Posters

P1 – Morphological adaptation of the subchondral bone plate in a rabbit knee joint with post-traumatic osteoarthrosis

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Description of bone architecture in the subchondral bone plate is important to understand the course of events of post-traumatic osteoarthrosis. The purpose of the present study was to evaluate morphological changes in the subchondral bone behind the previously found decrease in bone mineral density in a rabbit model with post-traumatic osteoarthrosis.

Methods: Fifteen rabbits were operated with meniscectomy in the right knee and a sham-operation in the left knee. Histomorphometric evaluation (subchondral bone thickness, porosity, bone volume, trabecular number and thickness) was compared to mineral density (DEXA) measurements of the subchondral bone. Operated animals were divided in three follow-up groups (13, 25 and 40 weeks after operation). Another five rabbits were used as normal control animals.

Results: Meniscectomized knees had an increased subchondral bone plate with a lower porosity than sham-operated (P=0.006) and control knees (P=0.03). However, the trabecular bone showed no morphological differences between the groups. Neither were there time-dependent differences between the groups.

Discussion: Our findings suggest that the first response after meniscectomy occurs in the cortical rather than the trabecular bone. Thus the mineralization process seems disturbed or prolonged in an early stage of post-traumatic ostearthrosis because the decrease in bone mineral density corresponded to a thicker and more compact bone plate at least during the first 40 weeks after operation.

P2 – Posttraumatic arthrosis of the wrist as a complication after distal radius fracture

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Degenerative changes of the carpus as the late complication of the distal radius fracture are still not fully appreciable and recognizable. The goals of this paper are evaluation of the following: 1) incidence of the degenerative changes of the carpus after distal radius fracture, 2) influence of that changes on the late end results of the treatment, 3) type of the etiologic factors of the posttraumatic degeverative changes of the carpus, and 4) intensity of carpal degenerative changes after distal radius fracture.

Retrospective investigation of 350 patients treated nonoperatively because of distal radius fractures was made. Mean time of follow-up was 8 years (range: 5–10 years). The control group have been constituted by healthy opposite wrists of the same patients.

In clinical and radiological evaluation were used: the Gartland-Werley scale and the Sarmiento scale, respectively. Degenerative changes of the carpus were measured by the Kirk-Jupiter scale.

There were 20% of the carpal degenerative changes in trial group in comparison with 2% in control group. Statistical analysis has confirmed correlation between past distal radius fracture with the residual deformity or incongruency and development of the carpal degenerative changes.

P3 – Inverse regulation of matrix molecules tenascin and aggrecan by interleukin-1beta—a possible explanation for changes in synthesis pattern of OA chondrocytes

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Our objective was to analyze the distribution of tenascin and proteoglycans in normal and OA cartilage, and to assess the effect of IL-1b on aggrecan and tenascin expression in human articular condrocytes in-vitro.

Methods: Nine normal and 30 osteoarthritic cartilage/bone-samples were obtained during total knee replacements or autopsies. After fixation, decalcification, paraffin-embedding specimens were sectioned perpendicular to the surface. After safranin-o staining, slides were graded according to Mankin subdivided in normal, mild OA, moderate OA and severe OA samples. Adjacent slides were immunostained for tenascin. Tenascin expression were confirmed by RT-PCR. In cell culture experiments, OA chondrocytes were treated with IL-1b. Relative quantitative RT-PCR for aggrecan, tenascin normalized to GAPDH were conducted to estimate the influence of IL-1b on latter transcript levels.

Results: Tenascin protein was immunodetected in normal and OA cartilage in different extensions. Tenascin expression by articular chondrocytes was confirmed by RT-PCR. In OA cartilage an increased tenascin deposition was present. A very strong tenascin staining was detected specifically in areas which show a major loss of proteoglycans. IL-1b treatment of OA chondrocytes in-vitro induced an inverse regulation of aggrecan- and tenascin-expression. Whereas aggrecan transcript levels were down regulated, tenascin levels strongly increased.

Conclusions: Our results strongly support the hypothesis that the inverse regulation of tenascin and aggrecan in-vivo, is regulated by IL-1b. It can be hypothesized that changes in the synthesis pattern of OA chondrocytes are influenced by proinflammatory mediators, diffusing from the joint cavity into the upper OA cartilage.

P4 – Expression of thrombospondin-1 and its receptor CD36 in human articular cartilage

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Thrombospondin-1 (TSP-1) a trimeric heigh-molecular weight glycoprotein is a multifunctional extra-cellular matrix protein. TSP-1 is involved in cell-matrix interactions of a various tissues. TSP-1 can bind to cells via different TSP-1 domains, its main receptors are CD 36 and CD51 (avb3-integrin). Nothern and western analysis showed the expression of TSP-1 in human cartilage, but its cellular source as well as the presence of its receptors CD36 and CD51 in normal and osteoarthritic cartilage are totally unknown.

Materials: We investigated 8 normal and 22 osteoarthritic cartilage samples on the expression patterns of TSP-1, CD36 and CD51, by immunohistochemistry and in situ hybridization.

Results: In normal cartilage we found TSP-1 to be present in the middle and upper deep zone. Predominantly chondrocytes of the middle zone showed RNA-expression. Also, its receptor CD36 was found mainely in the chondrocytes of the superficial and middle zone. In moderate osteoarthritic cartilage we found an increased number of TSP-1 expressing chondrocytes, as well as an increased pericellular immunostainig quite near to the surface. However, a small number of CD36 positive cells were observed across the whole OA cartilage. In severe osteoarthritic cartilage were observed a strong decrease in TSP-1 synthesizing chondrocytes by in situ hybridization as well as a strong reduction in the immunohistochemically matrix staining. In contrast to the decrease in TSP-1 we observed in 5 out of 8 these samples a overall enhanced number in CD 36 stained chondrocytes. Further, osteophytes with strong TSP-1 expression showed a large number of CD36 positive cells. However, CD51 positive chondrocytes could not be detected.

Conclusion: TSP-1 and its receptor are expressed in normal and osteoarthritic cartilage. The source of TSP-1 in normal cartilage are the middle zone chondrocytes, which also express the CD36-receptor. In early osteoarthritic cartilage an increase of TSP-1 was observed, whereas in later osteoarthritic cartilage TSP-1-synthesis is strongly decreased. It can be hypothesized that the strong enhanced number of CD36-stained chondrocytes in severe OA cartilage is a sign of chondrocytes frustrate efforts to contact the ECM, by binding to TSP-1.

P5 – Detection of eNOS in differenciated human chondrocytes

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The expression of iNOS is focus of many studies. Less is reported about the expression of the endothelial isoform eNOS. Its effect in various human organs has been investigated, but not in human articular cartilage.

The effects of eNOS are antiinflammatoric, anabol effects on tissues because of a low concentration of NO. We tried to show the expression of eNOS in human samples of articular cartilage.

Methods: After harvesting and digesting some cartilage flakes from human femoral heads we performed a normal monolayer-culture for about 12 days.

After confluence we cultured high-density-pellet-cultures in a special differentiating medium. After harvesting the pellets after 1 (t0), 7 (t1), 14 (t2) and 21 (t3) days we isolated the RNA of each pellet and produced the cDNA of the isolated RNA with RT-PCR and special primers (List 1).

List 1: Primers to detect eNOS

eNOS (forward):
5'-ACG TGC ACA GGC GGA AGA TG-3'
eNOS (reverse):
5'-GCA AGA GCT GAG GGC TGG GTG-3'

We showed the expression of eNOS in an electrophoretic analysis. As control we used human DNA-clones of eNOS-mRNA-sequence-insert in a pCMV-SPORT6-Vector. The DNA was also isolated, PCR-sequenced with the suitable pairs of primer and simultanously applicated to the electrophoresis.

Results: Our first results show the expression of eNOS in almost all of the 12 pellets of human samples of articular cartilage. Only one culture showed at t2 (14 days) no evidence of eNOS.

Conclusion: We detected eNOS in differenciated human chondrocyte-pellet-cultures with PCR and electrophoresis. We compared our results with a corresponding vectorized cDNA-clone simultanously applicated in the electrophoresis and found a correspondence with our results.

After the evidence of the presence and expression of eNOS in human articular cartilage the next step will show the influence of NO in the metabolic process in cartilage tissue.

P6 – Murine collagen induced arthritis: the pathogenetic influence of Fas, FasL and perforin

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Collagen induced arthritis (CIA) is the experimental murine model of rheumatoid arthritis (RA). One feature of RA preceding joint damage is synovial hyperplasia. Factors contributing to this phenomenon are unknown, however, imbalance between rates of cell proliferation and programmed cell death (apoptosis) has been suggested.

Methods: To evaluate the influence of two major pathways of cell death, perforin and FasL, in disease we determined the susceptibility to murine CIA of Fas, FasL, and perforin mutant mice. Mice carrying lpr (Fas), gld (FasL), and pfp (perforin) mutations were backcrossed onto the arthritis-susceptible DBA 1/J background for at least six generations. CIA was induced by immunizing mice with bovine collagen typ II in complete Freund's adjuvant. Arthritis was evaluated by clinical scoring and histopathological examination of the paw joints. CIA showed all the hallmarks of RA as synovial hyperplasia, pannus formation, cartilage destruction, and bone erosions each graded in a scale from 0 to 4.

Results: All three mutant strains mounted an immune response to collagen as evidenced by antibody titers and T cell proliferation assays. Contrary to expectation, DBA-lpr/lpr mice and gld/gld mice developed disease with incidence and mean date of onset comparable to control wild type littermates, however, arthritis was consistently milder in mutant mice: mean microscopic arthritis score 6 versus 7,6 in control mice at day 70 post immunization. DBA-pfp-/- mutant mice had delayed onset, day 49.9 as compared to day 41.5 in controls, whereas no differences were seen in incidence or clinical and morphologic disease severity.

Conclusions: Fas and perforin enhance the pathogenesis of murine CIA, but are not essential for disease induction. Our results suggest, that these major pathways of cell death do not play an essential role in the development of CIA.

P7 – Up-regulation of chondroadherin at the onset of articular cartilage degeneration in mouse model for osteoarthritis

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The aim of this study was to follow the expression of chondroadherin (CHAD) in mouse knee joints in normal and transgenic Del1 mice during knee joint degeneration and aging.

Methods: Transgenic Del1 mice with a short deletion mutation in Col2a1 gene develop early onset osteoarthritis in their knee joints (Saamanen et al. 2000). Knee epiphyses were collected at 2 to 15 months of age from Del1 mice, and from their nontransgenic littermates (controls). Northern analysis was used to follow the changes in CHAD mRNA levels and immunohistochemistry for protein distribution in normal and osteoarthritic articular cartilage.

Results: CHAD mRNA levels were up-regulated in Del1 mice at the age of 3 and 4 months, when OA lesions start to develop. At this age, major immunostaining for CHAD was seen in the intermediate zone of uncalcified articular cartilage, mainly in the interterritorial matrix. From 6 months onwards, major staining was seen in the calcified cartilage, with higher staining in the territorial than in the interterritorial matrix. Similar distribution patterns were seen in calcified cartilage of control mice, but at a much lower intensity.

Discussion: The results demonstrate up-regulation of CHAD mRNA and increased protein deposition in the superficial articular cartilage matrix of Del1 mice at the onset of cartilage degeneration, and later in the calcified articular cartilage. Earlier functional studies have shown that CHAD binds to collagen and promotes chondrocyte attachment on surfaces via a2b1 integrin. In Del1 mice up-regulation of CHAD production may result from attempts of chondrocytes to stabilize the extracellular matrix of degenerating cartilage.

Säämänen A-M et al. (2000) Osteoarhritis and Cartilage 8: 248-257.

P8 – Modifications of subchondral bone related to skeletal maturation: a 400 MHz quantitative ultrasound study in rat patella

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High frequency scanning acoustic microscopy provides images with a spatial resolution comparable to that of light microscopy. Using the Multi Layer Analysis method it is possible to obtain quantitative ultrasound (QUS) information, which can be related to mechanical and structural properties of the explored tissue. We have used this technique to investigate the age-related changes occurring in subchondral bone during rat skeletal maturation.

Methods: Sixteen patellae of 8 immature (7 weeks old) and 8 mature (11 weeks old) rats were fixed in methanol and cut in the sagittal plane of the patella using a high precision milling machine. Samples were then imaged in methanol at 400 MHz (3 µm spatial resolution) using a scanning acoustic microscope (SAM 2000). Acoustic impedance (Z) images of each sample were constructed using the local measurement of the reflection coefficient. Results were compared to histology.

Results: Qualitative analysis revealed that bone porosity decreased with age and the bone/cartilage interface became more regular. The mean impedance values of mature and immature samples were similar (5.0 ± 0.6 MRayl). However in mature bone, two regions of different reflection coefficients could be distinguished on 6 samples out of 8: a region just beneath cartilage where the lower Z (4.5 ± 0.6 MRayl) may indicate a low mineralized transitional zone between bone and cartilage; and a region of higher reflection coefficient (Z = 5.2 ± 0.7 MRayl) and probably higher mineralization in the deeper and earlier formed bone.

Conclusion: 400 MHz acoustic microscopy is sensitive to some changes occurring in subchondral bone during the maturation process. Qualitative images enable assessment of changes in bone structural organization and elastomechanical properties at a microscopic scale. Measurements of acoustic impedance are a new means to quantify the observed changes. The study of relationships between QUS microscopy and bulk properties will be the object of further works. QUS microscopy could therefore be a new method to investigate changes occurring in bone during the development of diseases like arthritis and osteoarthritis affecting bone and cartilage.

P9 – Aminoterminal propeptide of type I alfa1-homotrimer (hotPINP)

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At least two variants of type I collagen (classical form: alfa1x2-alfa2 and alfa1-homotrimer: alfa1x3) are found in vivo. During biosynthesis of these collagen variants also two different aminoterminal propeptides (abbreviated hetPINP and hotPINP) are formed. The available immunoassay for the intact PINP can not distinguish them. The ability to detect the synthesis of type I alfa1-homotrimer collagen is interesting since the Sp1 polymorphism of Col 1 A1 gene has been suggest to lead to its synthesis. Also in osteoarthritic bone has been found different ratios of alfa1/alfa2-chain (normal 2:1, OA bone 4:1-17:1). The alfa1-homotrimer collagen accounts for narrower disorganised collagen fibres and decreased mineralisation. The decreased tensile strength could explain the increased fracture risk. Aim of this study in to develop a specific assay for hotPINP.

Methods: The hotPINP was purified from human ascites and pleural fluids. In purification we used affinity chromatography (monoclonal antibody), DEAE-chromatography in low pH (to separate het- and hotPINP) and gel filtration. Intact PINP was estimated by radioimmunoassay, which measures both PINP variants equally. We selected a sandwich chemiluminescence immunoassay (CLIA) technology to distinguish if there are two or three similar alfa1-chains in the propeptide. At first thirteen different monoclonal antibodies against the PINP were tested both in capture and reporter position thus giving 169 combinations. The antibodies were biotinylated or labelled with acridiniumester. We used streptavidin coated 96-well plates. A flash-type chemiluminescence was analysed by a Victor2-multilabel counter.

Results: The correlation between CLIA and RIA for the intact PINP is 0.972 (n=210). Seven pairs reacts preferentially with the hotPINP. Since our hetPINP preparations contain some hotPINP, the exact determination of the cross-reaction needs further work. When we have analysed patient samples (elderly and children samples) hotPINP didn't correlate with intPINP. If intact PINP was low or near to low end reference range, percentage of hotPINP to intact PINP in some cases was high (20–80%).

P10 – Influence of cartilage biochemical components on 50 MHz ultrasound backscatter: a quantitative study in rat patella

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We have previously reported that quantitative 50 MHz ultrasonography was sensitive to subtle and progressive cartilage changes induced by experimental osteoarthritis in rat knee [1,2]. The goal of the current work was to identify the cartilage components that are responsible for

high frequency echographic signal changes.

Methods: The study was performed on male Wistar rat patellar cartilage. It included evaluation of both collagen and proteoglycan (PG) contents during maturation process and following cartilage PG depletion using a specific enzyme (hyaluronidase). Patellar cartilages were examined in vitro using a 3-D 50 MHz backscatter ultrasound scanner (25 μm axial resolution). US quantitative analysis was based on the evaluation of the cartilage surface and internal structure modifications using quantitative acoustic parameters: integrated reflection coefficient (IRC, surface) and apparent integrated backscatter (AIB, matrix). US data were compared to histological and biochemical findings.

Results: During maturation, US assessment revealed no changes in cartilage surface but a significant decrease in backscatter from cartilage internal structure. For PG depleted samples, no significant changes in US parameters was found between degraded and control cartilages. Correlation between US findings and biochemical and histological data demonstrated that PG variation has no effect on US signal but high frequency US backscatter is sensitive to collagen network organization

Conclusion: Current results demonstrate that changes in collagen network organization are responsible for variations of 50 MHz ultrasound backscatter. High resolution ultrasonography has potential to be used as a relevant quantitative technique for the evaluation of therapeutic effects.

[1] A. Saied et al., Assessment of articular cartilage and subchondral bone subtle and progressive changes in experimental osteoarthritis using 50 MHz echography. J Bone Min Res 1997; 12: 1378- 86.

[2] E. Cherin et al., Evaluation of acoustical parameter sensitivity to age-related and osteoarthritic changes in articular cartilage using 50-MHz ultrasound. Ultras Med Biol 1998; 24: 341-54.

P11 – Differential approach to the therapy of osteoarthrosis of the knee joint of the elderly patients

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Nowdays osteoarthrosis presents one of the most important problems of geriatric orthopedy. In the investigation 39 patients, age 57–74, with 1–2 stage of knee joint osteoarthrosis took part. Methods of study: orthopedic examination, x-ray of knee joint, visual analogie skale Mc Gill questionary, ultrasound densitometry, reovasography of shank, which was carried on before and after of the treatment course.

All patients were divided in three groups and dot basis therapy nonsteroid antiinflammatory drugs and vitamins. Besides this patients of the first group (n=25) got bioresonanse stimulation on knee joint during 10 minutes every day, 10 procedures for a cource. The patients of the second group (n=9) with the pathology of bloodrun in the chank wessels got intravenous in dropper 10 ml of actovegini in a day. The course of treatment consisted of 10 droppers. For the patients of the third group (n=5), in which according to the ultrasound densitometry data was diagnosed osteoporosis, miacalcic 100 IU in a day was included, 10 injections for a course. The investigations carried out after the treatment course showed a reliable improvement of the patients of all three groups, that manifested by the decreasing of pain syndrom. Besides this in the patients of the second group the improvement of periferal bloodrun in the shank was noted (the increasing of puls bloodfilling and the flexibility of vessels). Taking of miacalcic by the patients of the third group promoted the improvement of the structural-functional state of the bone system.

Thus, the differential approach to the therapy of osteoarthrosis of the knee joint taking into account the state of bone tissue and periferal bloodrun improves the quality of rehabilitation of the elderly age group patients.

P12 – Markers of bone turnover do not predict bone metastases in breast cancer

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Markers of bone turnover are often elevated in breast cancer patients with bone metastases (BM). To test whether bone markers could be used as early indicators of developing BM, we prospectively followed 112 postmenopausal women operated for primary breast cancer.

Methods: At the time of diagnosis/ study inclusion, none of the women had BM, other skeletal disease or bone active drugs. During follow-up (range 0.6–4 years, median 30 months), patients were seen every 3 months and timed blood/ urine specimens were obtained. Eleven patients developed BM (BM+) and each of these were matched to 4 women free of BM (BM-). The markers measured were serum (s) calcium, sTAP, sBAP, sOC, sPICP, urinary (u) PYD, uDPD, uNTX, sNTX, uCTX, sCTX. All analyses were done in single batches after study end.

Results: At any given point in time (including baseline), marker levels in the BM+ group did not differ significantly from those in the BM- group. Marker levels at baseline did not predict the later development of BM (OR 0.14–1.01, all NS). 93% of all changes in bone markers were below the least significant change,

as defined in an independent group of similar patients. The remaining 7% of values could not be associated in a consistent pattern with the occurrence of BM.

Conclusion: In patients with breast cancer, biochemical markers of bone turnover can not be used to predict or diagnose incident BM. This lack in diagnostic validity is mainly attributable to the high overall and long-term variability of the currently used bone markers.

P13 – A new histological score for the evaluation of osteolytic tumour burden in nude rats

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Radiographic and histological quantitation of bone related tumour burden is an important task when assessing experimental models of metastatic bone disease. Using a novel nude rat model of site specific metastatic osteopathy, we describe a semi-quantitative histological score for the evaluation of osteolytic tumour burden in bone.

Material and methods: Male nude rats (n = 12 per group) were inoculated with 100000 cells of the human breast cancer cell line MDA-MB-231. Cells were injected directly into the femoral artery of both legs. When radiographically visible osteolytic lesions appeared on d18, animals were either left untreated (group 1) or were treated with Ibandronate (IBN) s.c. 10 mcg P/kg/d until day 30 (group 2). Total animal X-rays were obtained on days 0, 6, 12, 18, 22, 26, and 30 and osteolytic areas (OA) were measured by computer based analysis (X-CBA). After sacrifice of the animals on d30, histological assessment of tumour burden was performed in both tibiae using a semi-quantitative score. This score involved the extend and specific localisation (cortical bone, bone marrow, growth plate) of metastatic infiltration, and the pattern of tumour growth. For comparison, metastatic tumour area (MA) was determined by histologic CBA(H-CBA).

Results: H-CBA correlated with the score sum at r= 0.762 (group 1) and r = 0.951 (group 2). X-CBA correlated with the score at r = 0.845 (group 1) and r = 0.854 (group 2), and with H-CBA at r= 0.815 (group 1) and r = 0.765 (group 2). When the score was correlated with the extend of metastatic infiltration only, correlations were r = 0.702 (group 1) and r = 0.970 (group 2). Dependent on the OA at treatment initiation, IBN prevented or reduced these lesions.

Conclusions: The score provides a simple and rapid histological estimate of metastatic tumour burden, which closely correlates with the quantitative histologic and radiographic methods.

P14 – Biomodels for bone metastases of hormone dependent cancer

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Bone metastases are common in breast and prostate cancer. Most breast cancer metastases are osteolytic whereas most prostate cancer metastases are osteosclerotic. Angiogenesis is acquirement for tumor growth and dissemination. Vessel formation is activated in response to a stimulus produced by tumor cells or adjacent stromal cells. Appropriate stimulus could be vascular endothelial growth factors (VEGFs), which have been found to be overexpressed in prostate cancer and have an important role in tumor angiogenesis and growth. Fibroblast growth factors (FGFs) are also produced by breast and prostate cancer cells and are strong candidates for mediating bone formation in cancer.

The aim of this study is to develop in vivo models to study breast and prostate cancer growth and metastases into bone. We will study the role of VEGFs and FGFs, especially FGF-8b, in bone metastases of prostate cancer.

Methods: We used the intracardiac and orthotopical implantation method in young athymic nude mice. Implanted cells were either native prostate cancer cells (PC-3 and DU-145 cells) or prostate cancer cells transfected with VEGF, VEGF-C or FGF-8b. After implantation we followed tumor growth by weighting animals and by taking X-ray pictures. Serum of mice was collected for TRAP measurement. We also took histological samples from bones and systemic organs for finding microscopical tumors.

Results: We have developed an in vivo model of prostate cancer. The intracardially injected cells metastasized to bone and local lymph nodes. The cells injected orthotopically into prostate developed into tumors that spread to local lymph nodes, but only a few bone metastases were observed.

Conclusion: We have established an appropriate model for studying the actual progression of prostate cancer.

P15 – Gait abnormalities after total knee arthroplasty—non-invasive three-dimensional kinematic analysis

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Preliminary investigation was made to evaluate gait of the patients after total knee arthroplasty with posterior cruciate ligament substituting or retaining AGC system.

The purpose of the study was 1) preliminary investigation of the functional status of the patients after total knee arthroplasty with PCL-substituting or -retaining system, and 2) preliminary gait analysis of the patients after total knee arthroplasty with PCL-substituting or -retaining system.

Materials: Between 2000–2001 17 consecutive total knee arthroplasties in 17 patients (14 women, 3 men) were made. There are following prosthesis implanted: with retaining PCL – 7, with substituting PCL – 10. All patients were evaluated. Patients' mean age was 64.3 years old.

Methods: Functional status according to HSS knee score at the 14 days and 6 months after procedure was regard as the end results for both types of TKR. Gait analysis was made in both group with computerized motion analysis system (VICON 250, Oxford Metrics) in Biokinematic Laboratory of Academy of The Physical Education.

Results: Our preliminary study does not support any functional advantage of the PCL-retaining design over the PCL-substituting design. Preliminary results of meticulous kinesiologic and kinematic gait analysis with three-dimensional computed image reconstruction are presented.

Conclusions: 1) there are no statistical differences between early functional results of the TKA with PCL-substituting or retaining system in our material, however the time of follow-up is very short and study group is very small, 2) until now, there are no differences between the TKA with PCL-substituting or -retaining system in our material during level walking with regard to basic kinematic gait parameters, angle changes of the knee joint, muscle length changes of the inferior extremity and the knee angular velocity, and 3) the study, however, is not accomplished (i.e. muscle strength or EMG activity were not included).

P16 – Characterization of functional responses to fluid shear stress in osteocyte-like cells

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Fluid shear stress caused by mechanical loading plays an important role in regulation of bone remodeling. Osteocyte has been considered to be a promising candidate to provide a cellular basis for mechanosensing in bone. In order to determine the role of the osteocytes in the mechanical loading, we examined the functional responses to fluid shear stress in osteocyte-like cell line MLO-Y4 culture. Cone-plate loading apparatus was designed for this purpose, where a tapered cone was rotated on the medium-filled culture plate, generating continuous laminar fluid flow over the cells. Cell number, area, and orientation of major axis in each cell were morphologically analyzed after applying 5-minute stimulations of 1Pa every 12 hours to the cells. The application of fluid shear stress made the cell align with the direction of fluid flow while the control cells without stimulation showed almost uniform orientation toward all directions. There were no significant differences in the cell number and the area between the control and fluid-sheared cells. Staining of microfilaments showed that the fluid shear stress induced cytoskeletal reorganization in the osteocytes, even in case when the cells were subjected only to brief periods of fluid flow. Additionally, cyclo-oxygenase 2 (COX-2) expression was examined in the fluid-sheared MLO-Y4 cells. COX-2 is a key enzyme for the formation of prostaglandins which may mediate the effects of mechanical loading on bone metabolism. Real time reverse transcription polymerase chain reaction (RT-PCR) method revealed that the osteocytes increased mRNA expression level of COX-2 in response to the fluid shear stress. Up-regulation of COX-2 related to mechanical stimulation was also shown by immunohistochemical observations. These findings may offer some insight into the role of osteocyte although further investigations are necessary to reveal the mechanism by which osteocyte senses mechanical loading and regulates the functions of osteoclast and/or osteoblast.

P17 – Validation of the remodelling parameters used for the simulation of cancellous bone resorption

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A dynamic stochastic simulation of cancellous bone remodelling that is integrated with a finite element package has previously been described and partially validated by examining the mesh density and model size. The two main stochastic remodelling parameters are activation frequency (a.f., the percentage of surface pixels to be activated) and activation length (a.l., the size

of each resorption pit). This paper investigates the effect of varying these parameters on the predicted properties of realistic 2D bone structures taken directly from mCT scans of cancellous bone.

The results show that whilst an increased activation frequency, and/or activation length obviously leads to a more rapid depletion in bone density (and therefore decrease in stiffness), the density/stiffness relationship remains unchanged. Despite the marked difference in stiffness for a particular step, when considering the stiffness vs. density relationship, the relationship remains unaltered irrespective of activation frequency. This was found to be true when considering the stiffness in both the x- and y- direction.

Variation of the chosen remodelling parameters for the simulation had no significant effect on the density/stiffness relationship for the particular structure examined. It is anticipated that the architecture of the structure will have an effect, in particular on the likelihood of perforation. Work is underway to quantify this, using different realistic bone structures and an in-house histomorphometry tool to evaluate and classify the architecture of samples as they are depleted.

P18 – The effect of strain adaptation on the anabolic remodelling of depleted cancellous bone structures

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The application of a 2D dynamic stochastic simulator of cancellous bone remodelling to anabolic therapy of depleted lattice bone structures has previously been described. The simulator has been extended to model actual bone sections, and now incorporates strain adaptation. Using finite element analysis, a two-dimensional human histological section produced from mCT was subjected to a complex loading pattern and strain-remodelling limits to produce an equilibrated, realistic bone structure. The structure was then depleted stochastically by 5% of its density and then either a) stochastically rebuilt, or b) strain-equilibrated and subsequently stochastically rebuilt. The density and x- and y-stiffness values of the structures (kx and ky respectively) were calculated for each run and compared. The resorbed structure had kx and ky values of 79% and 81% of the original structure. Equilibration of the resorbed structure resulted in a further decrease in density of 15%, with resultant stiffness values of 56% (kx) and 72% (ky) of the original. However when each of these structures were stochastically rebuilt to original density, the equilibriated structure had regained 95% (kx) and 104% (ky) of the original stiffness, whereas the totally stochastic structure had only regained 89% (kx) and 88% (ky). Regained original stiffness was achieved at 4% over original density for kx and at approximately rebuilt original density for ky for the equilibriated structure, but at 7% and 10% for kx and ky respectively for the stochastic structures.

Strain adaptation constantly optimises bone's load bearing capabilities and is an important consideration in bone remodelling simulations. This work shows its effect on simulations of anabolic therapies of realistic bone structures. The paper will present further investigation of the effect of strain adaptation at different stages of the depletion and rebuilding process, and also consider structures with greater initial depletions and differing degrees of trabecular thinning and perforation. Density, stiffness and histomorphometric data for the structures will be presented and discussed.

P19 – Use of stereolithography models as a validation tool for FEA of cancellous bone structures

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A stochastic simulation of cancellous bone resorption that is integrated with a finite element package has been previously described, and the mesh density, model size and remodelling parameters validated. The simulation was initially applied to two-dimensional idealised cancellous bone structures in a simulation of osteoporosis, and the finite element results were validated by the mechanical testing of stereolithography (STL) models of the depleted structures. This STL validation method proved to be promising and been extended into three-dimensions. This current paper will describe the extension of the method to realistic bone structures in three-dimensions, using micro-CT scan data from histological samples of human cancellous bone, and the subsequent mechanical testing of the STL models.

It is believed that the finite element results and STL models of the bone will allow us to fully explore the relationship between the stiffness and strength of the bone and its physical and geometric properties. The possibility of producing a number of samples of the same bone structure enables repeatability studies to be conducted and eliminates the end effects that can occur during machining of actual bone samples.

P20 – Dependence of FEXI (finite element analysis of X-ray images) derived stiffness upon resolution

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Mechanical stiffness derived from finite element analysis of conventional X-ray images (FEXI) has the potential to provide a superior prediction of fracture risk than conventional bone mineral density (BMD) assessment. FEXI may be applied to a range of X-ray based imaging modalities including microCT data, digitally scanned plane radiographs and conventional dual-energy X-ray absorptiometry (DXA) scans; and may be performed at various anatomical sites including distal radius, proximal femur and phalanx. However, the spatial resolution of these techniques varies significantly, typical values being 20 µm, 50 µm and 800 µm respectively for microCT, digitised plane radiographs and DXA. The dependence of FEXI derived stiffness upon spatial resolution was investigated in a 4 mm cubic sample of calcaneal sample bone that had previously been digitised via microCT, providing a 3D voxel map (141x141x141) at a resolution of 28 micro m. 2D projections simulating a radiograph or DXA scan were created for each of the three orthogonal directions. Maximum FEXI stiffness anisotropy was obtained with loading in the Y-direction, described by XY and ZY projections. The resolution of these 2D projections was gradually reduced by re-sizing the images. The degree of anisotropy decreased with reducing spatial resolution. Whilst the XY stiffness remained approximately constant, the ZY stiffness decreased significantly. This may be explained by the XY and ZY projections describing plate face surfaces and plate edges respectively; as image resolution is reduced, there is a more significant change in the finer detailed plate edge 'structure'. The clinical implications of these findings warrant further investigation.

P21 – Histomorphometry analysis—a novel application to simulation of strain-adaptation in cancellous bone

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In order to quantify the morphology of structures produced by our work on computer simulation of cancellous bone remodelling, a novel set of algorithms has been developed and applied. The set of metrics is organised in three themes. The first one deals with the direct derivation of basic metrics such as the trabecular thickness, spacing and number. The second deals with the area and volume distribution of bone and marrow, while the third considers surface roughness. The algorithms were tested on sample sections of cancellous bone and applied to a simulation of strain adaptation incorporating complex multi-axial loading. The metrics have been shown to be independent of structural anisotropy.

During the strain-adaptation simulation, BV/TV reduced steadily. However, detailed surface analysis demonstrated a reduction in perimeter and increase in surface roughness associated with trabecular perforation. Star area metrics were also sensitive to perforation. Marrow star area (SAD.M) demonstrated associated increase in both mean and SD values. In contrast, bone star area (SAD.B) remained fairly constant but with increasing standard deviation, probably indicating the presence of straighter and thicker trabeculae resulting from the strain-adaptation process.

P22 – Physical activity and exercise in adolescent girls influence parameters of quantitative ultrasonometry in weight-bearing (tibia) but not in non-weight-bearing bones (radius)

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In order to determine the effect of physical activity and exercise on QUS parameters in bone, we used the Sunlight OmnisenseTM Bone Sonometer (Sunlight Medical Ltd., Rehovot, Israel) for measurements of speed of sound (SOS) at different skeletal sites. In a cross-sectional study, we measured 335 healthy Caucasian girls—mean age 12.6 years (SD 3.13; range 6–18). Measurements have been performed at the tibia and the radius in order to analyze effects on weight bearing as well as on non-weight bearing bones.

A statistically significant correlation ($p < 0.001$) was found between age and maximum SOS at the radius ($r = 0.64$) and the tibia ($r = 0.65$). We observed also significant increases in the maximum SOS at the tibia between Tanner stages II-V and at the radius between Tanner stages III-V.

Additional information was obtained by questionnaire for physical activity. Thereafter, we compared 2 groups: 110 girls with high physical activity (regular sport activities—incl. athletics, gymnastics, ball game sports—for at least 3 hours/week) and 225 girls without any regular sport activities (other than school sports).

For the age groups before onset of puberty no effects of physical activity on SOS values were found for any measurement site. However, in adolescent girls aged 11 years and older we observed higher maximum SOS values at the tibia for the active group compared with the less active group, but did not found similar results at the radius.

Higher mean values of the maximum SOS at the tibia were significant for all Tanner stages ($p < 0.001$). The mean difference in SOS values for the entire group of active girls compared to the group of less active girls was about +85 m/s (0.78 SD).

In conclusion, in adolescent girls physical activity and exercise increase SOS values at the tibia (weight bearing) but not at the radius (non-weight bearing). This effect of bio-mechanical loading is consistent for the age groups above 11 years and for all puberty stages, indicating the possible role of puberty related to hormonal changes for the responsiveness of the bone for bio-mechanical impacts. However, we still do not know exactly which morphological or functional entity of the bone measured by QUS, this is still a subject of further research.

P23 – Hydroxylation of inert oxide ceramics enhances in vitro bioactivity

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Oxide ceramics are excellent materials for implants and prostheses because of their biomechanical stability. Additionally they do not interfere with magnetic resonance imaging, so it is possible to investigate, for example after spondylodesis, the surrounding soft tissue. However, oxide ceramics are bioinert, and after implantation failure rate is high. Aim of our study was to determine the influence of a surface modification via hydroxylation of oxide ceramics on the effects on human osteoblast like cells in vitro.

Methods: Alumina and zirconia ceramic plates were sintered at 1600 °C with a final diameter of 23 mm and the surface was polished to a mirror finish. The plates were then treated with 30% sodium hydroxide solution for 12, 24 or 48 hours (activation time) at 90 °C or were left untreated (control). X-ray diffraction pattern of activated plates showed parts of AlOH at the surface. Bending strength was examined in a four point bending test.

Human osteoblast-like cells were prepared from bone obtained from hip surgery per explant technique and cultured in DMEM medium (10% CS, 1% penicillin/streptomycin). Cells were seeded with $4x10^4$ cells / probe. We examined attachment, survival and apoptosis rate after 24 hours and protein, alkaline phosphatase (AP) and osteocalcin (OC) secretion as well as mineralisation rate after 7 days.

Results: There was no sign of cytotoxicity or growth inhibition. Adhesion was significantly enhanced up to 120% of control after 24, 48 and 96 hours activation time ($p <0.05$), AP secretion was enhanced up to 130% of control after 24 and 48 hours activation time ($p <0.05$). OC secretion showed no significant differences. Mineralisation was detectable at all probes.

Bending tests showed no differences.

Conclusion: Treating the surface of inert oxide ceramics with NaOH solution to produce biologically active OH-groups is a newly developed, simple and cheap technique. Bioactivity of alumina ceramics was clearly enhanced without cytotoxic effects or material changes. In conclusion, this method 'bioactivates' bioinert ceramics. In the future, it might be possible to design pure ceramic prostheses without the problem of early loosening.

P24 – Molecular biologic comparison of new bone formation and resorption on microrough and smooth bioactive glass microspheres

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In a recent in vitro study, chemical microroughening of bioactive glass surface was shown to enhance attachment of MG-63 osteoblastic cells to glass. The current study was designed to delineate the effects of microroughening on the gene expression patterns of bone markers during osteogenesis and new bone remodeling on bioactive glass surface in vivo.

Methods: Using a rat model of paired comparison, a portion of the medullary canal in the proximal tibia was evacuated through cortical windows and filled with microroughened or smooth bioactive glass microspheres. The primary bone healing response and subsequent remodeling were analyzed at 1, 2, and 8 weeks, respectively, using radiography, pQCT, histomorphometry, BEI-SEM and molecular biologic analyses. The expression of various genes for bone matrix components (type I collagen, osteocalcin, osteopontin, osteonectin) and proteolytic enzymes (cathepsin K, MMP-9) were determined by Northern analysis of the respective mRNAs.

Results: Paired comparison showed significant differences in the mRNAs levels for specific bone matrix components at 2 weeks: osteopontin was significantly higher ($p=0.01$) and osteonectin significantly lower ($p=0.05$) in bones filled with microroughened microspheres than in those filled with smooth microspheres. Bones filled with microrough microspheres also showed significantly increased ratios of cathepsin K and MMP-9 (both markers of osteoclastic resorption) to type I collagen ($p=0.02$ and $p=0.02$, respectively) at 2 weeks and a significantly

increased expression of MMP-9 at 8 weeks (p=0.05). The pQCT, histomorphometric and BEI-SEM analyses revealed no significant differences in the pattern of bone healing response.

Conclusion: Based on these results, microroughening of a bioactive glass surface could trigger temporal changes in the expression of specific genes especially by promoting the resorption part of new bone remodeling processes. Future studies are needed to evaluate if the observed changes of gene expression are directly related to the microrough surface of any biomaterial or biomaterial-specific.

P25 – Restoring the bone and cartilaginous tissue with the use of the material "LitAr"

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The material "LitAr" is an osteoconductive material for replacing the defects of the bone and cartilaginous tissue. The material has in its composition xenogenic collagen and calcium hydroxyphosphate (hydroxyapatite) which has been formed in the collagen matrix. The kind of method of making the material ensures a high level of the structural integration of the components, although there are no chemical bonds between them or at a level of hydrogen bonds. The compound has been successfully employed in otorhinolaryngology, maxillofacial surgery, traumatology and orthopedics.

In rhinosurgical practice the employment of the semisynthetic or synthetic implants does not ensure a total restoration of the cartilaginous tissue after performing the septum-operations.

We used the material "LitAr" for the first time for replacing the cartilaginous tissue of the nasal septum in the region of the tetragonal cartilage under the septoplasty conditions. The material biotransformation was checked with the use of computed tomography. Optical density in the defect region in the tomogram corresponded to 50–80 H—this value conformed with the native hyaline cartilage.

We used the material "LitAr" in surgical stomatology for replacing the jaw bone defects which resulted from the tumour-like diseases such as radicular and follicular cysts and fractures. In the event of fractures the material was introduced after the access to the defect zone as well as injectionally.

The dynamical scintigraphical investigation of the upper and low jaw after replacing the cystophorous cavity with the material "LitAr" was conducted with the use of the radiopharmcompound "Technephor" accumulation of the radiopharmcompound for the upper jaw was equal to 30 days. The following reduction of the ^{99m}Tc-accumulation level in the region of the replacement provided support for the bone tissue formation. According to the computed tomography data for the low jaw the maximum value of the same process fell within the 40th–50th day.

Thus, the application of the material "LitAr" is a promising line for replacing the postoperative defect of the bone and cartilaginous tissue.

P26 – Estimation of microstructural parameters of femoral cortical bone with microcomputed tomography

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The microstructure of cortical bone is influenced by age and osteoporosis. With microcomputed tomography (µCT) we measured parameters of cortical microstructure and compared them to histomorphological parameters of the femoral diaphysis.

Femoral cortical bone specimens from the mid diaphysis of 24 patients were harvested during the procedure of total hip replacement at the location, where normally one hole (Ø 4.5 mm) for the relief of the intramedullary pressure is placed. In vitro intracortical porosity and bone mineral density (BMD) measurements by µCT were compared with structural parameters assessed in histological sections of the same specimens. A strong correlation was found between intracortical porosity measured by µCT and histological porosity ($r = 0.95$, $p < 0.0001$). Also the fraction of porous structures, average pore diameter and av. pore area correlated well with µCT porosity ($r = 0.94$; $r = 0.88$ and $r = 0.90$; $p < 0.0001$) and with BMD ($r = -0.71$; $r = -0.66$ and $r = -0.73$; $p < 0.0001$).

Osteon density and fraction of osteonal structures were associated with µCT-Porosity ($r = -0,68$; $r = -0.63$; $p < 0.001$) and with BMD ($r = 0.58$; $r = 0.74$; $p < 0.0001$). We consider the measurement of porosity by µCT as a very potent procedure for assessing intracortical porosity and parameters related to porous structures of cortical bone nondestructively. This method could establish the possibility of estimating the morphological properties of cortical bone in small bone specimens by µCT.

P27 – Prediction of strength of cortical bone in vitro by microcomputed tomography

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The aim of this study was to evaluate the predictive value of bone mineral density and intracortical porosity measured by microcomputed tomography (μCT) for the strength of cortical bone biopsies.

Experimental study comparing the predictive value of bone mineral density and of intracortical porosity determined in vitro by microcomputed tomography for the mechanical properties of cortical bone cylinders

The assessment of cortical bone strength might be relevant for the prediction of fracture risk or the choice of suitable therapy strategies in orthopaedic surgery. The predictive value of cortical density for the mechanical properties is discussed controversially. The relevance of intracortical porosity measured by histomorphometry has been established, but the predictive value of porosity determined by microcomputed tomography remains to be explored.

Femoral cortical bone specimens from the mid diaphysis of 24 patients were harvested during total hip replacement procedure at the location, where a diaphyseal hole (Ø 4.5 mm) was drilled in order to reduce the intramedullary pressure. In vitro intracortical porosity and bone mineral density measurements by microcomputed tomography were compared with strength and elastic modulus assessed by a compression test transverse to the Haversian systems of the same specimens.

Significant negative correlations were found between porosity measured by microcomputed tomography scans and yield stress, stiffness and Elastic Modulus ($p < 0.001$), however, the positive correlations between bone mineral density and mechanical parameters were stronger ($p < 0.0001$). The mechanical parameter best predicted by mineral density as well as by porosity was yield stress ($r = 0.72$, $p < 0.0001$; $r = -0.64$, $p < 0.001$).

Bone mineral density determined by microcomputed tomography imaging in vitro may be a potent method to predict mechanical properties of cortical bone non-destructively. The application in vivo remains to be explored.

P28 – Cortical structure and bone volume as a non-invasive predictor for vertebral fracture risk

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The aim was to assess the value of cortical volume and cortical structure in CT images as a non-invasive predictor of vertebral fracture load.

Methods: 40 female patients, age 37–89 (median 58) were referred to us for suspected osteoporosis. For each patient, a lateral X-ray was available, on which the number of spinal fractures could be determined. BMD was measured in L1–L3 by QCT. The patients were divided into 4 groups based on WHO criteria: Group 1 (healthy, T-score > 1); Group 2 (osteopenia, T-score between -1 and -2.5); Group 3 (osteoporotic, T-score < -2.5, no fractures); Group 4 (osteoporotic, T-score < -2.5, fractures). 3 CT slices (2 mm) thickness, spanning a 6 mm mid- vertebral section were acquired and the cortical shell segmented using an automated algorithm. Cortical volume was determined by counting the voxels (0.2x0.2x2 mm) in the segmented corticalis. Quantitative parameters to describe cortical structure were obtained through the methods of low-BMD cluster counting and analyzing the gray-value profile of the cortical ridge. The values were related to age and compared between the Groups.

Results: The description of cortical microstructure by using the cortical ridge leads to significantly elevated parameter values in osteoporotic patients with fractures (Group 4). No differences were found within Groups 1–3. The parameter obtained by counting clusters of low BMD shows significant differences between Groups 2 and 3 (osteopenic / osteoporotic) and between Groups 3 and 4 (unfractured / fractured). Both structural parameters also increased significantly with age. Cortical volume was 8.5% lower in Group 4 over Groups 1–3, without statistical significance. No significant change of cortical volume was observed with age.

Conclusion: The load- bearing role of the cortical shell and the importance to include cortical properties in the diagnosis of osteoporosis is increasingly recognized. We found the hypothesis that cortical volume significantly changes with age and differs between osteoporotic and non-osteoporotic groups not to be true. Both BMD and parameters quantitatively describing cortical microstructure independently from BMD significantly change with age and the number of fractures, thus improving the prediction of fracture risk.

P29 – Determination of metric accuracy and uncertainty of micro-CT scans

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Cone beam micro-CT scans realize different voxelsizes by arranging different detector–tube as well as specimen–tube distances. According to geometric relations, voxelsizes can be determined and accuracy can be calculated from the pixelsize of the detector and the diameter and spatial stability of the x-ray spot. We tried to verify the calculated sizes by scanning specimens with certain dimensions.

Methods: Different manufacturers provided us with a hydroxylapatite foam of known bubble diameters, a sawed array of plastic pillars and metal wires. The objects were scanneded at different magnifications in the micro-CT. First, we determined their dimensions in a single planar picture and then we compared the sizes with those in the reconstructed volume. The objects were additionally measured by a calibrated reflected-light microscope and by another micro-CT.

Results: The measurements are still on the run and a first look at the results showed that the measured values of different methods were in comparable ranges. Repeated measurements in our microCT showed good reproducibility and the measured sizes did not vary reasonably at different magnifications.

Conclusion: The actual measurements turned out to be more difficult than expected. There were not only discrepancies between the values of different methods but the accuracy within the manufacturing process is uncertain as well. Furthermore, different materials show different partial volume effects. That is simple metal wires are not comparable with measured sizes of other materials. The apparent size depends on the gray scale adjustment. Normalization of the whole micro-CT system is mandatory, if reliable measurements are required.

P30 – Comparison of bone composition at six different skeletal locations by measures of complexity and bone mineral density

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The aim of the study was to compare the structural composition and deterioration of human bone tissue in osteoporosis at six different skeletal sites. The bones' composition was evaluated by measures of complexity.

Methods: Bone specimens of 29 human cadavers were examined. The distal radius, the proximal tibia, the vertebra L3, the femoral neck and the head, the calcaneus, and the midshaft of the humerus were scanned in high resolution mode (0.2 x 0.2 mm) in 1 mm slice thickness on a special configured XCT-2000 scanner (STRATEC, Germany). Our technique consist of four stages: 1) establishing the relation between pixel values and the BMD, 2) image preprocessing for standardized segmentation of a ROI from the rest of the image and splitting the ROI into trabecular bone and cortical bone, 3) symbol-encoding substitutes the value of every pixel by one of 5 different symbols, and 4) six measures of complexity assess the different aspects of bone architecture. The BMD is calculated from the very same areas which are used to quantify the architecture.

Results: Plots of bone density versus bone architecture complexity were used to analyze the organization and the loss of bone tissue. Skeletal sites can be distinguished from each other by both their BMD and their architecture assessed by the complexity measures. Despite the same BMD, bones from different skeletal locations have different complexities and different degrees of disorder. The rate of complexity change is defined by the skeletal location as well. Rank-Order correlation coefficients were used to compare different skeletal sites. The strongest correlation is found between the bones of the same upper and lower extremities. The lowest correlation is found between the vertebra and peripheral bones. The correlation between all skeletal sites of the whole bone is stronger than the trabecular bone alone.

Conclusion: The distinctions of architectural composition at different skeletal regions can be accurately assessed by measures of complexity. The femoral head is the most structural competent bone, opposed to the vertebral body, which has the least complex architecture.

P31 – Tartrate-resistant acid phosphatase functions as a protein tyrosine phosphatase in mouse lung tissue

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Tartrate-resistant acid phosphatase (TRACP) is an enzyme with unknown biological function. It is expressed primarily in bone-resorbing osteoclasts and activated macrophages. TRACP knock-out mice develop mild osteoporosis and show reduced clearance of the pathogen S. Aureus, suggesting an important role for the enzyme in bone resorption and immune defense system. We have used TRACP over-expressing (TRACP+) mice to identify potential biological substrates for the phosphatase activity of the enzyme. We performed Western-analysis to lung homogenates from wild-type (WT) and TRACP+ mice using phosphotyrosine and phosphoserine antibodies and quantitated band intensities with image analysis. The specific activity of TRACP was significantly higher in lung homogenates from TRACP+ mice compared with WT mice. Lung homogenates from TRACP+ mice contained significantly lower amount of phosphotyrosine, but the amount of phosphoserine was not different from WT mice. Two bands sized 120 kD and 180 kD were identified that contained 90% less phosphotyrosine in lung homogenates from TRACP+ mice. The band intensities showed a significant negative correlation with the specific activity of TRACP in lung homogenates from TRACP+ mice. These results suggest that TRACP functions as a protein tyrosine phosphatase, but not as a serine phosphatase, and that it may have at least two different natural substrates in lung tissue.

P32 – Short term neonatal treatment of male rats with diethylstilbestrol induces bone changes in adults

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Diethylstilbestrol (DES) is a nonsteroidal estrogen compound which was previously used in the prevention of abortion. However, it was found that DES exposure in utero causes abnormalities in genital tract of males and females, and the medical use of DES was denied. In order to study if neonatal DES treatment has any effects on skeleton, we studied bone changes in male rats (n=10) which were treated (subcutaneously) with 10 ug DES daily, for five days postnatally. At the age of 8 months, the body weight of DES treated rats was significantly higher compared to the control animals. As expected the development of testes in DES treated rats was abnormal. pQCT measurements showed that DES-treated animals had decreased the trabecular bone density in proximal tibia, although the ash weight of tibia did not reveal any difference between the treated and untreated groups. Results of bone histomorphometric analysis from proximal tibia were consistent with pQCT measurements, and confirmed lower trabecular bone area and thinner trabeculae in DES treated animals. The cantilever bending strength of the femoral neck was significantly lower in DES treated than in control rats ($p < 0.05$). These results suggest that even short neonatal or postneonatal diethylstilbestrol treatment can decrease the mechanical properties of bone and induce at least minor permanent changes in the skeleton of male rat.

P33 – Two independent mechanisms of parathyroid hypotensive effect

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The objective was to test if 13-34 and 39-68 fragments of human parathyroid hormone (h-PTH) preserve the hypotensive and phosphaturic effect of 1-34 PTH which might shed a light on mechanism(s) of acute profound hypotensive effect of intact PTH.

Design and methods: Male Wistar rats were thyroparathyroidectomized and were infused trough the jugular vein with 0.9% NaCl supplemented with [3H] inulin. Sequential increasing boluses of peptide: 10^{-10}, 10^{-9} and 10^{-8} Mkg.b.w. in the same rat were given i.v.. As a control 1-34 bPTH was used. Blood pressure was measured in carotid artery, urine was collected from cannulated urinary bladder.

Results: In contrast to 1-34 bPTH no significant phosphaturia was observed in 13-34 or 39-68 PTH treated rats. However, profound, almost identical hypotensive effect was observed at 10^{-9} M/kg b.w. of 1-34 and 13-34 PTH but no decrease of Mean Arterial Blood Pressure (MABP) in 39-68 PTH treated rats. Dose-dependent hypotensive effect of first two fragments was different: increased doses of 1-34 PTH resulted in more profound decrease in MABP while 13-34 PTH effect was less pronounced reaching -60 and -26 mm Hg, respectively, $p < 0.001$ at 10^{-8} M/kg b.w.

Conclusions: a) 13-34 PTH is hypotensive but not phosphaturic and b) its dose dependency of hypotensive effect is different from 1-34 PTH. The 39-68 sequence of aminoacids is unnecessary for PTH acute hypotensive effect. Since PTH induced phosphaturia is mediated by cylic AMP the results indicate that there are two mechanisms of hypotensive effect of 1-34 PTH: cyclic AMP-dependent (well known) and independent one (present results).

P34 – The effects of 1,25(OH)2D3 supplementation to the diet without vitamin D3 on mechanical and geometrical parameters of developing skeletal system of chickens

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The purpose of the present study was to investigate the influence of 1,25-dihydroxycholecalci ferol administered to the diet without vitamin D3 and low phosphorus and calcium on the mechanical and geometrical parameters of broiler chickens bones.

Methods: One-day-old chickens were used in two experiments lasted 21-d and 49-d. Birds were fed on the control positive diet D+ (2500 IU D3/kg feed), control negative diet D- (without vit. D3) and on the experimental diet E without vit. D3 but supplemented with 0.003 mg 1,25(OH)2D3/kg feed during the first three and seven weeks of life. On the 21st and 49th day, 10 chicks from each treatment were sacrificied and femora were isolated. Physical bone parameters (ultimate stress, elastic force) were determined by three-point bending test using an Instron 4302 Testing Machine. Structural parameters were also measured

Results: After 3 wk 1,25(OH)2D3 treatment chickens had significantly higher femur physical parameters than the control birds (ultimate stress: D+ 72N, D- 66N, E 88N). This was acccompanied by significantly higher values of structural parameters (cortical area-mm^2: D+ 23.9, E 28.2; thickness area-mm: D+ 2.4, E 2.8; the second moment of inertia-mm^4: D+ 49.3, E 70.6). After 7 wk of 1,25(OH)2D3 treatment, the strength of bones in experimental chicks was greater than positive and negative controls (D+ 254N, D- 163N, E 297–301N). Structural parameters in both experimental groups were similar to positive control and greater than in negative

control (cortical area-mm^2: D+ 37.2, D- 28.8, E 39.2–39.5; thickness area-mm: D+ 2.7, D- 2.3, E 2.8–2.9; cross section area-mm^2: D+ 26, D- 20, E 26–27).

Conclusion: Short (3 wk) and long (7 wk) term of 1,25(OH)2D3 supplementation improved strength of bones in comparison with diet containing adequate level of vitamin D3. The lack of significant differences between both experimental groups suggests that supplementation of 0.003 mg 1,25(OH)2D3/kg feed during first three weeks of life provided its optimal supply for growth of skeletal system of broiler chickens in the subsequent period as well.

P35 – Mature, fluorescent cross-links in ICTP antigens isolated from human bone, skin and leiomyoma

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The ICTP antigen has earlier been characterised from trypsin or collagenase digests of human bone and contains carboxyterminal telopeptides of alfa1-chains of type I collagen joined by mature trivalent cross-links. We have established a widely used immunoassay of this antigen using polyclonal antibodies. Divalently cross-linked or free telopeptides do not react in the ICTP assay. Matrix metalloproteinases (e.g. MMP-2, MMP-9, MMP-13) do not cleave ICTP, but cathepsin K destroys it.

Methods: We have now isolated the human ICTP antigen from bone, skin and uterine leiomyoma after trypsin digestion. In SDS-PAGE all the ICTP antigens behaved similarly (MW about 10,000), although with some variation. The fluorescent cross-links hydroxylysylpyridinoline (Pyd) and lysylpyridinoline (Dpd) as well as hydroxyproline were analysed and the total amount of the ICTP antigen detected with radioimmunoassay.

Results: The amount of hydroxyproline was identical in all the samples (12 nmoles/nmoles ICTP) indicating that the telopeptides were highly purified. However, the ratio of Pyd to Dpd was 5.8 in bone, 0.2 in skin and 33.3 in leiomyoma. Surprisingly, the molar amount of Pyd + Dpd was only 0.41 residues in bone ICTP, 0.03 residues in skin ICTP and 0.23 residues in leiomyoma ICTP. Digestion with cathepsin K produced five fragments from the alfa1-telopeptide, only one of which (MW about 2200) contained the fluorescent cross-links. These fragments could be separated into those containing the Pyd/Dpd and those with unidentified cross-links.

Discussion: The ratio Pyd/Dpd in ICTP from bone and leiomyoma was as expected. In skin the ICTP antigen, which is different from the more abundant telopeptide antigen with a histidinohydroxylysinonorleucine cross-link, only contained trace amounts of Pyd or Dpd. Also in bone and leiomyoma, the fluorescent pyridinolines did not explain all of the mature cross-links in the ICTP antigen (<1 residue/ antigen).

Conclusion: The peptide approach can be used to characterise the unknown cross-links.

P36 – The influence of alpha-ketoglutarate on growth, development and mineralization of the skeletal system during the postnatal life in the pig

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The study was undertaken to investigate the effect of exogenous AKG on the mechanical and geometrical properties of bone and on bone mineral density (BMD) during 35 days of life.

Material and methods: Experiments were carried out on piglets divided into a control group (C), which was administered physiological saline per os and an experimental group (E), which was administered AKG solution in doses of 0.4 g/kg b.w./day. The femora and humeri were isolated for measurements of the cross sectional area and the second moment of inertia. The three-point bending test and INSTRON 4302 apparatus was used to determine bone maximum elastic strength and bone ultimate strength. Bone mineral density (BMD) was analysed with use of dual energy x-ray absorptiometry (DEXA). Osteocalcin and 17-fl-estradiol in the plasma were also assayed.

Results: Cross sectional area of the femora and humeri showed higher values in piglets from experimental group than in controls (C: 49.9 ± 3.3 mm^2; E: 71.2 ± 4.9 mm^2). The values of the second moment of inertia of both bones presented higher values in experimental piglets than in the controls (C: 452.3 ± 49.2 mm^4; E: 769.4 ± 56.1 mm^4). The values of the maximum elastic strength of the both bones of the experimental piglets were higher than of the controls (C: 604.2 ± 26.2 N; E: 729.0 ± 37.7 N). Higher values of maximum ultimate strength of bones were observed in experimental piglets than in controls (C: 747.4 ± 3.89 N; E: 857.6 ± 45.4 N). Blood plasma osteocalcin was significantly higher at the age of 28 and 35 days in experimental piglets than in controls (C: 21.1 ± 2.2 ng/ml; E: 26.9 ± 1.9 ng/ml). Similarly higher values of 17-fl-estradiol were observed in piglets administred AKG. Bone mineral density (BMD) of both bones increased after AKG administration in comparison to controls (C: 0.43 ± 0.03 g/cm^2; E: 0.48 ± 0.02 g/cm^2).

Conclusion: AKG administered per os increases the bone mineral density and geometrical and mechanical properties of the femur and humerus in piglets. The study provides support for using the piglets as a model animal for investigation of development and mineralization of the skeletal system in human infants.

P37 – Effect of alpha-ketoglutarate (AKG) and l-alanyl-l-glutamine (Ala-Gln) on the development of the skeletal system in the postnatal life in the pig investigated on the model of ribs

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The study was undertaken to evaluate the piglet as an animal model for studying the influence of Ala-Gln and AKG on the developmental changes of geometrical and mechanical parameters of ribs in the period from birth to 56th day of postnatal life.

Material and methods: Ala-Gln and AKG were administered orally to the two groups of experimental piglets in the dose of 0.4 g/kg of b.w. per day, while the control piglets were treated with physiological saline. The piglets from control and experimental groups were sacrificed at the 3rd, 14th, 21st, 35th and 56th day of life. The most suitable ribs for evaluation of analysed properties in piglets were from the fourth to ninth rib. Therefore 12 ribs were isolated from every piglet for measurements. The three- point bending test and INSTRON 4302 apparatus was used to determine bone maximum elastic strength and bone ultimate strength.

Results: The values of the mechanical parameters were significantly higher in both experimental groups in comparison to control values during the whole period of observations. The values of the elastic strength in the control piglets at the age of 3 days amounted 133.0 ± 6.8 N and 139.2 ± 6.7 N in Ala-Gln and 146.4±5.0 N in AKG piglets and increased to 565.7 ± 33.0 N, 788.0 ± 22.0 N and 1071.0 ± 43.1 N at the age of 56 days respectively. The values of the ultimate strength increased at the same time from 177.8 ± 8.0 N to 705.5 ± 34.5 N in control piglets and from 188.1 N in both experimental groups to 932.8 ± 25.3 N and 1320.0 ± 5 3.1 N respectively. The differences in these mean values were statistically significant. The highest changes in the analysed parameters were observed between the 3rd and 14th and between the 35th and 56th day of postnatal life. The cross- sectional area of the ribs of experimental groups were higher than in controls.

Conclusion: AKG and Ala-Gln administered during the postnatal development increase geometrical and mechanical properties of ribs in the pig and ribs on this species may be used as a model bones for growth of the skeletal system.

P38 – The effects of alpha-ketoglutarate (AKG) on bones mineralization, geometrical and mechanical properties after fracture of ulna bone and denervation of the forearm in turkeys

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The aim of the study was to elucidate the effects of AKG administration on ulnar bone develpment and healing after experimental fracture.

Material and methods: The investigations were carried out on 24 turkeys at the age of 3 weeks and lasted up to 16 weeks of life. In the experimental group the turkeys were neurectemised (the radial and medioulnar nerve on right forearm were severed and the right ulna cut through in the midshaft). The fracture of the ulna was stabilized with orthopedic wire. The control group was shame operated. The AKG was administered directly to the crop during 13 weeks in the dosage of 0.4 g per kg/day. The turkeys were sacrificied at the 16th week of life. Using INSTRON 4302 apparatus and three points bending test, mechanical properties were estimated. BMD was determinated by dual-energy X-ray absorptiometry.

Results: AKG administration increased BMD of the ulna in experimental turkeys to 0.319 g/cm^2 versus 0.283 g/cm^2 in the control group. BMD in the ulna of the shame opperated turkeys after AKG administration amounted to 0.271 g/cm^2 versus 0.242 g/cm^2 in the controls. BMD of the left ulna in the turkeys which were administrated AKG amounted 0.303 g/cm^2, while in the controls were 0.272 g/cm^2. AKG administration influenced the values of ultimate strength that amounted to 530.5 N versus 446.5 N in the controls. Under the conditions of denervation of the forearm and fracture of ulna bone, there was an increase in cross section area, bone ultimate strength and weight of the ulna in comparision to untouched control bones, but lower values of these parameters in AKG group than in control group were observed. The histological examination showed that under denervation Haversian canals were doubled and Haversian systems were deformed. Moreover the bone trabecular system was irregularly formed with empty spaces none were present in the control turkeys.

Conclusion: Turkeys forearm bones are convenient for studying of bone denervation and fracture healing, and this model can serve for further study of bone repair processes.

P39 – The effect of alfa-ketoglutarate (AKG) on mineralization of femur in ovariectomized rats

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The studies were undertaken for determination of the effect of alfa-ketoglutarate (AKG) supplementation on mineralization of femur established by mechanical, geometric and morphometric parameters and also on bone mineral density in ovariectomized rats.

Methods: The experiments were carried out on 50 female Wistar rats at initial body weight of 250 g. The animals were maintained in controlled condition light/dark ratio, temperature and humidity and with free access to food and drink.

The rats were divided into 3 groups: group-A intact, group-B shame-operated and group-C ovariectomized. Seven day after surgery the animals were subsequently divided into groups received placebo and experimental drink including AKG. 60 days after the animals were killed using CO_2. Femora and blood serum were collected for further analysis.

Mechanical parameters were estimated by three point bending test for calculation of ultimate strength and resilience. Bone robusticity index and the weight-length index parameters of whole bone were measured. The following parameters, such as the second moment of inertia of the cross section in relation to the horizontal axis (Ix), the cross-sectional area (A) and the mean relative wall thickness (MRWT) were assessed. With the use of DEXA the bone mineral density was established.

Results: The obtained results of the study indicate that AKG supplementation decreases the body weight of ovariectomized rats in comparison to the ovariectomized rats receiving placebo (338.6 ± 8.7 g vs. 345.6 ± 7.5). AKG supplementation involves an increase of BMD in intact, shame-operated and ovariectomized rats in comparison to the AKG free groups by 12.5%; 9.4% and 10.4%, respectively.

Conclusion: The obtained results suggest the potential usefulness of alfa-ketoglutarate in the treatment of skeletal system disorders. This concept requires further research.

P40 – The influence of alfa-ketoglutarate (AKG) on mineralization of femur in rats with established osteopenia

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It is well documented that AKG involve an increase the synthesis of proline. This amino acid play key role in synthesis of collagen in skeletal tissue. The purpose of the present study was to investigate the effect of alfa-ketoglutarate (AKG), as an anabolic agent for the treatment of osteoporosis on the rat model with established osteopenia.

Methods: The experiments were carried out on 40 female Wistar rats at initial body weight of 250 g. The animals were maintained in controlled condition light/dark ratio, temperature and humidity and with free access to food and drink. The rats were divided into 2 groups, group-A shame-operated and group-B ovariectomized. Seven months after surgery the animals were subsequently divided into groups received placebo and experimental drink mixture including AKG trough sixty days, and later the animals were killed using CO_2. Femora and blood serum were collected for further analysis. Mechanical parameters were estimated by three point bending test for calculation of ultimate strength and resilience. Bone robusticity index and the weight – length index parameters of whole bone were measured. The following parameters, such as the second moment of inertia of the cross section in relation to the horizontal axis (Ix), the cross-sectional area (A) and the mean relative wall thickness (MRWT) were assessed. With the use of DEXA the bone mineral density was established.

Results: The obtained results indicate that 60 days of AKG treatment increased body weight in shame-operated group in comparison to the AKG free group by 5.1%, but in ovariectomized rats the AKG decreased body weight in comparison to the group received placebo by 9.5%. AKG supplementation increased BMD of femora in shame-operated and ovariactomized rats by 9.7 and 9.9%, respectively.

Conclusion: The obtained results indicated the AKG prevents the increase of body weight in condition of ovarian dysfunction and positively influences on bone mineral density in rats with established osteopenia.

P41 – Changes in bone metabolism during the menopausal transition

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In a prospective study covering 42 subjects a series of bone markers in serum and urine over a period of two years in pre-, peri-, and postmenopausal women were investigated. This allows a longitudinal comparison of the biochemical bone markers concerning their ability

to predict bone loss across the menopausal transition. The bone parameters are compared to the results of bone mineral density (BMD) examinations.

Methods: We applied the following panel of bone formation- and resorption markers: osteocalcin (OC), bone-specific alkaline phosphatase (BAP) and bone sialoprotein (BSP), pyridinoline (PYD), deoxypyridinoline (DPD), C- and N-terminal telopeptide cross-linked collagen type I (CTX, NTX), and tartrate-resistant acid phosphatase 5b (TRAP) at five time points during the course of two years in healthy pre-, peri-, and early postmenopausal women.

Results: CTX, NTX and TRAP, as well as BAP and OC were significantly increased from peri- to postmenopause. The pyridinium crosslinks indicated an increased degradation rate in the perimenopause. Significant inverse correlations with the two years changes of the BMD were found for BAP, CTX, OC and DPD in the perimenopausal group.

Conclusion: The measurement of a comprehensive panel of biochemical bone markers clearly depicted that metabolic changes in bone metabolism appear pronounced in the perimenopause, a time period still presenting satisfactory estrogen supply. The perimenopause is an important phase for a contingent development of osteoporosis.

P42 – Estrogen supplementation modulates effects of PCB exposure on trabecular bone in female rats

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In a previous study we showed that the endocrine disrupting organochlorine 3,3',4,4',5-pentachlorobiphenyl (PCB126) induced profound alterations in the long bones of the rat, leading to increased cortical thickness and decreased collagen content (Lind, Eriksen et al. 1999; Lind, Larsson et al. 2000).

The aim of this study was to investigate effects of estrogen administration on bone morphology in rats exposed to PCB126.

Methods: The 40 rats being exposed to PCB126 (i.p.) for 3 months (total dose 384 µ/kg bw,) were randomized to either OVX/sham operation (at week 0) or 17-beta-estradiol administration (i.p. 23 µ/kg)/vehicle (corn oil)) in a factorial design. As controls served OVX or sham operated rats and OVX rats injected with 17-beta-estradiol (n=10 in each group). Tibiae were prepared for histomorphometrical analysis.

Results: PCB126 exposure increased cortical thickness ($p < 0.05$) irrespectively of OVX or estrogen-supplementation ($p < 0.01$). However, regarding trabecular bone (Trabecular bone volume, TBV), a profound interaction was seen between OVX and estrogen-supplementation ($p < 0.001$) in PCB126-exposed rats.

Conclusion: In conclusion, this study showed that estrogen modulates PCB126 induced effects on trabecular but not on cortical bone in rats.

References:

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P43 – Experimental modeling of osteoporosis related to bilateral oophorectomy in female rats

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The loss of bone tissue after experimental oophorectomy in rats and the postmenopausal bone mass loss in women have many common features, namely: greater speed of bone remodeling with dominant resorbtion, greater loss of spongy versus compact bone tissue, similar skeletal response to estrogen, bisphosphonate, PTG, and calcitonin treatments, etc. Therefore, this study was undertaken to explore the biomechanic, osteometric and histomorphometric bone characteristics, its mineral composition, and teeth state in 30 female rats aged 18 months after bilateral oophorectomy. Methods used: osteometric method (the longest bone length, proximal epiphysis width, greatest diaphysis width, greatest front-back dimension of middle diaphysis, and distal epiphysis width); biomechanic characteristics of shoulder bone (specific sagging index, tensile strength, destroying moment, resiliency module and minimum bone destruction work); morphometry; bone composition (water, organic and mineral substances); bone biochemistry (calcium, phosphorus, sodium and potassium).

Results: Thus in the rats after bilateral oophorectomy the specific sagging index increased from 2.24 ± 0.17 to 2.71 ± 0.13 mcm/N ($p < 0.01$), the tensile strength decreased from 170.06 ± 14.39 to 163.60 ± 8.91 GPa ($p < 0.01$) and the minimum work of bone destruction decrease from 115.10 ± 9.68 to 94.25 ± 5.49 mJ ($p < 0.01$) compared to nonoperated rats.

Conclusion: The bilateral oophorectomy has worsened the biomechanic bone characteristics (bone destruction and bone strength) and reduced the osteometric indices (greatest width of diaphysis), and changed the biochemical bone properties (contents of calcium and sodium).

P44 – Influence of hot paraffin plaster on bone mineral quantity parameters in healthy population

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The problem of hot paraffin plaster influence on bone tissue is essential, especially because in medical bibliography one can find two different opinions to the point of harm influence of hot paraffin plasters on bone mineral quantity. No one had made any experiments with healthy or osteoporosis patients using hot paraffin plasters and one of the objective diagnostic methods e.g. DEXA, BUA or QCT to determined its influence on bone tissue.

The main purpose of our prospective controlled study is evaluation of hot paraffin plaster influence on bone mineral quantity parameters in healthy population.

In our experiment took place 44 healthy persons, 6 men and 38 women, aged 20–30 years, physiotherapy students. Everyone with the history of pain ailment of the feet, injuries of the feet, any metabolic illnesses, using same kind of medicines connecting with osteoporosis increase, irregular period in women, prolonged bed resting was excluded. Everyone have performed 30 hot paraffin plaster procedures on the right foot, one time a day, for 20 minutes, during 6 next weeks. The measurements of bone mineral quantity parameters were made on the heel using BUA method with ALOKA AOS 100 apparatus. Each patient has performed 4 measurements – before start of the hot paraffin plaster procedures and after 10, 20 and 30 procedures. The measurement was made on the both feet because the left foot was treated as a control group.

The statistical analysis was made with the STATISTICA 5.0 PL software.

The results were clear-cut: 1) there is no statistically important connection between hot paraffin plaster procedures of the feet and bone mineral quantity parameters of the heel evaluated by BUA in healthy population, 2) there is no statistically important difference in measurements of bone mineral quantity parameters made by BUA between the study group (the right feet after the hot paraffin plaster procedures) and control group (the left feet without any procedure).

Conclusion: Hot paraffin plaster procedures haven't any influence on the local bone mineral quantity parameters measured by BUA in healthy population.

P45 – Long-term variability of bone turnover markers in patients with non-metastatic malignancies

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Variability of bone marker measurements remains to be an issue of concern in the clinical application of these parameters. Most studies on marker variability have been performed in healthy subjects and over relatively short intervals of time.

Methods: As part of a prospective diagnostic study, we evaluated the long-term variability of various bone markers in 113 postmenopausal women diagnosed with primary breast cancer. None of the patients had bone metastasis, other skeletal disease or bone active drugs. During follow-up (range 0.6–4.2 years, median 30 months), patients were seen every 3 months and timed blood/ urine specimens were obtained. To minimise analytical variability, all analyses were performed after study end by the same technician, using well documented techniques and a single batch of reagents per analyte. The coefficient of variation was calculated as CV (%) = SQRT(SUM((CVi2)/n)) (CVi= SD/mean x 100; n= n of CVi). The least significant change (LSC) was then LSC (%)= Z x CV x SQRT(2). For a 95% confidence interval (LSC95), Z= 1.96. For a 80% confidence interval (LSC80), Z= 1.28.

Results: Lowest long-term CVs were recorded for serum calcium levels (5.1%), serum total alkaline phosphatase (12.1%) and serum bone alkaline phosphatase (14.0%). The calculated LSC95 for these markers were 14%, 33.1% and 38.8%, respectively. The LSC80 was 9.2%, 22.0% and 25.5%, respectively.

Highest long-term CVs were observed for the carboxyterminal telopeptide (CTX, "Crosslaps") measured in urine (55.9%) and serum (42.0%). The calculated LSC95 and LSC80 for these markers was 155 (urinary CTX) and 116.5% (serum CTX), and 101.2 (urinary CTX) and 76.1% (serum CTX), respectively.

Conclusions: In this "real life" study of breast cancer patients, long-term variability varied greatly between markers. For certain markers (e.g. urinary and serum CTX), the LSC was considerably higher than previously reported.

P46 – Bone mass changes in the diabetic food syndrome

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The changes in skeleton status in diabetic foot syndrome are still under investigation. Well documented low bone mass could be considered as the indication for initiation of early antiosteoporotic treatment. The aim of the study was to determine if there are some differences in bone mass in both lower extremities (with and without ulceration) and to assess the type of the bone changes, local or global, based on measurements in the feet, spine, and forearm.

Methods: The study population consisted of 30 subjects aged 22–75. Nine women and 21 men with diabetic foot syndrome. Ten subjects with diabetes type 1 and 20 with type 2. Bone mass was measured in both feet, the spine, and the forearm. Bone mass in the feet was measured using ultrasound (Achilles plus produced by Lunar), for assesment of the bone density in spine and forearm dual-energy X ray absorptiometry (DPX produced by Lunar) was performed.

Results: The Mean Stiffness value in feet with ulceration was (-1.72) SD T-score Young Adult, in feet without ulceration: (-1.21) SD T-score Young Adult. Bone mass below (-1.0) SD T-score Young Adult in foot with ulceration (mean value- (-2.33) was present in 22 subjects (73.3%).

In 17 patients (56.6%) bone mass was decreased (below 1.0 SD T-score) in both feet.

In these patients, density in spine and forearm was within normal limits. In persons with type 1 diabetes the mean bone mass density of the feet with ulceration was (-2.23) SD T- score Young Adult, and in type 2: (-1.48) SD T score Young Adult.

Conclusions: Bone mass was decreased in both extremities in diabetic foot syndrome. The measurements should be focused on distal part of the skeleton.

P47 - Bone mineral density changes in women without bone metabolic diseases

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The aim of the study was to analyse how the body mass index, the age, the amount of daily intake of calcium and vit.D are related to changes in women's heel bone mineral density, measured by ultrasound.

Methods: Fifty two women 35–55 years old not suffering from bone metabolic diseases (MBD) were examined and their bone mineral density was measured at the heel by the ultrasound device "Achilles" (Lunar).

The nutritional amount of Ca and vit.D, was calculated by evaluation of nutritional data, according to the methods supplied by the national Republic Nutrition Center.

All the examined women were distributed into three groups:

Group 1: 30 women (57.6%) being in fertile age,

Group 2: 9 (17.5%) women, their menopause began between the age of 40–45,

Group 3: 13 (25%) women, their menopause began past the age of 45.

Results:

Group 1	Group 2	Group 3
Age:		
44.6 ± 0.84	48.2 ± 1.0	53.1 ± 0.6
Body mass index (BMI):		
26.76 ± 0.93	26.64 ± 1.53	26.3 ± 1.08
Osteoporosis (T-score < -2.5):		
10%	11.1%	7.7%
Osteopenia (T-score -1.1 to -2.49):		
26.7%	33.3%;	30.8%
Normal BMD (T-score -1 to +1):		
63.3%	55.6%	61.5%
Ca daily intake (mg/p):		
530.6 ± 41.5	516.2 ± 122.8	445.8 ± 52.3

Conclusions:

Vit. D daily intake (mg/p): 1) 0.76 ± 0.11; 2) 0.87 ± 0.3; 3) 1.9 ± 0.77.

1. 9.6% of women, who stated that they were healthy were diagnosed as osteoporotic
2. 5.76% of women in fertile age were diagnosed as osteoporotic. 3.84% of women in postmenopausal age were diagnosed as osteoporotic.
3. There was a reliable correlation between BMI and heel area based on the ultrasound densitometric test T-level.
4. The daily amount of calcium and Vit.D intake was insufficient in all investigated groups.
5. There was no significant correlation between daily calcium intake and the densitometric t-level.
6. 73% of all women who had insufficient daily intake of calcium, didn't use any food with calcium supplement.

P48 – What is an appropriate age of the reference group in your area?

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The values of bone turnover and bone mineral density (BMD) of "young adults" are frequently used as one of the standards to assess the respective parameters in postmenopausal women.

The aim of the present pilot study was to examine the behaviour of the four biochemical markers of bone metabolism and the related radiological parameters in Estonian young women.

Material and methods: Thirty-six healthy women aged from 22 to 32 (median 26) years were examined. Bone turnover was evaluated by urinary desoxypyridinoline, (Dpd/crea), serum CrossLaps (S-CTx-1), S-bone ALP (B-ALP) and S-osteocalcin (S-Oc). The status of the subject's skeleton was evaluated by lumbar DXA and calcaneal quantitative ultrasound.

Results: Excretion of one of the resorption markers, Dpd/crea, turned out to be significantly higher (5–12, median 9 nmol/mmol) in comparison with the limits recommended by the manufacturer. All four markers had a significant inverse correlation with the age of the subjects. The values of CTx-1, Oc and BALP were obviously lower starting from age 29 years, those of Dpd/crea from age 30 years. Lumbar (2–4) BMD increased starting from age 30 years. The curve of the calcaneal stiffness values had a V-form, being the lowest between the age 26–28 years.

Conclusions: Estonian women seem to reach their peak lumbar BMD from age 30. This is preceded by a decrease in bone metabolic activity expressed both by the markers of resorption and formation. Their bone metabolism stabilizes after the age of 28 years.

Considering bone turnover and quality (density and elasticity), 'young adults' can form an inhomogenous group. In Estonia women between 29–32 years form a reliable reference group.If one includes younger subjects, the variability of the results would increase.

P49 – Difference in fracture incidence among attendees and non-attendees in a population based study of aged women

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All population-based studies have to address that a variable proportion of invited participants declines to attend. It is often assumed that the non-attendees represent a less healthy segment of the population. This study was undertaken in order to estimate the potential difference in fracture rate and fracture type between attendees and non-attendees in a population-based study.

Methods: Exactly 75-year old women were randomly recruited from the city population files, within the framework of the Malmö Osteoporosis Prospective Risk Assessment study (OPRA). Of the invited 1,604 women, 1,044 attended the investigation. Of the 560 non-attendees, 139 gave illness as reason not to participate, 376 declined to participate for reasons other than illness, 13 died shortly after the invitation, and 32 women were not reached despite repeated attempts. The Malmö University Hospital is the only one in the area treating fractures, with a unique radiology archive (all x-rays are kept since the late 1800s). We registered all previous fractures in the 1604 women by using the archive.

Results: Previous fracture at any time during their lifetime was registered in 448/1044 (42%) of attendees and 205/560 (36%) non-attendees ($p = 0.02$). The number of fractures per attendee was 0.81 and per non-attendee 0.75 ($p = 0.34$). 69 (7%) attendees had suffered a prior hip fracture, 178 (17%) a wrist and 78 (7%) a vertebral fracture, the corresponding results from the non-attendees was 43 (8%), 84 (15%) and 37 (7%) fractures, respectively ($p = 0.28$– 0.55).

Conclusion: In this study of aged women with a 65% response-rate, we observed more previous fractures among the attendees, suggesting that studies of osteoporosis are more attractive to women with previous fractures. However, there was no difference in the number of fractures per person or in number of typical osteoporotic fractures between the groups. This study indicates that non-attendees are not necessarily representing a frailer segment of the population, which should be considered when interpreting data from observational studies.

P50 – Inflammatory bowel diseases and osteoporosis

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Osteoporosis is a frequent finding in patients with inflammatory bowel diseases (IBD). The cause of the connection has not been defined completely. Probably the chronic inflammation, steroid therapy and malnutrition contribute to the osteoporosis.

The aim of the study, materials and methods: The aim of this study was evaluation femoral neck Bone Mineral Density (BMD) by dual-energy X-ray absorptiometry (DEXA) in 16 women with IBD (6 with colitis ulcerosa and 10 with Crohn's disease) age from 40 to 65 years (mean age 49.7 years). The mean time of duration of IBD was 11.0 years (from 5 to 20 years).

Results: The mean value of femoral neck BMD was 0.694 ± 0.160 g/cm^2 ($74.3 \pm 18.7\%$ -T-score: -1.99 ± 1.54; $85.3\% \pm 18.4\%$ -Z-score: -0.99 ± 1.19). Osteoporosis was found in 25% women, osteopenia in 37.5% women with IBD. The mean value of Body Mass Index (BMI) was 18.80 ± 2.37 (14.4 to 20.5) and correlated to BMD. The mean value of serum protein and serum albumin were: 5.64 ± 0.83 g/dl and 2.84 ± 0.49 g/dl. There was no correlation between serum protein, albumin and BMD. There are first part of clinical assessment BMD

and risk factors of osteoporosis in patients with IBD.

Conclusions: Osteopenia and osteoporosis are frequent in IBD women. Women with IBD should have bone density assessment.

P51 – Anthropometric indices and osteoporotic fractures in postmenopausal women

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Relationship between anthropometric indices and the emergence of osteoporotic fractures in the postmenopausal women. Material and methods: 107 postmenopausal women aged 50 to 87 years (average age 66.5 ± 1.2 years) were examined. Depending on the presence of osteoporotic fractures in their anamnesis, the women were divided into two groups. The prevalence of osteoporotic fractures in the 1st group (n=39) was as follows: Colles' fractures(32%), vertebrae (6%), femoral neck 4.5%), ribs (9%), tibia (9%), fibula (6%), metatarsus (12%), etc. The subjects without osteoporotic fractures were ascribed to a 2nd group (n=77). The structural-functional state of the bone tissue elasticity, density and firmness) was determined by an ultrasound densitometry (Achilles+). The anthropometric study was performed by using the Bunak's method in Shaparenko's modification (1994).

Results: In the women with osteoporotic fractures we found a statistically significanct reduction of the following anthropometric indices: hip length, hand circumference, narrow part of the ankle circumference and face height. Likewise, an ultrasound densitometry index, such as the wide-band ultrasound weakening, reflecting not only bone density but also the number and spatial orientation of the trabeculae, was reduced in these patients. In addition, the women with osteoporotic fractures showed a tendency to reduction of such anthropometric indices as hip circumference, head length, hand width and hip width.

Conclusion: The anthropometric indices should be taken into consideration while identifying a risk group for osteoporotic fractures among women at the postmenopausal period.

P52 – Geometry of the hip in patients with Turner Syndrome in relation to healthy controls

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Patients with ovarial agenesi (Syndrome of Turner) have an increased risk of developing secondary osteoporosis due to estrogen deficiency. Most patients are substituted with estrogen during puberty and adolescence to facilitate pubertal development and prevent secondary osteoporosis.

It has been suggested that the geometry of the hip is a predictor for hip-fractures independent of bone mineral density (BMD). The purpose of the present study was to investigate the variation of the geometry of the hip in Turner-patients in relation to healthy controls. Using newly developed software, we measured hip-axis-length (HAL), neck-width (NW), neck-shaft-angle (NSA), and femoral head-radius (HR) from DXA-scans performed on a Hologic QDR-1000 densitometer. The study population consisted of 58 patients with Turner Syndrome and 60 age-matched healthy adults, aged 21–67 years. The effects of height and weight on the measured parameters were investigated using multiple regression analysis, backwards (level of significance $p < 0.10$) (SPSS).

The height was 146.6 (6.9) and 167.1 (6.2) cm and body weight was 17.4 (13.9) and 62.3 (8.3) Kg in Turner Syndrome and controls, respectively. The hip axis length was smaller, neck width and neck shaft angle slightly increased, and femoral head radius smaller in Turner syndrome compared with controls. After correction for height and weight, however, hip axis length was normal, neck width was increased ($P=0.06$), and femoral head radius smaller ($p < 0.01$). In conclusion, our data supports other dataset about disproportional growth in Turner Syndrome. Hip geometry, however, cannot explain the increased risk of fracture in Turner Syndrome.

P53 – Lumbar spine and proximal femur bone mineral density measurements in patients with low back pain

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The bone cells, osteocytes, react with outer forces, causing compression and extending. These forces have influence on bone trabeculae architecture, thus improving bone resistance against load. Loads exerted on the sceleton, especially axial, have an influence on the bone mass and the bone quality.

Disturbance of function of motor segment, caused by low back pain, restricts flexion, extension, lateral flexion and rotation of lumbar column under normal axial load. Disturbance of function of motor segment also creation of degenerative changes.

The aim of this study was to assess BMD in lumbar spine (L1, L2, L3, L4) and proximal femur (neck and Wards triangle) in patients with low back pain.

Methods: In 109 patients BMD was measured using Lunar DPX-plus measurement system. The results of measurement were expressed as number of S.D. multiplications from mean value of the norm for young adult (T-score).

The patients were divided in two following groups, with or without lumbar spine degenerative disease: 1) under 45 years (55 persons), and 2) over 45 years (54 persons).

Results:

1. In group of patients aged over 45 years BMD is lower than under 45 years.
2. In group of patients aged over 45 years, without degenerative disease, BMD becomes lower, decreasing from L1 to L4.
3. In group of patients aged over 45 years, with degenerative disease, BMD increases in L3 and L4 in comparison with L1 and L2.
4. In group of patients over 45 years, with degenerative disease, BMD value in femoral neck and in Ward triangle is higher than in patients without degenerative disease.
5. In patients with intervertebral disc herniation measuring of mineralisation in Ward triangle seems to be more useful than in lumbar vertebrae.
6. In group of younger patients, under 45 years, no evident abnormalities were found.

Conclusions: The analysis of the results indicates that:

1. In the second group (over 45 years), without lumbar spine degenerative disease, BMD growingly decreases from L1 to L4. That may be connected with movement limitation of lower segments in patients with low back pain.
2. The assessment of the degree of Ward triangle mineralisation is more useful than in lumbar spine in patients with low back pain.

P54 – Relationships between polymorphisms of the VDR, COLIA1, and ER genes, and bone mineral density in warsaw population

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Osteoporosis is a common disease characterized by reduced bone mineral density and increased fracture risk. Genetic factors play an important role in the pathogenesis of osteoporosis, and recent studies have shown that a polymorphism of vitamin D receptor (VDR) gene, collagen type I a1 (COLIA1) gene and estrogen receptor (ER) gene are associated with bone mineral density (BMD) in several populations.

The aim of this study was to investigate relationships beetween bone mineral density in lumbar spine and femoral neck and polimorhisms of the VDR, COLIA 1, and ER genes.

Methods: The study group comprised 400 subjects, female and male in age range 20–80 years, randomly selected from the Warsaw population. The gene polymorphisms were evaluated by restriction fragment length polymorphism (RFLP) analysis, using the restriction enzymes: Bsm I (VDR; n=358), Van91 I (COLIA1, n=390), and PvuII and XbaI (ER, n=382). BMD of the femoral neck and lumbar spine were evaluated by DXA (Lunar DPX-L).

Results: The frequency of VDR genotypes was as follows: bb (36.03%), Bb (44.97%), BB (19.00%), the frequency of COLIA1 genotypes (G to T polymorphism in Sp1 site) was GG (69.5%), GT (27.7%), TT (2.8%) and that of ER haplotypes was px (51.6%), PX (35.3%), Px (13.1%). The investigation of correlations between BMD and polimorphism of VDR, COLIA1, and ER genes is in progress and will be presented.

P55 – Crosscalibration of Achilles apparatuses— in vitro and in vivo study

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It is well known that differences between ultrasound apparatuses concerning measured values exist. The aim of this study were: 1) to state how big they are between seven Achilles apparatuses; 2) to answer if they are the same with evaluations using phantoms and humans. Seven Achilleses were used. Group of 33 patients of both sexes (20–64 years) participated in the study, both healthy and with low bone mass. All apparatuses were placed in the same room to avoid possible influence of air temperature on measurement results. Sequence of Achilles apparatuses used for measurements in each participant was random. Phantoms (3 Vancouver oil phantoms and 1 neoprene standard) were measured with 40 min. intervals. Between measurements phantoms were placed in bath filled with room temperature water. Achilles having Stiffness values the nearest of mean of all Achilleses was chosen as reference. Maximum difference, coefficients of correlation and equations of regression lines were calculated, separately for humans and phantoms. Maximum difference was 8.3 (13.8%) for humans for low values and 2.4 (2.1%) for high values. For phantoms they were 18.0 (30.0%) and 8.7 (7.6%), respectively. Coefficients of correlation (r) were from 0.9835 to 0.9929 for humans and from 0.9958 to 0.9999 for phantoms. Coefficients of regression lines were different in humans and phantoms, too.

Our results confirm the existence of significant difference between ultrasound apparatuses. It has been

suggested, that even the same type of apparatuses are investigated, the proper way to crosscalibrate it is using humans instead of phantoms.

The authors are deeply indebted to EPOLOS Study Group members for their cooperation and generous access to Achilles apparatuses used in the study.

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P56 – Ultrasound bone measurements in adult polish population—the EPOLOS study

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Quantitative Ultrasound has been recognized as an useful method in fracture risk assessment. The method is radiation free, noninvasive and low cost. The aim of this study was to state SOS, BUA and Stiffness values in adults from Polish population as a part of the EPOLOS study. 1470 adults (938 female, 532 male, aged 20–80 yrs) were randomly recruited from Polish population, based on Ministry's of Home Affairs and Administration rolls. The response rate was 12%. The EPOLOS study was approved by local ethical committee and written informed consent was obtained from all participants. Achilles apparatuses were used for ultrasound heel measurements. SAS software was used for processing and analyzing data. Studied group was divided into 5-year sex groups. Mean value and SD were calculated for each group for SOS, BUA and stiffness. Mean SOS and SD (in m/s) in women were from 1578 (26.14) in 20–25 year group to 1519 (27.81) in 75-80 year group and in men from 1582 (33.02) to 1536 (30.18), respectively. Mean BUA and SD (in dB/MHz) were in women from 115.7 (11.04) to 99.01 (9.80) and in men from 123.0 (10.97) to 114.0 (11.32), respectively. Mean Stiffness and SD (in %) in women were from 98.96 (12.84) to 70.02 (10.48) and in men from 100.1 (16.87) to 96.07 (15.18), respectively.

*The Epolos Study group: Adamczewski Z.5), Bielecka L.1), Danska A.8), Gesing A.5), Gloskowska-Koptas R.5), Jedziniak A.7), Jedrzejuk D.7), Karczmarewicz E.1), Kasprzyk M.4), Kobylinska M.1), Koptas W.5), Marcinkowska M.5), Matusik H.1), Michalak M.8), Nizynska A.7), Olejnik M.8), Olszewski K.3), Pawlowski P.3), Pludowski P.1), Sewerynek E.5), Skorupa E.1), Skowronska-Juzwiak E.5), Suchowierska J.6), Sniegowski M.3), Swierczynska-Machura D.5), Talalaj M.6), Wiktorska J.5), Wolanski R.3), Zacharska G.6), Zasada K.5), Ziajska A.7), Zygmunt A.5).

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P57 – Bone mineral density measurements in adult polish population—the EPOLOS study

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Osteoporosis is recognized as a significant salubrity problem. In many communities morbidity is becoming higher. The aim of this study was to state bone mass in adults from Polish population as a part of the EPOLOS study. 1470 adults (938 female, 532 male, aged 20-80 yrs) were randomly recruited from Polish population, based on Ministry's of Home Affairs and Administration rolls. The response rate was 12%. The EPOLOS study was approved by local ethical committee and written informed consent was obtained from all participants. Lunar DPX apparatuses were used for Ap spine and femur bone mineral density measurements. The same measurement modes were used in all centers, based on

manufacturer recommendation. Scan file were collected and analyzed by one technician. Checking on DPX apparatuses were done twice, at the beginning and at the end of the study with using of ESP phantom. There were no significant drift and no significant differences between apparatuses. SAS software was used for processing and analyzing data. Studied group was divided into 5-yrs sex groups. Mean BMD and SD were calculated for each group for total femur, femoral neck and Ap spine (L2–L4). In women mean BMD and SD (in g/cm2) were from 1.026 (0.110) in 20-25 yrs group to 0.842 (0.116) in 75-80 yrs group for total femur, from 1.044 (0.121) to 0.770 (0.109) for femoral neck and from 1.165 (0.093) to 1.006 (0.198) for Ap spine (L2–L4), respectively. In men mean BMD (SD) was from 1.141(0.146) to 0.968 (0.154) for total femur, from 1.174 (0.171) to 0.871 (0.141) for femoral neck and from 1.194 (0.134) to 1.156 (0.242) for Ap spine (L2–L4), respectively.

*The Epolos Study group: Adamczewski Z.5), Bielecka L.1), Danska A.8), Gesing A.5), Gloskowska-Koptas R.5), Jedziniak A.7), Jedrzejuk D.7), Karczmarewicz E.1), Kasprzyk M.4), Kobylinska M.1), Koptas W.5), Marcinkowska M.5), Matusik H.1), Michalak M.8), Nizynska A.7), Olejnik M.8), Olszewski K.3), Pawlowski P.3), Pludowski P.1), Sewerynek E.5), Skorupa E.1), SkowroÒska-JÛzwiak E.5), Suchowierska J.6), Sniegowski M.3), Swierczynska-Machura D.5), Talalaj M.6), Wiktorska J.5), Wolanski R.3), Zacharska G.6), Zasada K.5), Ziajska A.7), Zygmunt A.5).

P58 – Ultrasound bone measurements in children with chronic asthma treated with inhaled steroids—follow-up study

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The aim of the study was to assess a bone status: bone structure and quality in 45 children with chronic asthma. The visualization of bone structure has been investigated by several techniques. Ultrasound bone measurements were done using Omnisense (Sunlight) and Achilles (Lunar). Bone mineral density measurements were done using DPX-L apparatus (Lunar). There were two groups of children: I – 28 children, aged 6–11 years (mean=8.25, SD=1.82), treated with inhaled steroids for one year; II – 17 children, aged 6–11 years (8.49 ± 1.78) who had never received any exogenous steroids (control group). They had measurements four times at 3 and 6 month intervals. Z-scores were calculated for each individuals. Mean Z-score (SD) for total body BMD changed from 0.12 (0.87) to 0.11 (0.66) in treated group and from 0.07 (0.57) to –0.05 (0.69) in controls, during one year period. Mean Z-score for Ap spine BMD changed from –0.12 (0.87) to –0.13 (0.91) and from –0.30 (0.55) to –0.32 (0.59) respectively. Bigger changes were observed for ultrasound bone measurements. Mean Z-score for Stiffness changes from 0.16 (1.22) to 0.50 (1.14) in treated group and from 0.45 (0.87) to 0.04 (0.84) in control group. Mean Z-score for tibial SOS changed from –0.30 (1.03) to –0.80 (1.20) and from –0.29 (0.86) to –0.50 (0.86) respectively. Mean Z-score for radial SOS changed from –0.43 (0.69) to –0.66 (1.16) in treated group and from –0.20 (0.84) to –0.15 (0.78) in control group. It seems, there were not big differences between studied groups. One year treatment with low dose of inhaled steroids does not seem to be associated with an increased risk of development of osteoporosis in children with chronic asthma.

P59 – Simulation of ultrasound propagation—a tool for the assessment of associations between quantitative ultrasound and bone structure

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Quantitative Ultrasound (QUS) propagation is complex and affected by different bone properties like density and structure. Different QUS variables might be associated with different bone properties in different ways which opens up the potential for selectively determining specific bone properties, independent of bone mineral density. During disease or therapy density and structural aspects might change in different ways with different impacts on QUS variables. Using simulation of ultrasound propagation we investigated the impact of different patterns of bone loss on QUS variables in trabecular and cortical bone.

We used "Wave2000 Pro" (CyberLogic Inc, NY) to simulate ultrasound transmission through examples of trabecular bone (calcaneus) and cortical bone (finger phalanx). Starting with a natural bone structure, assessed using μ-CT, structural changes were induced artificially and ultrasound variables were calculated before and after inducing loss in bone mass. In trabecular bone the impact of thinning, loss of trabeculae, and loss of cross-links on Speed of Sound (SOS) and Broadband Ultrasound Attenuation (BUA) was calculated. In cortical bone loss was simulated as either endosteal resorption or as increased porosity.

In trabecular bone, a 27% loss in bone mass caused a 30% decrease in BUA similar for all mechanisms while for SOS the magnitude of the loss differed: 1.6% in case of loss of trabeculae, 2.3% in case of thinning and 3.5% in case of loss of cross-links. In cortical bone, a 20% loss in bone mass induced a 50% reduction in signal amplitude similar for both mechanisms, while SOS responded differently: 2.2% loss in case of endosteal resorption and 5.7% in case of increased porosity.

Simulation studies indicate that ultrasound variables respond differently to variable patterns of bone loss. BUA in trabecular bone and signal amplitude in cortical bone follow bone loss independent of the mechanism, while SOS in both types of bone strongly depends on the type of structural changes. If the validity of these results can be confirmed in experimental studies, these simulation studies may lead to a more specific assessment of skeletal changes by means of QUS.

P60 – Compliance with bisphosphonate treatment in post-menopausal osteoporosis—a cross-sectional questionnaire study

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In randomised placebo-controlled studies (RCTs), pharmaceutical treatment of post-menopausal osteoporosis reduces the incidence of fractures with 40 to 50 percent. In these studies, compliance with treatment has ranged from 50 to 89 percent. Participants RCTs are, however, highly selected, motivated, and receive special care. We investigated the compliance with bisphosphonate treatment for post-menopausal osteoporosis in a consecutive series of patients in the clinical setting of a secondary referral centre.

Methods: During the 2-year study period 353 patients were diagnosed with osteoporosis. All patients were issued a self administered questionnaire. 110 patients received bisphosphonates and chose to participate in the study. Sixty-seven patients did not respond, 5 had died, 24 lost to follow up, 147 patients were treated with other drugs and were excluded from the study. No patients had emigrated.

Results: At 1, 2, and 3 years compliance with bisphosphonates was 96%, 62%, 42%, respectively. Seventy-six percent stated that they had received information on treatment from the prescribing doctor. These patients were significantly more compliant than those who felt less informed ($p < 0.001$). Moreover, the level of education was significantly ($P=0.04$) related to compliance. Compliance was similar among patients on etidronate (66%) and alendronate (72%) ($p=0.55$) and in patients with (76%) and without (61%) previous osteoporotic fractures ($p=0.09$). The proportion of patients with adverse events were similar between compliant and non-compliant patients ($p=0.66$). Finally, neither the retail price of the drugs, disability-score, or age were significantly different among compliant and non-compliant patients.

Conclusion: Our study confirms that compliance with bisphopshonates is much lower in a clinical setting than in RCTs. Carefully provided information at the time of prescription is the most important predictor of compliance. Also, the level of education is significantly related to complicance. We suggest that specific education of the patients may increase compliance with treatment.

P61 – Compliance and adherence are related to the effectiveness of bisphosphonate treatment

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Bisphosphonates (BP's) are an effective treatment of Osteoporosis (OPO). In clinical practice with unselected patients the effect has yet to be evaluated. The objective of this study was to compare the effect of 4 different BP's on BMD and bone turnover changes in unselected patients. Methods:161 female patients underwent spinal QCT and vertebral morphometry and were thus diagnosed suffering osteoporosis. The patients were divided in four groups: 2 groups with oral BP treatment (group I: etidronate (ETD); 14 days 400 mg/d, 76 days 500mg calcium/d), (group II: alendronate (ALN); 10 mg/d) and 2 groups with I.V. BP-treatment (group III: pamidronate (APD); 30 mg every 3 months) and group IV: ibandronate (IBD); 2 mg every 3 months). The I.V. treatment was chosen in patients with severe comorbidity and/ or on multiple drug therapy (>3 drugs). Characteristics of the 161 female patients:

	n	Age years	Preval. FRX (%)	BMD mg/ml	Comorb/ pat (%)
Group I	39	63.3	27.5	73.2	36
Group II	21	58.9	22.5	75.2	20
Group III	61	66.0	37.7	68.3	59
Group IV	40	65.7	16.4	64.7	52

Bone turnover was significantly decreased after 3 and 12 months in group III and IV using I.V. BP`S: APD ($p < 0.01$) and IBD ($p < 0.07$). In the orally treated patients there was no significant change in bone turnover observed.

	BMD (mg/ml)	BMD 1 year (mg/ml)	BMD 2 years (mg/ml)
ETD	72.2	81.0	90.2
ALN	71.5	73.3	59.8
APD	69.5	67.3	69.1
IBD	63.5	58.7	63.7

Although the I.V. treatments with BP's decreased the in bone turnover significantly, no increase in BMD could be observed, but the BMD was stabilised. The difference between the orally treated patients- BMD increase in group I and BMD decrease in group II may be to due compliance and adherence problems.

P62 – Risedronate reduces vertebral fracture risk independent of pre-treatment bone turnover

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Previous studies have shown that patients with vertebral osteoporosis and accelerated pre-treatment bone resorption (PBR) have greater gains in bone mass during anti-resorptive treatment than patients with lower PBR. To investigate whether these observations are also relevant with regard to fracture incidence (FI), we studied the effect of PBR on vertebral FI in the Risedronate (RIS) phase III clinical trials, including 1196 postmenopausal osteoporotic women from both the VERT and HIP programs. Bone resorption rate was assessed by urinary deoxypyridinoline (uDPD; normative median (NM) = 15.4 nmol/mmol creat). FI was evaluated by annual vertebral radiographs Patients received 5 mg RIS or placebo, 1000 mg calcium and, if needed, 500 IU Vitamin D per day.

Results: As expected, the cumulative new vertebral FI in patients receiving placebo was sig-nificantly higher in those with PBR > NM compared to subjects with PBR < NM. In the pooled data set, RIS reduced the risk of new vertebral fractures after year 1 of treatment irrespective of PBR (RR < NM DPD 0.28, $p = 0.032$ vs. RR> NM DPD 0.33, $p < 0.001$; treatment by PBR interaction $p = 0.808$). The NNTs after one year were 15 in the group with high PBR and 25 in the patients with low PBR, the difference being driven by the higher vertebral fracture risk of patients with high PRB in the placebo group. Risedronate also significantly reduced the risk of new vertebral fractures after 3 years of treatment ($p = 0.002$ high PBR, $p = 0.026$ low PBR), again irrespective of PBR high/low status. The NNTs after 3 years wre 13 for high PBR and 16 for low PBR.

Conclusions: High baseline PBR increases vertebral fracture risk. 5 mg risedronate reduces the risk of new vertebral fractures in postmenopausal osteoporosis irrespective of pre-treatment bone resorption.

P63 – Potential health benefits of phytoestrogens in the case of postmenopausal osteoporosis

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This study was designed to investigate the possible role of phytoestrogens in the reduction of osteoporosis as abrupt decrease in estrogen secretion in postmenopausal women accelerates bone loss.

Methods: The study population consisted of 42 non-smoking women (60–75 years old). Their bone mineral density (BMD) was measured by dual energy X-ray absorptiometry (DXA) and body mass index (BMI) was calculated. The osteoporosis was diagnosed in all patients (T-score by DXA was -2.6 to -3.2). Diet history, especially daily intake of milk and dairy products was ascertained by questionnaire and analyzed the average daily intake of Ca and Mg. Main Ca intake from dairy products formed only one third from recommended intake. The first - patients were treated with sodium alendronate (Fosamax) 10 mg/day during 28 days. Parallel to Fosamax the patients were supplemented with phytoestrogens + minerals Ca, Mg, Zn and vitamins D, C and K for one year.

Results: After 12 months treatment/supplementation we examined the positive changes in BMD: the average of BMD was changed +2.2 to +8.1%.

Conclusion: Supplementation of phytoestrogens, minerals Ca, Mg and Zn and vitamins C, D and K has been proven effective in the reduction of postmenopausal osteoporosis.

P64 – Tibolon in treatment of osteoporosis related to bilateral oophorectomy (experimental research)

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This work has aimed to study the biomechanic, biochemical and osteometric bone data of rats belonging to two age groups (5 and 18 mo.) after surgical bilateral oophorectomy and Tibolon therapy. Altogether 60 white female rats.

Results: Due to bilateral oophorectomy, the bone biomechanic characteristics worsened (bone destruction and bone strength), the osteometric indices decreased, and the biochemical bone properties underwent changes.

Following a six-week Tibolon therapy, there occurred an improvement in the biomechanic, biochemical and osteometric bone data of rats in both groups. Thus in the rats (18 mo. aged) after Tibolon treatment the specific

sagging index decreased from 2.71 ± 0.13 to 2.17 ± 0.15 mcm/N ($p < 0.01$), the destruction moment increased from 161.25 ± 9.15 to 207.00 ± 8.42 GPa ($p < 0.01$) and the minimum work of bone destruction grew from 94.25 ± 5.49 to 121.70 ± 5.05 mJ ($p < 0.01$) compared to operated rats, pointing to the improvement of biomechanic bone properties. Identical changes, albeit statistically insignificant, were observed in the young animals group.

Under effect of Tibolon therapy, the increased values were registered in the following osteometric bone indices of old rats: longest bone length, proximal epiphysis width, greatest diaphysis width, greatest front-back dimension of middle diaphysis, distal epiphysis width ($p < 0.05$).

Conclusion: The Tibolon has proved to be effective in eliminating the structural-functional disturbances and in treating the osteoporosis related to bilateral oophorectomy.

P65 – Hormone replacement therapy and risedronate in postmenopausal women with bone mass loss

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The objective was to determine if the combined use of hormone replacement therapy and risedronate influences on bone mass loss in postmenopausal women.

Material and method: We studied for 12 months 18 women who were 47 to 64 years old at base line, were within 2 and 11 years of menopause, and had a bone mineral density at the lumbar spine between 145 mg/cc and 65 mg/cc measured by the QBMAP system with a spiral CT Picker PQ-S densitometer at L2, L3, L4 and L5. Of all the women, 10 were assigned to transdermal therapy with 50 µg/day of 17 beta-oestradiol on a intermitent cyclic regimen (28 out of 35 days) combined with 100 mg/day of micronized progesterone, 800 IU of vitamin D3 and 1 g of calcium carbonate supplementation. 8 were treated with 5 mg of risedronate, were assigned to transdermal therapy with 50 µg/day of 17 beta-oestradiol on a intermitent cyclic regimen (28 out of 35 days) combined with 100 mg/day of micronized progesterone, 800 IU of vitamin D3 and 1 g of calcium carbonate supplementation. The SPSS programme was used for statiscal analysis.

Results: The characteristics of the women recruited for both groups were similar. Mean mineral bone density at the lumbar spine was between 1 and 3 DS below the mean value for 30 years old normal premenopausal women. After a treatment of 12 months statistically significant difference was found among both groups as for the bone mineral density at the lumbar spine.

Conclusions: It is necessary to carry out a wider and longer study but it seems that the combination of HRT and risedronate contribute advantages to decrease the bone mass loss in postmenopausal women, at least, at lumbar spine.