Oral presentations

O1 – Abnormalities at the chondroosseous junction in an osteopetrotic rat mutation suggest a role for septoclasts in longitudinal bone growth

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A variety of cells participate in the advancing osseous front at the chondroosseous junction (COJ) through activities that contribute to the longitudinal growth of long bones. A recently identified cell, the septoclast, has been shown to participate in the removal of the last transverse septum of the hypertrophied chondrocyte column. This cell is long and slender, lies just outside the invasive capillary sprouts, and stains intensely for the cysteine proteinase cathepsin B. The apex of the septoclast ends on the transverse septum in a structure that resembles the ruffled border of osteoclasts.

Retarded bone growth is a characteristic feature of osteopetrosis, a metabolic disease characterized by reduced bone resorption. In one osteopetrotic mutation in the rat, toothless (tl), retarded bone growth at the COJ is accompanied by suppressed angiogenesis and the absence of osteoclasts. Given the structural and functional similarities of septoclasts and osteoclasts, we used this mutation without osteoclasts at the COJ to determine if septoclasts were also missing. To test the hypothesis that osteoclasts and septoclasts have a co-dependent origin at the COJ we compared this site in the proximal tibia of tl rats and normal littermates with respect to number, distribution and cathepsin B activity of septoclasts.

In the normal COJ septoclasts were present as thin cells regularly aligned along the axis of the bone and exhibited strong staining for cathepsin B. These cells were present, but fewer in number, irregularly distributed, and not as cathepsin B-positive in tl rats. Ultrastructural evaluations are in progress.

The presence of septoclasts in an osteoclast-free region in the tl skeleton suggests that these two catabolic cells at the COJ are not under identical developmental controls. However, the aberrations in number, alignment, and enzymatic activity of septoclasts in tl rats with retarded bone growth suggest that septoclasts may play a significant role in bone growth at the COJ.

O2 – Quantitative genetics of biochemical indices of bone and cartilage metabolism

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The present research was driven by a clinical problem of osteoporosis (OP) and osteoarthritis (OA). The major aim of the study was to reveal the contribution of the genetic factors into interindividual differences in circulating levels of the number of active molecules relevant for bone and cartilage biology. Our research results obtained until the present moment can be divided into 2 sections: A) Genetic analysis of bone mass and size characteristics and traits related to OA, and B) Pedigree based study of plasma concentrations of calciotropic hormones, growth factors, cytokines and biochemical indices of bone remodeling. In this part of the study we used some 150 nuclear pedigrees of ethnically homogenous Caucasian origin. Model fitting techniques of the quantitative genetic analysis was implemented to reveal effects of age, sex, sex hormones, latent environmental factors and genetic sources on variation of each of the studied variables and covariation between them. The obtained results indicated strong involvement of the putative genetic factors in determination of circulating levels of the majority of the studied biochemical indices. Thus, narrow sense heritability estimates for PTH, 25(OH)D, osteocalcin, PICP, TIMPs, IGFBP-3, TGF-b, TNF-a and M-CSF, ranged between 0.30 and 0.60, but was virtually zero for IL6. Major gene effect was clearly inferred for some of these variables especially for 25(OH)D, PICP and IGFBP-3. Genetic relationships between the studied biochemical indices, as well as between them and bone aging traits were complex. The pairwise genetic correlations varied very substantially in extent (from 0.00 to 0.60). Further extensive research is needed in this field to clarify the complex relationships between the biochemical factors and bone/cartilage characteristics.

O3 – Acid phosphatase and oxygen radical-generating activities of type 5 tartrate-resistant acid phosphatase are functionally independent

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Type 5 tartrate-resistant acid phosphatase (TRACP) is an enzyme with unknown biological function that is expressed by bone-resorbing osteoclasts and activated macrophages. Active site of the enzyme contains a binuclear iron center with two irons, one of which is redox-active. In addition to its acid phosphatase (AcP) activity TRACP is also capable of generating reactive oxygen species (ROS) in vitro. Both activities require the redox-active iron in the active site. We have studied the two activities using wild-type and mutated forms of recombinant rat TRACP. Single amino acid mutations were generated by site-directed mutagenesis targeted to the amino acids predicted to be important for the AcP activity. The mutated amino acids were either in the substrate-binding pocket of the enzyme or in the alternative glycosylation sites, but not in the amino acids coordinating the two iron atoms in the active site. The AcP activity was determined using 4-NPP as substrate, and ROS activity either by monitoring the formation of malondialdehyde acetal from degradation products of deoxyribose or by detecting the fluorescence of 7-hydroxycoumarin-3-carboxylic acid (7-OHCCA) after hydroxyl radical attack. The pH-optimum of wild-type recombinant rat TRACP was 4.5 for the AcP activity and 6.5 for the ROS generating activity. All the mutated TRACP enzymes had significantly decreased AcP activity compared to the wild-type enzyme, while the ROS generating activity was decreased, not affected or increased. The changes in the AcP and ROS generating activities did not correlate with each other in individual mutants. These results suggest that not only the substrate availability and specificity, but also the pH of the environment may regulate the function of TRACP. We conclude that the two activities of TRACP may have independent mechanisms and function in different cell types and different intracellular compartments.

O4 – Development and evaluation of a stochastic simulation of cartilage bone osteogenesis

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Both long and irregular bones tend to form via endochondral ossification and are referred to as cartilage bones. Based upon the hypothesis that bone grows and forms as a semi-deterministic /semi-chaotic system, it should be possible to accurately model the osteogenesis of cartilage bones using a stochastic simulation. A thorough review of the literature has been undertaken enabling the cell types and tissues to be identified and a set of simulation rules to be established. The operation of the simulation has been evaluated longitudinally, reporting bone and blood vessel structures as the simulation develops to completion with fusion of the epiphyses; the simulation variability has been assessed by repeat runs using the same default conditions; and the effect of independently modifying key simulation parameters has been studied. This is thought to be the first report of a stochastic simulation of cartilage bone osteogenesis. The developed structures accurately follow the growth and form of irregular cartilage bones such as the vertebrae or calcaneus. The future of the simulation is now dependent primarily upon its potential utility in the field of bone metabolism and disease.

O5 – High frequency ultrasonic assessment of the effects of anti-inflammatory drugs on cartilage using zymosan-induced arthritis in rats

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The effects of anti-inflammatory (AI) drugs on articular cartilage and subchondral bone are controversial. The current study investigates the ability of 55 MHz quantitative ultrasound (US) to detect the effects of Naproxen (NPX) and Dexamethasone (DEX) on cartilage and subchondral bone when administered orally to relieve experimental rat arthritis.

Methods: A subacute arthritis was induced in rat knees by intra-articular injection (iai) of Zymosan (ZYM) which induces synovitis and reversible cartilage damage. 96 rats were divided in 3 groups. Each one included 8 control (saline iai), 8 ZYM (2mg ZYM iai), 8 NPX (ZYM iai + 10 mg/kg/day NPX) and 8 DEX rats (ZYM iai + 0.1 mg/kg/day DEX). Patellae were explored in vitro 5, 14 and 21 days after ZYM injection (D0) using a 3-D US microscope. US assessment was based on the analysis of acoustic parameters: integrated reflection coefficient (IRC, from cartilage surface) and apparent integrated backscatter from cartilage matrix (AIBc) and cartilage/bone interface (AIBb). Results were compared to histology.

Results: ZYM induced articular surface fibrillation, that resulted in a decrease in IRC at all times ($p < 0.05$), and a regularization of the cartilage/bone interface at D5 and D14 that induced an increase of AIBb ($p < 0.05$). NPX treatment had no effect on surface alterations (significant decrease of IRC) while DEX administration prevented them (IRC value was similar to that of controls). NPX partially compensated the AIBb increase due to ZYM ($p < 0.05$) at D14. AIBb of DEX rats was similar to that of controls at D14 but increased significantly (p

<0.05) at D21. Cartilage matrix echogenicity decreased with time for all groups due to maturation (p <0.05) except for DEX-treated rats.

Conclusion: Quantitative 55 MHz US allows detection of arthritic cartilage and bone lesions as well as the beneficial or deleterious effects of AI drugs. At this dose-regimen, DEX appears to prevent articular surface and subchondral bone alterations but also normal maturation of the matrix. NPX seems to be unable to repair cartilage surface and only partially efficient on bone.

O6 – Sensitive quantitation of cartilage degeneration using novel ultrasound indentation technique

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The aim was to investigate the capability of ultrasound indentation and imaging techniques to detect early stage osteoarthrotic changes in mechanical and acoustic properties of bovine articular cartilage in vitro. Furthermore, relationships between mechanical, acoustic, histological and biochemical properties were studied.

Methods: The ultrasound indentation instrument consists of an ultrasound transducer installed on the tip of an arthroscopic indentation instrument (Artscan 200). Ultrasound B-mode images were obtained with a 20MHz Dermascan-C instrument. Osteochondral samples (n=30, dia 19 mm) of variable degenerative state were prepared from the bovine patellae. The grade of cartilage degeneration was determined according to Mankin scoring. Dynamic moduli of samples were measured with the novel instrument by several manual instantaneous compressions (prestress 215kPa, strain 4%). Thickness and deformation were calculated from the ultrasound signal using the time-of-flight principle and a predetermined sound velocity (1627 m/s). Ultrasound reflection coefficient was determined from the cartilage surface. Ultrasound images were acquired using the imaging instrument. A high-resolution material tester was used for reference mechanical measurements. Finally, water and uronic acid contents of the samples were measured.

Results: A high linear correlation was established between ultrasound indentation modulus and reference dynamic modulus (n=30, r=0.993, p <0.001). Correlation between ultrasound reflection coefficient and Mankin score, uronic acid content or reference dynamic modulus was positive (r=0.847, r=0.834 and r=0.863; p <0.001), while the correlation between ultrasound reflection coefficient and water content was negative (r=-0.662, p <0.001). Ultrasound imaging detected sensitively structural changes in the superficial cartilage. Distinct changes were observed both in the ultrasound reflection amplitude and surface roughness.

Conclusion: The ultrasound indentation instrument distinguished accurately between different degenerative grades. Ultrasound reflection and B-mode imaging provided a sensitive method for evaluating cartilage structural and functional properties.

O7 – Magnetic resonance techniques for the early detection of cartilage degeneration in osteoarthritis

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There is a genuine need for the non-invasive detection of early osteoarthritis (OA). Magnetic Resonance (MR) is the method of choice, but standard morphologic MR shows only macroscopic cartilage lesions. Recently a number of dedicated MR methods have been suggested (and mainly tested in vitro), which aim at assessing biochemical parameters indicative of early OA. Such parameters include the proteoglycan (PG), collagen and water content (WC), and collagen structure. This paper reviews how dedicated MR methods might contribute to the early diagnosis of OA.

The loss of PG in early OA can be detected after injection of negatively charged contrast agents (e.g. Gd-DTPA), the accumulation of which causing a signal change in the affected area. The reduction of PG content can also be visualised using technically more challenging 23Na imaging methods. Both methods demonstrated differences in healthy volunteers and OA patients.

The collagen content might be assessed using magnetisation transfer (MT) methods. The obtained image contrast is thought to reflect the exchange of water molecules with the collagen constituents of cartilage. The status of the collagen network is also reflected by the apparent diffusion coefficient (ADC) of cartilage water at long diffusion times, which probes for structural parameters in the um range. At short diffusion times, the ADC is a (non-linear) measure for the WC. The WC could also be assessed using T2 mapping of cartilage. This method is based on the correlation of T2 and WC, with T2 being a very sensitive measure for the WC. Finally, cartilage WC has recently been determined from T2-corrected proton density maps. Although quantitative, this method might lack the sensitivity to small changes in WC that are typical in early OA.

Conclusion: All of the dedicated MR techniques show image contrast superior to morphologic MR methods, thus providing a basis for the detection of early OA. The potential of combining the above techniques remains to be evaluated in large scale patient studies.

O8 – Assessment of superficial articular cartilage degeneration with quantitative magnetic resonance imaging

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The aim was to investigate the capability of two potential MRI techniques, T2 relaxation time and Gd-DTPA(2-)-enhanced T1 imaging, to indicate the enzymatical damage of superficial articular cartilage.

Methods: Cartilage samples were prepared from bovine patellae. For T2 measurements, the superficial collagen network was digested with collagenase (n=8), proteoglycans (PGs) were cleaved with chondroitinase ABC (n=7), and compared to control samples (n=9). For Gd-DTPA(2-)-enhanced T1 imaging, adjacent cartilage samples were prepared (8 pairs). One sample served as a control while the other was treated with chondroitinase ABC. Digestion times were 44 hours. T2 relaxation time was determined from a series of high-resolution T2-weighted spin-echo measurements at 9.4 Tesla. In Gd-DTPA(2–)-enhanced T1 imaging, T1 relaxation time was measured from cartilage samples after balancing them in 1mM Gd-DTPA(2-) contrast agent solution for at least 2.5 hours. T1 relaxation time was determined from a series of T1-weighted saturation recovery experiments Relaxation time at each image pixel was determined and their mean value in the first 78um of tissue was determined.

Results: Superficial T2 of 40 ± 5ms was measured for the control tissue. After collagenase and chondroitinase ABC digestions T2 were 360 ± 130ms ($p < 0.01$, Mann-Whitney U-test) and 42 ± 13ms, respectively. T1 relaxation time in the presence of Gd-DTPA(2-) was 330 ± 50ms and 300 ± 80ms ($p < 0.05$, Wilcoxon signed-ranks test) for control and chondroitinase ABC treated tissue, respectively.

Conclusion: The present high resolution MRI experiments indicate that T2 relaxation time of superficial cartilage is sensitive and specific for collagen damage, while Gd-DTPA(2-)-enhanced T1 imaging can identify the PG cleavage of superficial cartilage. The present MRI techniques could possibly be used to identify the early stages of osteoarthritis, typically involving changes in the superficial extracellular matrix components.

O9 – Osteoprotegerin inhibits the loss of periarticular bone mass in the rat antigen-induced arthritis

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The aim was to investigating the influence of osteoprotegerin (OPG) therapy on inflammation activity and histomorphometric parameters of periarticular and axial bone in the animal model of antigen-induced arthritis (AIA) in rats.

Method: AIA was induced by standard methods in 20 female Lewis rats. 10 animals remained untreated. One half of the AIA rats (10 animals) were treated with OPG, the other half was treated with PBS. The dose per i.p. was 3 mg OPG/kg (0.6 mg/rat). Starting on the day of arthritis induction, animals were treated 10 times: day 3, 8, 10, 13, 15, 17, 20. Animals were killed on day 23, 3 days after the last injection. The following parameters were measured: swelling of joints on days 3, 7, 17 and 23; histomorphometric measurements: bone volume, structure and cellular rearrangement of the periarticular bone (right tibial head – arthritis joint) and axial bone (3rd lumbal vertebra) on day 23.

Results: Untreated AIA was associated with a significant reduction of the trabecular bone volume of the tibial head compared with the bone of healthy animals. Treatment with OPG resulted in a significant reduction of bone turnover at the tibial head and at the vertebra in comparison with untraeted AIA. Osteoid surface ($p < 0.001$ and $p < 0.001$), osteoid surface with osteoblasts ($p < 0.001$ and $p < 0.001$) and resorption surface with osteoclasts ($p < 0.01$ and $p < 0.001$) were significantly reduced at both, tibial head and 3rd lumbal vertebra, in OPG-treated animals. In addition to the reduction of cellular bone turnover, OPG had an inhibitory effect on the periarticular arthritis-associated bone mass loss. The trabecular bone volume showed significantly higher values in OPG-treated animals than those found in untreated AIA ($p < 0.01$), but was still lower than in healthy animals ($p < 0.001$). Moreover, the treatment with OPG reduced swelling of the joint on day 23 by 22% as compared to the untreated AIA animals.

Conclusion: Treatment with OPG effectively inhibits the arthritis-related periarticular bone mass loss in the animal model of AIA rats. This protective effect is connected with an intense suppression of bone turnover including resorption of bones.

O10 – Idiopatic juvenile osteoporosis— lean body mass and bone mineral content relationship

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The purpose of this study was the estimation of relation

between muscle and bone in IJO children in comparison to healthy children.

Patients and methods: 61 children confirmed by Dent criteria as IJO were measured using densitometry method. Total Body measurements were performed to evaluate Lean Body Mass (LBM) and Total Body Bone Mineral Content (TBBMC). The ratio between those parameters were calculated and compared with normative values established in healthy children.

Results: Significant differences were stated both between acute phase and chronic phase of IJO and when compared to healthy children.

Furthermore, sex related differences were also stated.

The mean value of LBM/TBBMC ratio in healthy girls was 18.96 (3.08) and was significantly lower when compared to value calculated for acute phase (26.14 ± 5.83; $p < 0.001$) and chronic phase (19.94 ± 3.09; $p < 0.01$) in IJO girls.

The mean value of LBM/TBBMC ratio in healthy boys was 19.82 ± 2.54 and was significantly higher when compared to healthy girls ($p < 0.001$) and lower in comparison to acute phase (23.46 ± 3.00; $p < 0.001$) and chronic phase (20.73 ± 2.75; $p < 0.05$) calculated in IJO boys.

Conclusion: The examined ratio between Lean mass and TB BMC reflects the changing bone - muscle relationship in healthy children. In accordance to mechanostat theory, the marked malfunction of the muscle- bone interaction was stated in IJO patients, specially during acute phase of the disease. On the basis of this study we can differ the acute phase and chronic phase of IJO using studied ratio.

O11 – Short-term bioactivity of titanium alloys is enhanced by covalent and adsorptive immobilisation of the RGD binding sequence

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Because of their biomechanic properties, titanium alloys are the gold standard in cementless endoprosthetics. In the past, there were lots of investigations to enhance the bioactivity of the titanium surface, leading to a better ongrowth of the surrounding bone, resulting in a higher survival rate of the prosthesis over the time. The RGD binding sequence enhances cell adhesion of osteoblast-like cells. Aim of our study was to enhance the bioactivity of the titanium surface via binding of the RGD binding sequence.

Methods: TiAl4V6 alloy surfaces were coated by chemical vapor deposition (CVD) polymerization with functionalized para-cyclophanes. The RGD binding sequence was either bound adsorptive or covalently using the spacer molecule hexamethylene diisocyanate (HDI). Binding of RDG and the resulting surface modification were tested with atomic force microscopy (AFM) and surface MALDI-TOF-MS.

Bioactivity of the modified and non-modified alloys (titanium alloy, titanium alloy + HDI, titanium alloy + HDI + covalently bound RGD, titanium alloy + HDI + adsorptive bound RGD) was tested with human osteoblast-like cell cultures, taken from hip surgery by explant technique. 1×10^4 cells were cultured on the probes with DMEM medium + 10% FCS, 5% CO_2. After 24 hours, adhesion rate, spreading, cell vitality (fluorescein diacetate) and cell proliferation (BrDU) were tested.

Results: Cell adhesion rate and cell spreading were clearly enhanced through covalent and adsorptive binding of RGD compared with untreated titanium alloys. Cell vitality was not influenced. Proliferation rate of the human osteoblast-like cells was enhanced to 140% after adsorptive immobilisation and 200% after covalent immobilisation of RGD peptides compared to untreated titanium alloys.

Conclusion: Both covalent and adsorptive immobilisation of the RGD binding sequence enhances the early response of human osteoblast-like cells to titanium alloy surfaces. Covalent binding of RGD doubles the proliferation rate. Long-term investigations of these surface modifications are in progress to test the influence of both binding types over time.

O12 – Bone remodeling 18 months after total hip arthroplasty (THA) with Exeter stem

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Operation with implantation of a THA changes the strain distribution pattern of the proximal femur, with a massive loss of stress in the proximal part of the femur and an increase of stress at the distal part of the femoral component. The purpose of this study was to quantify the changes in bone mineral density (BMD) after insertion of the collarless, two side conical, cemented Exeter stem.

Methods: 26 patients were included. They all had a THA with Exeter stem. BMD was measured in 7 ROI's according to Gruen's zones, using DEXA (Dual Energy X-ray Absorptiometry) scanning postoperatively and at 18 months' followup. At the same time the contralateral hip and spine was scanned.

Results were tested for difference using the nonparametric Wilcoxon matched-pairs signed-rank test.

Results: A significant loss of 7.4–13.6% was present in Gruen zones 2, 3, 6 and 7. No statistically significant changes were seen in Gruen zones 1, 4 and 5, or in the hip or spine scannings.

Conclusion: In the proximal, lateral zone 1 BMD is low before and after THA. This zone is a 'low stress

zone' with following low BMD regardless of the circumstances.

Under and medial of zone 1 we found zones 2, 3, 6 and 7 in which bone remodelling pattern in the study period showed a significant decrease in BMD. This indicated that there is a lower amount of stress in these zones following THA.

In zone 4, at the tip of the stem, and in zone 5, medial and proximal of zone 4, there was no change in BMD during the study period.

These findings, for the Exeter stem, correspond with findings done by others concerning other types of stem. However, we would have anticipated to find a rise in BMD in zone 4 at the tip of the stem.

O13 – Prediction of distal forearm fracture by finite element analysis of DXA images (FEXI)

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Bone mineral density (BMD) assessment by dual energy X-ray absorptiometry (DXA) is the current preferred method of assessing osteoporotic fracture risk. Other physical factors however contribute to the overall risk of fracture including anatomical geometry and the spatial distribution of bone. Finite element analysis (FEA) is a widely used technique for the computer modelling of structures that is inherently sensitive to geometry and material distribution. FEA has been applied to conventional DXA-derived spatial BMD images of the forearm (Lunar Expert fan-beam densitometer) to calculate the mechanical stiffness of the radius. 10 female subjects who had previously suffered a distal radius fracture were compared with a control group of 10 non-fracture female subjects matched for handedness. BMD at the ultra-distal radius (UDBMD) and lumbar spine (LSBMD) were not significantly different (Independent Sample Test) for the two cohorts, with p values of 0.14 and 0.91 respectively, FEA stiffness providing superior discrimination (p=0.057). Receiver Operator Characteristic (ROC) analysis was undertaken with area under curve (AUC) values (95% confidence interval) of 0.69 (0.437, 0.943), 0.525 (0.236, 0.814) and 0.770 (0.555, 0.985) obtained for UDBMD, LSBMD and FEA stiffness respectively. In this cross-sectional pilot study, finite element analysis of forearm DXA images (FEXI) appears to provide a superior means of identifying distal radius fracture risk than conventional BMD of the ultra-distal radius and lumbar spine. The technique has the potential to be applied to other fracture scenarios, for example, the proximal femur.

O14 – Fracture discrimination by structural parameters is superior to bone density: an in vitro study

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Bone Mineral Density (BMD) can be used to discriminate groups of subjects with and without vertebral fractures. However, the overlap in BMD of these groups indicates, that there is room for improvement. We investigated whether microstructural assessment may offer better discriminational power compared to BMD.

46 complete T12 vertebrae were taken from female human cadavers. 16 of them were taken from subjects with vertebral fractures (other than T12) as classified by an experienced skeletal radiologist (age 85.2 ± 6.9), whereas 30 vertebrae were taken from subjects without signs of vertebral fractures (age 79.9 ± 8.8).

High resolution computed tomography (HRCT) images were captured by a FanBeam microscope (Stratec, Pforzheim, Germany) with an isotropic voxel size of 160 microns sidelength. Structural parameters were calculated by in house developed software using a fixed threshold for binarization of the bone and marrow phases. A central rectangular region of interest was defined within the trabecular core in dorsal-ventral direction with a distance of 12 mm, while the remaining dimensions were maximized with respect to the volume and the limits given by the geometry of the vertebrae.

Trabecular BMD was obtained by Quantitative Computed Tomography (QCT) of L2-4. Trabecular BMD ($p < 0.05$) and bone volume fraction (BV/TV, $p < 0.03$) differed significantly between fractured and unfractured groups. Discrimination was better for trabecular number (Tb.N, $p < 0.003$) and trabecular separation (Tb.Sp, $p < 0.005$), while trabecular thickness (Tb.Th), bone surface to volume ratio (BS/TV) and the degree of anisotropy (DA), calculated from the Mean Intercept Length, showed no significant differencies. The difference in Tb.N was significant even after adjusting for difference in BMD.

Our results demonstrate that fracture discrimination was possible by those structural parameters that are less susceptible to partial volume effects. Trabecular Number was a significant contribution independent of BMD in discriminating fracture groups. Our results indicate the potential for HRCT measurements in vivo as improved method for fracture discrimination and perhaps prediction.

O15 – Femoral neck fractures: reasons for the most common location of the fractures

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The subcapital cervical fracture of the proximal femur is the most common fracture of all femoral fractures. The morphological reason for this epidemiological observation has not been described yet. The purpose of this study was to find an anatomical substrate that can explain the high fracture frequency at the subcapital region of the femoral neck.

Methods: Ten proximal femora from human cadavers were scanned in high-resolution mode (0.2 x 0.2 mm) and in 1 mm slice thickness on a special configured XCT-2000 pQCT-scanner (Stratec, Germany). 80 slices were acquired from each specimen. The data were visualized by the 3D platform Amira. The BMD of each slice was calculated based on a calibration curve obtained from the scanner using an EFP (European Forearm Phantom). The structural composition was quantified by measures of complexity.

Results: The Structure Complexity Index (SCI), which quantifies the local complexity of the structure, the Trabecular Net Index (TNI) measuring the trabecular richness, and the Index of Global Ensemble quantifying the overall dynamics within the architecture are specifically decreased in the transition area from the femoral head to the femoral neck. The lowest BMD of all slices, regardless of their average femoral neck BMD, was found in the same anatomical region. 3D visualization of the architecture demonstrated the trabecular arrangement of the femoral neck and was able to show additional information not available in common 2D radiographs of the hip.

Conclusion: 1) The anatomy of the femoral neck cannot be studied precisely on 2D radiographs. The study of the trabecular arrangement needs the information of the third dimension. 2) The architecture of the proximal femur has a weak area that is in the transition area of the femoral head to the femoral neck. This can be quantified by several structural parameters and by the BMD. 3) The results indicate that the subcapital region of the femoral neck is a favored location for fractures due to structural incompetence.

O16 – Low metacarpal index predicts hip fracture—a population study

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To date bone mineral density has mostly been measured using dual-energy X-ray absorptiometry. There is recent evidence, however, that an old method of measuring metacarpal index (MCI, combined cortical thickness divided by bone diameter) from hand radiographs could be quite accurate. We studied MCI for its associations with alleged risk factors for osteoporosis and with the risk of hip fracture in an extensive health survey.

Methods: From 1978 to 1980, a representative population sample of 8,000 Finns aged 30 years or over was invited to a comprehensive health examination; 90% complied. Hand radiographs were taken from a subsample of 3,595 subjects. MCI could be determined from 3,562 of them from the midpoint of the second metacarpal bone. Record linkage to the national hospital discharge register identified 117 subjects in the cohort who were hospitalized for hip fracture by the end of 1994.

Results: At baseline, a multitude of factors independently of each other were significantly associated with high MCI: young age, male sex, body mass index, short height, non-smoking, physical activity, cardiovascular disease and chronic neck pain. Adjusted for these factors and other major chronic diseases at baseline, the relative risk of hip fracture in those with MCI under 0.5, 0.5, 0.6 and 0.7 or more was 1.00, 0.63 (95% confidence interval, 0.40–0.98), 0.60 (0.34–1.05) and 0.29 (0.10–0.86), respectively.

Conclusion: Since low MCI shows expected associations with alleged risk factors for osteoporosis and strongly predicts hip fracture, and since hand radiographs are easily available and cheap, the application of this peripheral measure of bone status should be considered for screening of osteoporosis and for epidemiological research.

O17 – Calcaneal DXA and ultrasound: comparison of DXL Calscan, Lunar PIXI and Hologic SAHARA instruments

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DXL Calscan is a new bone scanner for the determina-

tion of calcaneal BMD. The instrument supplements X-ray absorption measurement with a laser measurement of total heel thickness, and thereby, can theoretically reduce the uncertainty related to variable composition of soft tissue. For Calscan, we investigated its precision, agreement with Lunar PIXI, and ability to predict BMD in proximal femur, lumbar spine and total body. For comparison, Hologic SAHARA ultrasound scanner was tested, too.

Methods: For 39 subjects (age 59.6 ± 9.2 years, 18 men, 21 women), DXA measurements using DXL Calscan and Lunar PIXI as well as ultrasound measurements with Hologic SAHARA were conducted. BMD in the femoral neck, lumbar spine (L2–L4) and total body was determined using Lunar DPX-IQ axial DXA scanner. In addition, 24 subjects were measured with Calscan three times to indicate the short-term precision of the instrument.

Results: The in vivo precision (CV%) of DXL Calscan was 1.24 (standardized CV% = 1.51). Calcaneal BMD values by Calscan were 19% lower than those of PIXI (0.452 ± 0.093 g/cm^2 vs. 0.559 ± 0.118 g/cm^2, p <0.01). Interestingly, the difference of BMD-values by Calscan and PIXI increased as a function of body mass index (BMI) (r=-0.43, p <0.01, n=38). However, a high linear correlation between Calscan and PIXI BMDs was revealed (r=0.84, p <0.01, n=39). Both instruments predicted equally well axial BMD in femur, spine and total body (r=0.73–0.86, p <0.01, n=39), and more accurately than BUA or SOS by SAHARA (r=0.54–0.67, p <0.01, n=39). Mean heel thickness, as measured with Calscan, was 5.3 ± 0.4 cm.

Conclusion: In vivo precision of the DXL Calscan measurements was similar to that of Lunar PIXI (CV% <1.5). Also, both instruments predicted axial BMD equally. The BMD-values of Calscan were lower than those of PIXI, especially in subjects with high BMI. This suggests that the elimination of soft tissue effects is different in these instruments. Theoretically, the elimination should be more accurate in Calscan, equipped with a laser measurement of total heel thickness and, thereby, enabling correction for both lean and adipose tissue.

O18 – Dynamic, quantitative assessment of the propensity to fall—the Hull Stabilometer

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The propensity to fall is second only to low bone density as a predictive risk factor for osteoporotic fracture, and any attempt to improve fracture prediction by DXA or other means would benefit from the quantitative assessment of fall propensity. Current methods of fall propensity assessment provide only a coarse measure of fall risk and are often qualitative in their application, making them both unsuitable for small subject groups and too insensitive to assess the effectiveness of fall prevention therapies upon individuals.

The Hull Stabilometer is a new device intended to determine a subject's propensity to fall from their dynamic response to an applied stimulus. In operation, the subject stands upon a force platform, which triangulates the centre of pressure (COP) through three internal force transducers. A precise movement is then applied to the platform to simulate a 'trip' or other 'jolt'. The fully variable movement is controlled by two stepper motors connected by drive belts to a linear rail system upon which the platform is mounted.

The response of the subject to the movement is detected by the platform transducers and sent to the controlling PC, where it is matched up with 'real-time' positional information obtained from transducers connected to the motors and linear rail system. This allows the response of the subject to be exactly matched with the time, direction and degree of the applied impulse, making the Stabilometer a powerful research tool for those wishing to assess the influence of fall risk upon fracture.

O19 – What do the Danish population and patients know about osteoporosis? A cross-sectional questionnaire study

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The patient's knowledge on risk factors for osteoporosis is crucial for their decision to seek medical advice and for compliance with pharmacological treatment. The aim of our study was to develop and test a questionnaire and assess the knowledge on osteoporosis in patients and the general population.

Methods: The questionnaire was developed from approximately 100 questions proposed by patients or doctors and other staff members. Also, questions from previously published studies were considered. A number of questions regarding epidemiology, symptoms, risk factors, diagnostic work-up, and treatment were selected. After, testing and rephrasing, the resulting questionnaire comprised 28 multiple-choice questions. Finally, questions on background information were included. The questionnaire was issued to 95 women and 15 men referred for evaluation in an osteoporosis clinic and 81 controls anticipated to differ with respect to their knowledge. Moreover, a slightly revised version of the questionnaire was issued to 1124 females and males aged 45–55 years via the Internet with the assistance of an opinion poll institute.

Results: In the control group, doctors, nurses, day-care workers, high school students, and industrial workers

scored 22.6; 22.4; 13.7, 12.4; and 11.1 of 24 possible points, respectively. In comparison, patients scored 17.0 (males 15.5 versus females 17.2).

In the patients (and population), 84% (90%) and 85% (80%) knew that osteoporosis is responsible for the majority of hip fractures and vertebral fractures in the elderly, respectively. Also, 67% (66%) knew that smoking increases the risk of osteoporosis. Moreover, 66% (65%) and 70% (53%), knew that early menopause and osteoporosis in first-degree relative, respectively, are important risk factors. Finally, 69% (38%) knew that vitamin-D is important for bone health.

Conclusions: Our data show that knowledge on osteoporosis can be measured using the proposed questionnaire. As anticipated, our data show that the population and patients are well informed in general terms. However, we found that both the population and patients lack specific knowledge on important risk factors for osteoporosis. We suggest that this may impair self-care and affect patients decision to seek medical advice.

O20 – Mechanisms that contribute to risedronate's rapid anti-fracture efficacy

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Risedronate therapy has been shown to rapidly reduce vertebral fracture risk. The positive effects on reducing fracture risk at one year have been observed prior to the zenith of BMD changes at 3 years (Harris 1999, Reginster 2000). This suggests that other factors beyond BMD are important to fracture protection.

Reducing bone turnover appears to be a major factor for risedronate to reduce fracture risk. Recent data with risedronate suggests that reduced bone turnover is associated with reduction in vertebral fracture risk, however, there appears to be a "threshold" beyond which further marker reduction is not associated with better fracture protection (Eastell 2001). While significant changes in bone markers and BMD are associated with treatment efficacy, they do not completely explain how fracture protection is achieved.

Three-dimensional micro computed tomography (micro-CT) has led to insights in understanding mechanisms by which risedronate preserves bone microarchitecture. Studies in growing, ovariectomized mini-pigs demonstrated that risedronate preserved trabecular architectural elements in vertebral bodies (Dufresne, 2002). Mechanical testing showed preservation of architectural elements contributed to overall bone strength apart from the contribution made by overall mass. The combination of mass and architectural elements explained over 90% of the observed strength in these samples.

Investigations with micro-CT have also examined the short-term effects of risedronate on bone architecture in early postmenopausal women with normal bone mass (Borah, 2002). In women treated with calcium supplementation, significant loss of trabecular elements were seen within 12 months. Women in this group showed decreases in bone volume (BV/TV) and trabecular number, as well as increases in trabecular separation compared with the risedronate group. Women receiving risedronate (5 mg daily) had a maintenance of 3D trabecular structure; bone volume and the architectural parameters were preserved compared to baseline.

In summary, these data demonstrate that risedronate reduces bone turnover to preserve bone microarchitecture contributing to its rapid effects on bone strength.

O21 – Risedronate provides sustained vertebral and nonvertebral fracture benefits over 5 years

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Risedronate has been shown to be effective in the treatment of postmenopausal osteoporosis. In three year clinical studies, risedronate, a pyridinyl bisphosphonate, reduces vertebral fractures by up to 49% (1) nonvertebral fractures by up to 39% (2), and hip fractures by up to 60% (3). No placebo-controlled data beyond 4 years of treatment with bisphosphonate were available prior to a recent 2-year extension of a 3-year, placebo controlled vertebral fracture study reported with risedronate (4). Aim of this extension study was to determine effects of 5 years risedronate treatment.

Methods: Patients in the extension study continued receiving oral risedronate (5 mg) or placebo daily. Spinal radiographs were taken yearly during the first 3 years and at year 5 and nonvertebral fractures were collected as adverse events.

Results: In total, 265 patients entered the extension (135 on risedronate 5 mg and 130 on placebo). In years 4–5, risedronate significantly reduced the incidence of new vertebral fractures by 59% (risedronate, 13.8; placebo, 28.2%; p=0.011). The risk of osteoporosis-related nonvertebral fractures was reduced by 41% (risedronate, 5.2%; placebo, 8.5%; p=0.29, NS). In a Kaplan-Meier "time to first event" analysis of the overall study population over 5 years, risedronate treatment resulted in a significant reduction of both vertebral fractures by 50% (95% confidence interval, 30% to 64%, $p < 0.001$) and nonvertebral fractures by 37% (95% confidence interval, 6% to 58%; p=0.022).

Conclusion: This study provides the first controlled data for any bisphosphonate demonstrating sustained

vertebral and nonvertebral fracture benefits through 5 years of treatment.

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O22 – Alendronate prevents bone loss in men with primary osteoporosis

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Alendronate administration is a highly effective therapy for osteoporosis, however, the drug in question—as a majority of available pharmacological agents used for those purposes—has been registered with indication for postmenopausal women and for treatment of steroid-induced osteoporosis. The aim of the study was to evaluate the effect of treatment with alendronate in men suffering from primary osteoporosis. 75 men with primary osteoporosis, diagnosed by densitometry (DEXA-DPX, Lunar) of the femoral bone neck vs. WHO criteria, were included into the study; the mean age of the patients was 65.5 years and the anamnesis of 29 patients revealed a history of fractures. All the patients were divided into two groups. The patients of Group I were administered sodium alendronate (in a dose of 10 mg daily), calcium preparations (calcium carbonicum, 1000 mg daily, as converted into elementary calcium), and vitamin D3 (cholecalciferol in doses of 1000 U daily). The patients of Group II received calcium preparations and vitamin D3 only, administered in the same doses as in Group I. Following 12 months of the therapy, densitometric examination was repeated in order to evaluate bone mineral density (BMD) changes. In Group I, BMD increased by 0.04 g/cm^2 ($p < 0.05$) between the first and the second densitometric evaluation, while decreasing by 0.01 g/cm^2 in Group II, however, not attaining—in the latter case—the threshold of statistical significance. No fresh fractures occurred in all the studied patients, the tolerance to the applied treatment being fairly good in both groups.

Conclusion: Sodium alendronate, when applied to men with primary osteoporosis, increases BMD.

O23 – Cortical bone formation and strength are increased in OVX rats given simvastatin twice daily

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It has been shown that some of the statins possess bone anabolic properties. Statin increased expression of the BMP-2 gene in osteoblasts resulting in increased cancellous bone volume and a minor decrease in osteoclast number of 3-month-old rats (Mundy et al., Science 286: 1946-1949, 1999). Simvastatin increased the vertebral cancellous bone mass and compressive strength of one-year-old female rats (Oxlund et al., Calcif Tissue Int 69: 299-304, 2001). In the present study the effects of statin on tibia cortical bone of OVX (ovariectomized) rats were studied. Sixty Wistar female rats, 4 months old, were allocated to 4 groups: 1) baseline control, 2) sham + placebo group, 3) OVX + placebo, and 4) OVX + simvastatin. Simvastatin MSD (20 mg/kg) or placebo were given twice daily at 9 a.m. and at 3 p.m. by a gastric tube for 3 months. The rats were injected with tetracycline i.p. at day 11 and calcein i.p. at day 4 before sacrifice of the 7 months old rats. The mechanical properties of the tibia diaphysis were studied by a 3-point-bending test. The breaking strength of the OVX + statin group (111.2 ± 2.1 N, mean ± SEM) was increased ($2p < 0.02$) compared with the OVX group (102.4 ± 2.8 N), and increased ($2p < 0.001$) compared with the sham + placebo group (97.5 ± 2.2 N). Cross-sections were cut and mineral appositional rates (MAR) and bone formation rates (BFR)at the different aspects and in different regions of the tibia were calculated from the fluorescent labels. At the tibia mid-diaphysis for example, the periosteal BFR of the OVX + statin group (0.67 ± 0.11 mm^3/day) was increased ($2p < 0.05$) compared with the OVX group (0.30 ± 0.04 mm^3/day), and increased compared with the sham + placebo group (0.42 ± 0.10 mm^3/day). In conclusion, simvastatin given perorally twice daily increased the bending strength of the tibia diaphysis of OVX rats. The bone formation rates were increased both at the endosteum and periosteum of OVX rats given simvastatin. The new cortical bone exhibited a normal lamellar structure. Simvastatin increased bone formation, but seems to respect the regional pattern of bone resorption, formation and drift.

O24 – Simvastatin improves fracture healing in mice

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The purpose of this study was to investigate the effect of high-dose systemic treatment of simvastatin on fracture healing in mice.

Methods: Femur fractures stabilised with marrow-nailing were produced in 70 mature male Balb-C mice. 35 mice were given a diet prepared with simvastatin, so that each mouse received an approximate dose of 128

mg per kg of body weight and day. The remaining mice received the same diet without the simvastatin. Bilateral femurs were harvested at 8, 14 and 21 days post-operative. Biomechanical tests were performed by way of three-point bending (beam length 6 mm). Histological specimens were prepared using standard techniques. For statistical analysis, ANOVA and Scheffé was used.

Results: At 8 days, the fracture callus was too soft for meaningful biomechanical testing. At 14 days, the callus of the simvastatin treated mice had a 53 % bigger transverse area than controls (p=0.001), the force required to break the bone was 63% greater (p=0.001) and the energy uptake was increased by 150% (p=0.0008). Stiffness and modulus of elasticity were not significantly affected. At 21 days, the fractures were histologically healed and the mechanical differences had disappeared. At this time, the specimens had approximately 70% of the strength of the contralateral unbroken bone.

Conclusion: These results point to a new possibility in the treatment of fractures. The doses used were exceedingly high, but this study still demonstrates a valuable principle: that it might be possible to use statins at doses which improve healing of a fracture without deleterious effects to other bone-locals. Most of the simvastatin would have been sequestered in the liver. If statins specifically engineered towards bone and not the liver were to be developed, doses at which an effective treatment could be achieved would be substantially less.

O25 – Quantitative ultrasound for the assessment of juvenile bone status

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Quantitative Ultrasound (QUS) can be used in the assessment of adult fracture risk. As a radiation-free method QUS is also interesting for measurements on children. However, several children specific tasks have to be announced like creation of a specific reference database, adaptation of the method to the growing skeleton and consideration of specific error sources. The sensitivity to skeletal growth, and especially to disorders of growth, has to be proven and peculiarities of different QUS approaches have to be taken into account.

Three methods are used in adults and children, trabecular transverse transmission (calcaneus) and cortical transverse (phalanges) and cortical axial transmission (radius, tibia). Reference databases, which can be easily created with radiation-free QUS, are available for the cortical measurements and in part for the trabecular measurement. Most parameters depict an age-dependent increase, strongest pronounced in cortical transverse transmission mode. SOS on the calcaneus does not or only slightly increase with age nor does it correlate with pubertal stage. All other parameters show significant associations with height and pubertal stage. Cortical measurements seem to be closer associated with skeletal than with chronological age and also show gender specific differences during and after puberty.

Different disorders show an impact on QUS measurements. Rheumatoid diseases are associated with lower QUS parameters in all methods. Examples for disease impact on calcaneus ultrasound are anorexia nervosa and Rett's syndrom. Disorders of growth and puberty affect QUS parameters of the phalanges. On tibia and radius QUS parameters demonstrated type-specific positive correlations with physical activity level. Measurements in preterm infants, which can be easily performed using QUS in cortical axial transmission (radius, tibia) and transverse transmission (humerus) show lower QUS results as in term children.

These studies demonstrate the impact of skeletal growth and disorders on QUS parameters. Taking into consideration children specific tasks QUS measurements might become a useful, radiation-free tool for the assessment of juvenile bone status.

O26 – Structural-functional state of bone tissue in ukrainian children and adolescents for results of ultrasound densitometry

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The aim of this research is a study of structural-functional state of bone mass, anthropometric parameters and physical development of children and teenagers populating in different regions of Ukraine. 1729 children and adolescents without disease influencing bone tissue metabolism, from 7 to 17 years (696 boys and 1033 girls) were inspected during the study. To evaluate structural-functional state of bone tissue, ultrasound densitometer Achilles+ (Lunar Corp., Madison, WI) was used. Speed of ultrasound spreading (m/s), broadband ultrasound attenuation (dB/MHz), and Stiffness index of bone tissue (%) were determined. The means and standard deviation (SD) of anthropometric (weight and height) and ultrasonic parameters were calculated. For results of ultrasound densitometry the normative data of ultrasound parameters depending of age for Ukrainian children population were presented. The main characteristics of the physical development are gradual growth without depending on age. The parameters of bone mineral density were correlated with age, weight and height. The normative data of ultrasound parameters can possible be used to diagnosing decrease of BMD in Ukrainian children in period of peak bone mass forming.

O27 – Systematic review of the role of bisphosphonates in metastatic disease: skeletal morbidity

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The aim was to systematically review the role of bisphosphonates in the reduction of skeletal morbidity in patients with bony metastatic disease.

Method: We identified randomised controlled trials (RCTs) by searching electronic databases, scanning reference lists, and consultation with experts and pharmaceutical companies. Foreign papers were included. The inclusion criteria were: RCTs, patients with proven malignant disease and bony metastases, oral or intravenous bisphosphonate in the experimental arm, compared to another bisphosphonate, placebo or standard care, and one outcome of skeletal morbidity.

Results: Fifty papers describing 33 studies, fulfilled the inclusion criteria. Data extracted from 21 studies were eligible for inclusion in the meta-analyses. Bisphosphonates, compared with placebo, significantly reduced the odds ratio (OR) for all skeletal morbidity endpoints, except for spinal cord compression (SCC). The following pooled OR (95% CI) were calculated: vertebral fractures 0.742 (0.633–0.868); non-vertebral fractures 0.714 (0.597–0.854); combined fractures 0.710 (0.608–0.827); radiotherapy 0.681 (0.598–0.776); SCC 0.714 (0.470–1.083); orthopaedic surgery 0.681 (0.508–0.913) and hypercalcaemia 0.544 (0.364–0.814). Bisphosphonates significantly increased time to first skeletal related event (SRE). No survival benefit was observed.

Conclusion: Bisphosphonates significantly decrease all skeletal morbidity endpoints, except SCC. Bisphosphonates significantly increase the time to first SRE and should be started early when bone metastases develop.

O28 – Active tartrate resistant acid phosphatase (TRAP) 5b as serum marker of bone metastasis in human breast cancer

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The osteoclast specific active TRAP 5b isoform is detectable in serum and claimed to be a specific marker of bone resorption. The present study was undertaken to evaluate the usefulness of TRAP 5b as serum marker of bone resorption in breast cancer patients with bone metastases.

Methods: TRAP 5b serum levels were measured in 192 samples from patients with breast cancer with and without bone metastases and in 53 healthy pre- and postmenopausal women using the enzyme immunoassay Bone-TRAP.

Results: Serum levels of TRAP 5b were significantly higher in patients with breast cancer and clinical signs of bone metastases before therapy than in healthy women (5.2 ± 2.0 U/l vs. 3.4 ± 0.9 U/l; $p < 0.0001$). There was also a significant difference between patients with bone metastases before and during bisphosphonate therapy (5.16 ± 2.0 U/l vs. 3.89 ± 1.9 U/l; $p < 0.01$), indicating a reduction of bone alteration under this treatment. The subgroup with progression of bone metastases under bisphosphonate therapy showed the highest difference in TRAP 5b concentrations compared to patients with stable disease (7.2 ± 1.8 U/l vs. 3.38 ± 1.36 U/l; $p < 0.0001$).

Conclusion: Serum TRAP 5b levels are elevated in patients with bone metastases and breast cancer. The TRAP 5b levels decline under bisphosphonate therapy when no progression is detectable. When progress of the bone metastases occurs, TRAP levels rise again. Therefore, active TRAP 5b seems to be a useful serum marker for bone metastases in breast cancer patients, especially to detect progressive disease under bisphosphonate treatment. Further studies with larger numbers of patients have to confirm these data.

O29 – P-25-hydroxyvitamin D in danish patients with primary hyperparathyroidism: effect on clinical presentation

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It has been suggested that vitamin D deficiency / insufficiency is associated with a more severe clinical picture of primary hyperparathyroidism (PHPT).

Our aim was to investigate the metabolism of vitamin D in PHPT. Furthermore, possible relations between plasma 25(OH) vitamin D and the severity of PHPT were investigated.

Methods: The study was performed as a cross-sectional study. We collected biochemical, histological and osteodensitometric data from patients with PHPT admitted to the Department of Endocrinology C, Århus Amtssygehus from January 1994 to July 2001 (N = 302). A group of 251 medical patients served as a reference group for plasma levels of 25(OH) vitamin D.

Results: Mean plasma level of 25(OH) vitamin D (mean (95% CI)) was lower in patients with PHPT compared with the reference group (29.5 nmol/l (7.0–82.0) versus 42.5 nmol/l (12.4–103.1), $P < 0.001$). Plasma levels of 1,25(OH)2 vitamin D was elevated in 20%

of the patients and correlated positively to P-25(OH) vitamin D ($r = 0.20$, $P < 0.005$) and creatinine clearance ($r = 0.31$, $P < 0.001$). The levels of P-25(OH) vitamin D showed inverse correlation to P-PTH ($r = -0.42$, $P < 0.001$) and parathyroid adenoma weight ($r = -0.32$, $P < 0.05$). Bone mineral density, given as z-score (mean ± SD), was reduced in the hip (-0.41 ± 1.18, $P < 0.001$) and forearm (-0.96 ± 1.19, $P < 0.001$) but not in the spine (0.11 ± 1.51, $P < 0.09$). A positive correlation was observed between P-25(OH) vitamin D and BMD in the hip ($r = 0.23$, $P < 0.05$), forearm ($r = 0.28$, $P < 0.05$), and whole body ($r = 0.47$, $P < 0.05$). No significant correlation was observed between BMD in the spine and P-25(OH) vitamin D.

Conclusion: Patients with PHPT have a lower vitamin D nutrition state compared with other medical patients. Vitamin D deficiency in patients with primary hyperparathyroidism is associated with a more severe clinical picture of primary hyperparathyroidism. Finally, renal function and supply of 25(OH) vitamin D are the major determinants of 1,25(OH)2 vitamin D production in PHPT.

O30 – Relations between vitamin D and calcium/creatinine clearance ratio in patients with familial hypocalciuric hypercalcemia (FHH) and primary hyperparathyroidism (PHPT)

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The majority of FHH- and PHPT-patients present with asymptomatic hypercalcemia. The Calcium/creatinine clearence ratio is used to differentiate between FHH and PHPT. However there is a considerable overlab in the ratio for the two conditions.

Aim: The aim of this study was to investigate whether there is a correlation between vitamin D and the calcium/creatinine clearence ratio in patients with FHH and PHPT.

Methods: In a retrospective design we compared 20 FHH-patients with 92 patients diagnosed with and operated for PHPT. The patients were not treated with either thiazide diuretics, lithium, or clucocorticoids. We registered the following biochemical parameters: Plasma- Ca, PTH, creatinine, 25-vit-D, 1,25-vit-D, and 24-h urinary calcium and creatinine.

Results: In the FHH- compared to the PHPT-group, plasma 25-vit-D levels were higher (43.7 vs. 29.2; $p=0.001$), whereas 1,25-vit-D levels were lower (87.8 vs. 134.5; $p<0.0001$). There was a positive correlation between 1,25-vit-D and calcium/creatinine clearence ratio in the PHPT-group ($r=0.275$; $p=0.012$), but not in the FHH-group. Similary 1,25-vit-D correlated to 24-h urinary calcium in the PHPT-group ($r=0.447$, $p<0.0001$), but not in the FHH-group. In a multiple regression analysis, the only independent predictors of 1,25-vit-D were group, age, and 25-vit-D.

Conclusion: Calcium/creatinine clearence ratio does not seems to be influenced by vit-D status in patients with FHH. However, vit-D levels do differ between patients with FHH and PHPT.

O31 – Induction of high numbers of resorbing osteoclasts from human peripheral blood mononuclear cells

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Peripheral blood mononuclear cells (PBMC) isolated from different species have been shown to differentiate into active osteoclasts under appropriate conditions. The combination of soluble forms of growth factors, receptor activator of NF-kB ligand (RANKL, also called TRANCE/ODF) and macrophage colony-stimulating factor (M-CSF) is generally known to induce osteoclast formation and resorption activity in cultures containing osteoclast progenitors. By culturing isolated human PBMC for 14–21 days on bovine bone slices (106 cells/bone slice) in the presence of RANKL (20 ng/ml) and M-CSF (10 ng/ml), 20–100 tartrate-resistant acid phosphatase (TRAP)-positive multinucleated cells (MNC) were formed. Since, the osteoclast formation capacity and the resorption activity were quite low, we decided to study how additional growth factors, such as TNF-a and/or dexamethasone, affected osteoclast differentiation and resorption. The combination of RANKL, M-CSF and TNF-a (10 ng/ml) induced the formation of 286.3 ± 28.5 (mean ± SEM, $n = 6$) TRAP-positive MNC/bone slice in a 14-day culture. The resorption marker, C-terminal telopeptide of type I collagen (CTx; CrossLaps, Osteometer) concentration in the culture media at the end of the culture was 8.8 ± 1.5 nM. Dexamethasone at 10^{-8} M further enhanced both osteoclast formation and resorption 4-fold and 8-fold, respectively. In the culture, there were 1215.7 ± 153.9 TRAP-positive MNC/bone slice and the CTx concentration was 68.0 ± 7.9 nM. To find out how this culture system could be used for studying the effects of resorption inhibitors on bone resorption, bafilomycin A1 (10^{-8} M) and calcitonin (10^{-8} M) was added at day 10 to the culture and cells were cultured for 4 additional days. At the end of the culture, the culture media were collected and analyzed for CTx. Both inhibitors significantly ($p < 0.05$) inhibited the resorption activity of human PBMC-induced osteoclasts compared to control cultures. This data suggest that high numbers of resorbing osteoclasts can be induced from human PBMC. This assay can also be used as a resorption assay for screening bone resorption inhibitors and studying their mechanism of action on osteoclasts.

O32 – Analysis of osteocalcin released during osteoclastic bone resorption in vitro

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Osteocalcin is the most abundant non-collagenous protein in bone matrix. It is produced by osteoblasts and considered as a marker of bone formation both in serum and in cell culture. However, osteocalcin incorporated into bone matrix must also be released during osteoclastic bone resorption. We have studied if osteocalcin immunoassays could thus be utilized to detect osteocalcin molecules of resorptive origin in osteoclast cultures.

Primary osteoclasts were obtained from rat or mouse long bones and cultured on bovine bone slices. In addition, human osteoclasts differentiated from peripheral blood mononuclear cells (PBMC) were used. Release of osteocalcin into medium during bone resorption was evaluated with several in-house immunoassays for detection of different molecular forms of osteocalcin. For comparison, a commercial osteocalcin assay was also used. The heterogeneity of osteocalcin released into medium was determined using reverse-phase HPLC.

Primary rodent osteoclasts as well as human PBMC-derived osteoclasts released immunodetectable osteocalcin from bone matrix into medium. The amount of osteocalcin was increased in the presence of stimulators of resorption, e.g. 1,25(OH)2D3 or PTH, and was decreased in the presence of inhibitors of resorption, e.g. a specific inhibitor for vacuolar type proton ATPase, bafilomycin A1 and a cysteine protease inhibitor, E64. Osteocalcin level in the medium correlated with the concentration of well-characterized marker of bone resorption, C-terminal telopeptide of type I collagen (CTx), with r-value > 0.9. Several molecular forms of osteocalcin, including intact molecule, were isolated and identified from the conditioned medium.

We conclude that detectable osteocalcin is released from bone matrix during bone resorption as well as it is synthesized by osteoblasts during bone formation. Thus, osteocalcin could be used as an index of bone resorption in rodent osteoclast and human PBMC-derived osteoclast cultures.

O33 – Overexpression of cathepsin K in transgenic mice results in decreased amount of the metaphyseal trabecular bone and in increased amount of the diaphyseal cortical bone

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Cathepsin K is a lysosomal cysteine proteinase expressed abundantly in osteoclasts. Several recent reports indicate that it is a major proteinase responsible for degradation of bone extracellular matrix. We have recently described that overexpression of cathepsin K in mice leads to metaphyseal osteopenia at the age of seven month. The purpose of this study was to further characterise the bone phenotype of these transgenic mice.

Methods: Female mice homozygous and negative for the transgene locus were studied at the age of 1, 3, 7 and 12 months. Hind limbs were analysed by peripheral quantitative computed tomography (pQCT). The femurs were used for histomorphometry and the tibias for three-point bending test. Other metaphyseal and diaphyseal bone samples were collected for total RNA extraction, and subsequent analysis of gene expression levels of various bone proteins by Northern analysis.

Results: In growing transgenic mice, osteopenia of metaphyseal trabecular bone was confirmed by pQCT analysis at the age of 1 and 3 months and by histomorphometry at the age of 3 months. Up-regulated metaphyseal mRNA levels for gelatinase-B (MMP-9) referred to the enhanced osteoclastic bone resorption. Surprisingly in diaphyses, the pQCT measurements revealed increased amount of cortical bone in transgenic mice. This was already observed at the age of 1 month, where histomorphometry demonstrated that the thickening occurred endosteally. At the age of 3 months, increased ultimate failure load provided a functional consequence to the thickening. Down-regulated mRNA levels for core-binding factor alfa1, osteocalcin and carbonic anhydrase II suggested that bone turnover might even be decreased in diaphyseal regions.

Conclusion: The study confirmed the osteopenia of metaphyseal trabecular bone in transgenic mice. Surprisingly, cathepsin K overexpression also resulted in endosteal thickening of the diaphyseal cortical bone. This finding suggests differential regulation of bone turnover in trabecular and cortical bone. Since the phenotype was already observed at earliest time point studied, the endocortical bone resorption might be even decreased during early skeletal development.

O34 – Incerased bone mineral density, altered cross-sectional morphology and decreased bone resorption in cathepsin K deficient mice

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Cathepsin K (Ctsk) is a major protease in resorption of bone matrix molecules. Ctsk deficient mice develop severe osteopetrosis due to impaired bone resorption. The aim of our study was to further evaluate skeletal

changes in cathepsin K deficient mice.

We generated our own cathepsin K knockout mice by removing exons 2 to 5 of the Ctsk gene in embryonal stem cells. Obtained wild type (wt) and homozygous (Ctsk-/-) mice for the inactivated Ctsk allele were studied with peripheral quantitative computed tomography (pQCT) to evaluate changes in bone architecture and density at the age of 8 weeks. Bone specimens were taken for histomorphometry and serum samples were collected for analysis of bone breakdown products. To study bone resorption in vitro, primary wt and Ctsk-/- osteoclasts were cultured on bovine cortical bone slices for 3 to 4 days. The culture media were analyzed for bone degradation products.

pQCT measurements of femurs from male wt and Ctsk-/- mice showed increased cross-sectional area and increased total and trabecular bone mineral density (BMD) in the metaphyseal region of Ctsk-/- mice. The cortices were thickened and bone marrow cavities were smaller. Periosteal perimeter was increased and endocortical perimeter decreased in the metaphysis of the Ctsk-/- mice. Also the cross-sectional morphology of the metaphyseal bone was changed in the Ctsk-/- mice. In diaphyseal region the cortices were thickened and total BMD was increased in the cathepsin K deficient mice. However, there was no significant difference in the size of bone marrow cavity or the endocortical perimeter between Ctsk-/- and wt mice referring that endocortical bone resorption is not severely affected. The amount of medium C-terminal telopeptide of type I collagen (ICTP), degradation products of C-terminal telopeptides of type I collagen (CTX) and bone derived osteocalcin fragments were significantly decreased in the media from Ctsk-/- cultures.

This data indicates that cathepsin K is needed for normal bone development. However, our results on Ctsk knockout and overexpressing mice suggest that cathepsin K may have a different roles in the resorption of trabecular and cortical bone.

O35 – Comparison of serum tartrate-resistant acid phosphatase 5b with other markers of bone turnover in monitoring alendronate therapy

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The purpose of this study was to compare the usefulness of serum tartrate-resistant acid phosphatase isoform 5b (TRACP 5b) with other markers of bone turnover in monitoring alendronate therapy. The subjects of this one-year double-blinded intervention trial were 164 healthy postmenopausal women who were within 5 years after onset of menopause and with no previous use of medication related to bone metabolism. The subjects were randomly assigned into two groups, one receiving 5 mg alendronate daily, and the other receiving placebo. All subjects received a daily supplement of calcium and vitamin D. The bone markers, including the resorption markers serum TRACP 5b, total urinary pyridinoline (PYR) and deoxypyridinoline (DPD), and the formation markers serum total osteocalcin (OC), bone-specific alkaline phosphatase (BAP) and procollagen I N-terminal propeptide (PINP) were assessed at baseline and at 3, 6 and 12 months after start of treatment. Bone mineral density (BMD) of the lumbar spine was measured at baseline and at 12 months. Compared with the placebo-group, BMD increased significantly more, and the markers decreased significantly more in the alendronate-group ($p < 0.0001$ for each parameter). Least significant change (LSC) was determined for each marker based on their analytical and biological variability. Those subjects that showed a decrease of more than LSC were defined as responders for the treatment. Clinical specificity of each marker was determined as the percentage of non-responders in the placebo-group, and clinical sensitivity as the percentage of responders in the alendronate-group. The specificity and sensitivity, respectively, of the markers were: TRACP 5b 89.6% and 81.6%; DPD 90.9% and 60.5%; PYD 84.4% and 59.2%; BAP 93.3% and 55.3%; PINP 83.1% and 86.8%; OC 68.9% and 75.0%. When the specificity of each marker was multiplied with its sensitivity, the results were as follows: TRACP 5b 0,73; DPD 0,55; PYD 0,50; BAP 0,52; PINP 0,72; OC 0,52. These results suggest that serum TRACP 5b is a specific and sensitive marker for monitoring alendronate treatment. Of the other markers measured, only PINP was comparable with TRACP 5b.

O36 – Seasonal variation in bone mass in postmenopausal women

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A seasonal variation in bone biochemical markers has been described. Whether such a variation also has an impact on bone mass has yet to be resolved.

Methods: We investigated seasonal variation in bone mass by cross-sectional designs in three large cohorts of women (n = 3,742) from two geographical regions in Sweden. One cohort was strictly population-based (all 75 years old, n = 1,044), and two cohorts were patient-based

(age >55 years, mean age 67, n = 1,293 and mean age 69, n = 1,405). Each woman was assessed once and the inclusion was for the population-based sample continuous throughout almost all days of the year. Bone mineral density (BMD) of the total body, hip (neck, Ward, trochanter) and lumbar spine was determined by means of DXA.

Results: For all three cohorts the BMD was similar in those assessed throughout the summer half year (April–September) compared to those assessed throughout the winter half year (October–March). This absence of summer-winter difference was evident for all skeletal regions and remained after controlling for small differences in age and weight.

On the other hand, when women who had been assessed during autumn (July–December) were compared with women assessed during spring (January–June) we found the BMD to be up to 5% lower in autumn in all skeletal regions in the population-based sample, and 4% lower at the Ward's triangle and trochanter in one of the patient-based samples but not in the other. After correction for small differences in age and weight, women who had been assessed throughout the autumn period still had lower BMD compared with women assessed during spring at the hip (neck, Ward and trochanter) in the population-based sample and at the trochanter in one of the two patient-based samples.

Conclusion: We found no summer/winter variation in bone mass in these three cohorts of post-menopausal women. Although our data are not conclusive we found indications that BMD may be a few per cent lower during the autumn half of the year compared to the spring half.