provide measurements of so called areal or surface bone mineral density (BMD, in g hydroxyapatite per cm^2). Generated by this technique values are dependent upon size and integrated mineral density of the scanned segments. In the overview of overview of available techniques areal BMD has been shown to be directly related to bone strength. On the same the mean volumetric mineral density (g hydroxyapatite per cm^3) can be noninvasively determined by quantitative computer tomography or by calculation of density in two projections as so called bone mineral "apparent" density (BMAD in g/cm^3). These later ones are contributing significanty in mechanical resistance evaluation when taking into account size component measurements (SSI-strenght strain index). Nevertheless even when areal BMD values are informative in general strength prediction its interpretation are affected by several additional factors. The skeleton of growing child is heterogenous in dynamics of its development. In undertaken analysis must be considered sex, calendar and bone age as well as pubertal stage and sex data. Important adjunct for these issues is the development of ultrasound data with utilization of possible discrimination of cortical and trabecular bone data.

I21 – Male osteoporosis

AH Holmberg

Department of Orthopaedics, Malmö University Hospital, Sweden

Osteoporosis and osteoporotic fractures are a world wide socioeconomical problem. 1990 we had 1.26 million hip fractures throughout the world, in 2050 the number estimated is 4.5 million.

Today about 1/3 of all hip and vertebral fractures occur in men.

Although the number of osteoporotic fractures in men is smaller, the comorbidity for men is higher compared to women and also the mortality the first year after a hip fracture. Secondary osteoporosis is more common in men. Common causes are hypogonadism, alcohol abuse, glucocorticoid excess, gastrointestinal disease, endocrine disorders and chronic renal failure.

Risk factors for osteoporotic fractures are mainly the same for men and women, for example high age, low bone mass, earlier fracture, low BMI, low physical

activity, fall tendency, malnutrition and alcohol abuse. The higher incidence of fracture in women results from quantitative differences in risk factors.

The best way to diagnose osteoporosis and assess risk for osteoporotic fractures in men today is risk factor analysis and Bone Mineral Density (BMD) measurements. Studies show that men and women have similar absolute risk for hip and spinal fracture at the same areal BMD value. This suggests using the same reference material and BMD cut off for intervention and treatment of osteoporosis in both sexes. A much smaller proportion of the men compared to women will have BMD values below the cut off.

At present, the only available treatment for men is bisfosfonates. Studies have shown BMD increases in both hip and spine, similarly to women, and in glucocorticoid treated patients also spinal fracture reducing effect.

Parathyroid hormone (PTH) has in a not yet published study produced a considerable rise in BMD both in hip and spine, during the first 12 months. This effect is similar to that in women, and hence may also have the same fracture reducing effect.

Male osteoporosis is at last getting the attention it deserves. Many questions have been answered but more remain. We need studies to help us understand more about male osteoporosis, and make intervention and prevention of future osteoporotic fractures possible.

I22 – Male osteoporosis

Ove Torring

Karolinska Institute and Department of Medicine, Södersjukhuset, Stockholm, Sweden

Osteoporotic fractures in men is increasing in frequency and occur later in life compared with women. Men also have a higher peak bone mass (PMB) to start with, higher muscle mass throughout life, less frequency of falls, larger bone size and less tendency to trabecular discontinuations. After PBM age-related decline in bone density occurs more gradually in men than in women. The testosterone levels also decline successively in this period but boneloss in men is more related to the levels of estradiol than testosterone. Recent data also suggest a significant role of estradiol in bone metabolism of elderly men.

Among predisposing factors for osteoporosis in men are low bone density, high age, increased risk of falling, history of previous fractures, low body mass, and smoking. In about half of the patients a previous or current diseases or treatments which predispose to osteoporosis can be found. Among such factors are hypogonadism, severe alcoholism, GI-diseases with malabsorption, thyrotoxicosis, hyperparathyroidism, after organ transplantation, chronic inflammatory diseases, immobilisation and use of glucocorticoid treatment.

Treatment involves calcium and vitamin D supplements more or less rutinously since many patients are dietary deficient in vitamin D and calcium. Testosterone in hypogonadism. Antiresorptive treatment with bisphosphonates should always be considered. Alendronate increases BMD and reduce the risk for new fractures as effectively as in women. Promising results has been achieved with PTH treatment in men.